

European Zebrafish Meeting 2026

ABSTRACT BOOK

ORAL PRESENTATIONS

EMERGING TECHNOLOGIES I

305

Light-mediated control over gene expression for lineage tracing and clonal cancer modeling

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Topic

2 Emerging Technologies

Abstract Content

Tightly regulated expression of genes at the correct time and place is instrumental for proper vertebrate development and deviations can lead to diseases. To investigate normal development and disease-related processes, in vivo interrogation methods with high spatiotemporal resolution are ideally required. Recently, several optogenetic tools to manipulate gene expression with exquisite control in time and space have emerged, but in vivo applications of such systems have remained challenging. Here, we present the novel transgenic zebrafish strain zHORSE with inducible expression of a light-controlled Cre recombinase. We show that zHORSE endows spatiotemporal control over gene expression at different developmental stages down to single cell resolution. We demonstrate that zHORSE can be applied for lineage tracing and for targeted expression of oncogenes. zHORSE is compatible with existing Cre/loxP zebrafish effector strains and will enable applications ranging from dissecting and manipulating development to clonal cancer modeling.

71

Error-free and efficient prime editing in absence of maternal Polθ

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Topic

2 Emerging Technologies

Abstract Content

Faithfully introducing patient-derived pathogenic mutations remains an inefficient process in zebrafish. The standard strategy relies on homology-directed repair (HDR) of double-strand breaks (DSBs) induced by CRISPR-Cas9. However, this approach typically yields edit rates below 10% and is frequently plagued by unintended mutations. To circumvent these limitations, prime editing—a CRISPR-based method utilizing a Cas9 nickase fused to a reverse transcriptase—can be employed to introduce edits encoded in a prime editing guide RNA (pegRNA) extension. While prime editing and other single-strand break tools were designed to minimize the collateral damage associated with DSB repair, prime editing in zebrafish embryos remains notably imprecise, often generating more unintended indels than precise edits.

In this study, we demonstrate that virtually all unwanted mutations are generated by DNA polymerase θ (Pol θ) dependent microhomology-mediated end-joining (MMEJ). We show that the loss of maternally deposited Polθ abolishes these unintended mutations and can increase precise edit rates to over 50%, resulting in near-perfect precision. By eliminating the primary source of error during CRISPR prime editing, our work resolves a major obstacle, enabling the routine and rapid phenotyping of pathogenic mutations in zebrafish larvae.

261

Leveraging CRISPR integration to build intracellular signaling sensors

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Topic

2 Emerging Technologies

Abstract Content

To self-organize into tissues and organs, cells coordinate by exchanging information through signaling pathways. Biosensors that report signaling activity in real time can provide key insights into this process. Unfortunately, traditional reporters of signaling activity suffer from shortcomings such as poor kinetics, positional effects, and lack of specificity. A system to overcome such limitations has been reported in *C. elegans* and consists of an effector protease fused to the endogenous Notch protein and a nuclear-localized sensor. When signaling is active, the effector localizes to the nucleus and cleaves the sensor, resulting in a quantifiable change in its subcellular localization. To adopt this system, we developed sensor and effector constructs that function when expressed transiently in zebrafish embryos. However, detection of physiologic signaling activity requires endogenous genomic

modification of the corresponding transcription factors, which is generally low in efficiency and technically challenging in zebrafish. To achieve this, we successfully piloted a modification to the SALSA system using the short epitope tag ALFA, which we found can be integrated into the relevant loci with high efficiency. This increase in efficiency allows us to generate sensors with relative ease and extend the approach to other signaling pathways, such as Wnt. Taken together, this suite of sensors allows us to report intracellular signaling in a developing vertebrate in real time, with high accuracy and temporal resolution.

394

Nano-pulling promotes axon regeneration in vivo

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Topic

2 Emerging Technologies

Abstract Content

Mechanical force is a powerful inducer of axon growth. Our team developed and validated a technique called nano-pulling, which uses magnetic nanoparticles (MNPs) internalized by neurons and guided by external magnetic fields to apply controlled mechanical forces. This method has been shown to promote axon elongation, directional growth, synapse maturation, and neuronal differentiation. Encouraged by these findings, we extended our investigation to an in vivo model of neural regeneration. Zebrafish represent a powerful platform for studying the regeneration of the central nervous system (CNS): unlike mammals, they mount a highly regenerative response after spinal cord injury (SCI) reactivating pro-regenerative pathways and promoting robust axonal reconnection. This capacity is especially pronounced in larvae, which restore spinal cord continuity and locomotor function within few days, providing an accessible system to track the dynamics of CNS regeneration.

Here, we use the zebrafish model to investigate whether nano-pulling can stimulate axon growth and regeneration in vivo. We optimized a SCI model in zebrafish larvae, assessing survival and physiological functions. MNPs were injected in the spinal cord of 1 day post fertilization larvae. Confocal microscopy confirmed that MNPs remained localized within the spinal cord for several days after injection. Four hours after MNPs administration, the lesion was performed. Motor recovery was assessed at 1 and 4 days post lesion using the Visual Motor Response assay, which measures locomotor responses to dark-light transitions.

We found that, in SCI larvae, the group subjected to nano-pulling showed a significantly improve motor function compared to control groups, indicating faster functional recovery of spinal circuitry.

Overall, our data support the hypothesis that nano-pulling can promote axonal regeneration in vivo. Given the established clinical use of iron oxide nanoparticles and magnetic fields in medicine, future studies will further investigate the translational potential of this technology for the treatment of CNS lesions.

326

Genome editing with programmed short DNA integration using Prime Editors to establish a zebrafish model for Robinow syndrome

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Topic

2 Emerging Technologies

Abstract Content

CRISPR/Cas9-mediated genome editing has advanced target mutagenesis in zebrafish; however, stochastic integration of random insertions and deletions limits precise genome manipulation. Prime Editors, modified Cas9 proteins fused with reverse transcriptase, enable programmed short DNA modification *in vitro* and *in vivo*. In this study, we tested the functioning of a nickase- and a nuclease-based Prime Editors for the precise insertion of a 3 bp stop codon into the endogenous target gene to generate a mutant allele that produces a precisely truncated protein. The Wnt cognate receptor ROR2 regulates embryonic development and organ formation, and mutations in the ROR2 gene are associated with Robinow syndrome in humans. By injecting Prime Editor mRNA or ribonucleoprotein complex with prime editing guide RNAs, we integrated the programmed 3 bp insertion into the zebrafish *ror2* gene to establish a model for Robinow syndrome. We found that the nuclease-based Prime Editor (PEn) outperformed nickase-based PE2 in editing efficiency, whereas PEn also induced a significantly higher amount of random indels. The prime edit modification was transmitted to the next generation with high efficiency, and the stable mutant fish showed shorter tails in hatching larvae and cranio-skeletal abnormalities in adults. To further evaluate our approach, we also inserted a 30 bp nuclear localisation signal into a reporter transgene and translocated GFP signals to the nucleus. These results demonstrated that prime editing can efficiently generate genetically modified zebrafish without using exogenous donor DNA.

52

An orthogonal Cro/O_R3 binary system for targeted gene expression

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Topic

2 Emerging Technologies

Abstract Content

Background: Precise spatiotemporal control of gene expression is essential for dissecting complex biological systems and understanding cell-type-specific functions in vivo. Although several binary expression systems are available, orthogonal platforms that function robustly across species remain limited.

Methods: We developed a phage-derived Cro/O_R3 binary transcriptional activation system and evaluated its performance in zebrafish and human cells. A Cro repressor fused to the VP16 activation domain (CroF2) was used to drive expression of O_R3 operator-linked genes. Cell-type specificity was achieved using enhancer-trap and promoter-controlled zebrafish Cro driver lines. As a proof of concept, we targeted glutamate transporter Glast/Slc1a3b-expressing cells in the central nervous system and expressed a human ataxia-associated, uptake-deficient Glast/Slc1a3b variant. In parallel, Gal4/UAS-mediated labeling was used to visualize neurons in the spinal cord.

Results: The CroF2 transcription activator robustly activated OR3-linked transgenes in both zebrafish and human cells. The CroF2/O_R3-mediated expression of the uptake-deficient Glast/Slc1a3b variant impaired astrocyte development and resulted in uncoordinated spastic locomotion. Concurrent Gal4/UAS-mediated visualization of motor neurons revealed markedly disrupted positioning, demonstrating their selective sensitivity to extracellular glutamate homeostasis during spinal circuit assembly.

Conclusion: The Cro/O_R3 system functions as a robust and orthogonal platform for targeted gene expression across species. This technology enables precise mechanistic dissection of cell-type-specific functions and intercellular interactions underlying complex biological processes.

DISEASE MODELS I

246

A constitutively active variant of the adhesion GPCR Adgrg6 (Gpr126) with novel ear defects and precocious peripheral myelination

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The adhesion class G-protein-coupled receptor (aGPCR) ADGRG6 (GPR126) is expressed in multiple tissues and cell types in the developing vertebrate embryo, and has conserved roles in peripheral myelination. In humans, recessive loss-of-function mutations in *ADGRG6* are causative for a lethal congenital neurological disease characterised by joint contractures. Conversely, over-expression of *ADGRG6* mRNA is associated with several cancers, suggesting that mis-regulated receptor activity in later life is oncogenic.

We have used CRISPR/Cas9 mutagenesis in zebrafish to disrupt the Adgrg6 GAIN domain, a highly conserved extracellular motif with autoproteolytic activity. An in-frame 3-base-pair insertion mutation, predicting the introduction of a single amino acid, converts the protein into a constitutively active, gain-of-function form. Zebrafish embryos heterozygous for the mutation show a fully penetrant dominant phenotype, characterised by precocious expression of the fusion plate marker *nr4a3* in the inner ear, and the myelination marker *mbpa* in Schwann cells of the posterior lateral line nerve. Phenotypic defects are potentiated by increased temperature and pharmacological treatments that raise levels of cyclic AMP. Juvenile and adult heterozygous fish have behavioural deficits characteristic of vestibular dysfunction, including loss of balance and circling. Structural modelling of the folded protein indicates that the mutation disrupts a region of the GAIN domain positioned directly adjacent to the GPCR Proteolytic Site (GPS) and the Stachel sequence, an encrypted intramolecular agonist.

We hypothesise that the amino acid insertion either facilitates GPS cleavage, promotes protein dissociation after cleavage, or destabilises encryption of the Stachel sequence within the GAIN domain, resulting in uncontrolled receptor signalling through $G_{\alpha}s$, adenylyl cyclase and cAMP.

352

A synthetic genetic interaction screen in zebrafish uncovers novel modifiers of developmental ocular disorders

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Topic

1 Disease Models

Abstract Content

Variable expressivity and penetrance are pervasive in developmental genetics and human disease, often reflecting background modifiers that enhance or suppress phenotypes. Yet systematic identification of genetic modifiers in vertebrates has been limited by the lack of efficient *in vivo* interaction screens. In congenital ocular disorders that are compatible with life, such as microphthalmia, anophthalmia and coloboma (MAC), our knowledge of genetic modifiers is sparse, despite well-documented partial penetrance and unilaterality.

We established a paradigm for synthetic genetic interaction (SGI) mapping in zebrafish using CRISPR F0 ("crispant") knockouts in combination with background alleles that show mild and/or low-penetrance ocular phenotypes. The screen enabled rapid assessment of single, double and triple mutant/crispant combinations affecting early eye development. An initial digenic screen testing >150 combinations revealed infrequent (~12% of tests), but specific interactions between genes (including *nr2f5/epha4a* showing fully penetrant bilateral coloboma and *mab21l2/cxcr4a* enhancing microphthalmia), in addition to uncovering novel single-gene phenotypes (*epha4a*, *efna5a*, *nr2f5* and *cxcr4a*). Some alleles, such as *yap1*^{nl13}, interacted with many other genes to consistently increase coloboma penetrance and severity, suggesting the presence of interaction hub genes as proposed for interaction networks in other model systems. To test the robustness of ocular development to higher-order perturbation, we performed a trigenic interaction screen, uncovering frequent (~67% of tests) but often incompletely penetrant interactions.

Together, these results show that vertebrate eye development is highly resilient to genetic perturbation and reveal an interaction network underlying MAC severity. Our study demonstrates the feasibility of crispant-based genetic interaction mapping in vertebrates and nominates several human orthologues (*NR2F1/2*, *HES1/4*, *CXCR4*, *EPHA4*, *EFNA5*, *FAM43A*, *FABP4*, *MAB21L2*, *YAP1*) as candidate genes and genetic modifiers in MAC and related ocular disorders. Additional contributors to this project will be acknowledged in the presentation.

118

Role of myeloid cells and leukotriene signalling in Uveal Melanoma progression

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Topic

1 Disease Models

Abstract Content

For many years, genetic lesions have been regarded as the primary drivers of tumour initiation and progression. Tumours were traditionally viewed as isolated entities, to be directly targeted for therapeutic benefit. However, recent findings on the immune cells as active players in tumorigenesis and the success of immunotherapy for tumour treatment have shifted this perspective. Tumour is now considered a complex disease involving interactions between neoplastic cells and their surrounding environment, commonly referred to as the tumour microenvironment (TME). Mutual communication between tumour cells and the TME appears to be critical for the development of many malignancies.

By combining single-cell transcriptomics with *in vivo* live imaging, we investigated the critical role of the TME and the crosstalk between tumour cells and myeloid cells in driving tumour progression. Our models include transgenic zebrafish models of uveal melanoma (UM) and larval human-zebrafish xenografts.

We observed the activation of YAP signalling, an oncogenic pathway turned on in the majority of UM, in zebrafish transgenic cancer models induced by mutant Gαq or Gα11, alpha subunits of trimeric G-proteins associated to cysteinyl leukotriene receptors (CYSLTRs). Notably, leukotrienes, eicosanoid inflammatory mediators released in the TME, induced nuclear translocation of YAP and upregulation of YAP targets in primary UM cells and in zebrafish UM explants. Analysis of the single-cell transcriptome of zebrafish UM models identified both tumour and myeloid cells, mostly neutrophils, as producers of leukotrienes. Transplantation of human UM cells in zebrafish revealed a pro-active interaction between cancer cells and neutrophils, which promoted tumour progression.

Ongoing studies using our zebrafish UM models and transplantation assays aim to elucidate the mechanics of tumour cell-neutrophil communication, with particular focus on the leukotriene–CYSLTR2 axis. Our findings underscore the importance of cell-to-cell communication in tumour progression and provide a foundation for future investigations into microenvironment-targeting therapies.

61

Ultra-rare biallelic THAP12 variants cause loss of function and underlie severe epileptic encephalopathy

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Topic

1 Disease Models

Abstract Content

Background: Developmental and epileptic encephalopathies (DEEs) are severe childhood-onset neurological disorders frequently caused by rare genetic variants disrupting brain development and neuronal excitability. Despite advances in genome-wide sequencing, a substantial proportion of DEE cases remain without a molecular diagnosis.

Methods: Whole-genome sequencing was performed in two affected siblings presenting with infantile spasms evolving into Lennox–Gastaut syndrome. Functional consequences of identified variants were investigated using in vitro assays and in vivo modeling in mice and zebrafish. Transcriptomic profiling of larval zebrafish brains was conducted to assess downstream molecular effects, and rescue experiments were performed using wild-type and patient-derived human THAP12 mRNA.

ResultsWe identified compound heterozygous variants in THAP12 associated with reduced protein abundance, consistent with a loss-of-function mechanism. Mouse models carrying either patient-specific allele exhibited embryonic lethality in both homozygous and compound heterozygous states, demonstrating an essential and dosage-sensitive role for Thap12 during early development. Zebrafish loss-of-function models recapitulated key features of the human phenotype, including microcephaly, brain hypoplasia, abnormal neuronal activity, and increased seizure susceptibility. Transcriptomic analyses revealed dysregulation of cell cycle and apoptotic pathways, consistent with increased cell death and reduced proliferation observed in mutant embryos. Importantly, overexpression of wild-type human THAP12 mRNA rescued these phenotypes, whereas the patient-derived allele did not.

Conclusion: These findings establish THAP12 as a novel gene underlying autosomal recessive DEE and demonstrate that biallelic loss-of-function disrupts early brain development and neuronal survival. This work expands the spectrum of genetic causes of DEE and highlights the importance of investigating ultra-rare variants to improve diagnosis in unresolved cases.

91

Compound inheritance of EHHADH and MASP1 mutations in non-syndromic cleft lip: familial genomic analysis and functional characterisation in zebrafish

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Topic

1 Disease Models

Abstract Content

Background. Non-syndromic cleft lip with or without cleft palate (NSCL/P) affects 1–7 per 1,000 live births and remains genetically unexplained in most familial cases. Single-gene models account for only a fraction of observed heritability, pointing to oligogenic mechanisms involving rare variants acting in combination. We identified a Polish family with three affected individuals and linked the phenotype to mutations in two adjacent genes on chromosome 3q27.

Methods. Whole-genome sequencing was performed across all family members. Candidate variants were prioritized by population rarity, predicted pathogenicity, and familial co-segregation. Functional consequences were assessed in zebrafish by mRNA microinjection, craniofacial phenotype scoring, and Alcian Blue cartilage staining. Ongoing studies extend this work using Sox10 immunofluorescence to assess NCC fate in vivo, and scratch assays with peroxisome morphology analysis in HEK293 and BJ fibroblast cell lines.

Results. We identified compound heterozygous variants in *EHHADH*, encoding a peroxisomal bifunctional enzyme, and *MASP1*, a lectin complement serine protease — both co-segregating with the cleft phenotype across the family. Zebrafish co-injection of both mutant forms produced more severe craniofacial malformations than either variant alone, including cleft-like defects of the ethmoid plate. Expression analysis revealed sequential activity of *MASP1* during early NCC migration and *EHHADH* during cartilage maturation.

Conclusion. Our results support a compound inheritance model in which disruption of peroxisomal β-oxidation and complement-mediated NCC guidance act together to exceed the developmental threshold for normal palatogenesis. We are currently investigating whether *EHHADH* loss drives NCC apoptosis while *MASP1* disruption impairs directed migration, to establish the mechanistic basis of their interaction in craniofacial morphogenesis.

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GERMLINE & EARLY DEVELOPMENT I

59

Capturing the dynamics of DNA repair across embryogenesis with light-activated CRISPR

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Topic

9 Germline and Early Development

Abstract Content

Background

Accurate and timely repair of DNA double-strand breaks (DSBs) is essential for genome maintenance in all cells. Embryos are particularly vulnerable to DSBs. During zebrafish development, a single fertilized cell undergoes rapid divisions to form an embryo of 50,000 cells in the first 24 hours, subjecting its genome to intense replication stress and the inevitable formation of genomic DSBs. While we know that failure to repair these breaks can result in embryonic lethality, the kinetics, fidelity, and pathway choice of DSB repair during embryogenesis are not well understood.

Methods

Here, we used light-activated CRISPR to generate targeted genomic DSBs across zebrafish embryo development. Importantly, DSB induction occurs within seconds after light stimulation, enabling precise measurements of repair kinetics within a single cell cycle.

Results

We found that DSBs were repaired within minutes during the early, rapid-division stages. At later stages, the pace of DNA repair declines as the cell cycle slows. By leveraging mathematical modeling and mutants that disrupt DNA repair pathways, we uncovered a developmental transition from error-prone microhomology-mediated end joining to more faithful non-homologous end joining that correlates with the gradual shift in repair kinetics.

Conclusion

To our knowledge, this is the first study to resolve DSB repair dynamics with high temporal resolution during embryo development. Our study establishes a framework

for systematically interrogating the cellular responses to DNA damage in living organisms.

389

The physical biology of time and tempo in embryonic pattern formation

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Topic

9 Germline and Early Development

Abstract Content

A central problem in development is to understand how the tempo of embryogenesis is set. Embryos can adjust their developmental speed according to the environment, resulting in sped-up development in warm and slowed-down development in cold conditions. However, the mechanisms that control the tempo of the underlying patterning processes are still unclear. One of the first patterns established during vertebrate development is the formation of the three germ layers. This process is controlled by diffusible morphogens, whose distribution in the embryo is crucial for correct patterning. How do extracellular morphogen gradients adapt to different developmental speeds? The classical Arrhenius equation postulates that reactions scale exponentially with changes in temperature, resulting in a 2-3-fold change in reaction speed with every 10°C. In contrast, the Einstein-Stokes equation states that diffusion changes linearly with temperature in Kelvin – for a morphogen that experiences a 10°C difference at physiologically relevant temperatures, this would translate into a mere change of 3%, incompatible with the observed changes in growth. To address this conundrum, we used automated high-throughput measurements to quantify developmental speed in medaka embryos across a broad temperature range. We then systematically quantified temperature-dependent changes in production and degradation using photoconvertible morphogens, and measured morphogen mobility at local and global tissue scales using fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP). Interestingly, while developmental speed, morphogen production and degradation scaled in an Arrhenius-like manner, morphogen diffusion remained unchanged despite proportional rescaling of the gradients themselves. Our mathematical simulations suggest that this robustness arises because the system operates in a reaction-dominated regime, in which changes in reaction and growth kinetics – rather than diffusion – are sufficient to rescale morphogen gradients. Together, our experiments and simulations provide new insights into how morphogen gradients adjust to maintain tissue patterning across changes in developmental time and tempo.

175

Zygotene cilia control meiotic chromosome pairing and germ cell development from zebrafish to humans: a new framework for human infertility

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Topic

9 Germline and Early Development

Abstract Content

Developing germ cells must accurately organize their chromosomes for successful fertilization and embryonic development through the program of meiosis. Hallmarks of meiosis are homologous chromosomal pairing and recombination. Errors in these processes during gametogenesis are a major cause of aneuploidy, infertility, miscarriages, and developmental disorders. Chromosomal pairing is controlled by a cytoplasmic cytoskeletal machinery. Telomeres tether to Sun/KASH complexes on the nuclear envelope and associate with perinuclear microtubules that are organized from the centrosome. Dynein-mediated telomere motion around the nuclear envelope drives chromosome homology-searching movements. Ultimately, pulling of telomeres towards the centrosome forms the "chromosomal bouquet" configuration. However, despite the bouquet discovery in 1900, the underlying biomechanical regulation has remained elusive.

We recently discovered the zygotene cilium in zebrafish oocytes, which mechanically anchors the centrosome to facilitate chromosomal pairing dynamics and is essential for female meiosis. Here, we show that cilia also regulate chromosomal dynamics in zebrafish male meiosis and are essential for testis morphogenesis and fertility. Using germ cell-specific conditional elimination of zygotene cilia in mice, we establish previously unrecognized ciliary functions in chromosomal pairing, gametogenesis, and fertility in mammals. Furthermore, we document the occurrence of zygotene cilia in human meiotic germ cells by identifying ciliary structures as well as gene expression signature through single-cell transcriptomics. Finally, we have identified high impact variants in ciliary genes in women and men with unexplained premature ovarian insufficiency or crypto-/azoospermia, indicating that zygotene cilia defects may underlie their reproductive failure, and expanding the spectrum of ciliopathies.

Together, our findings uncover a conserved fundamental mechanism in the regulation of chromosomal dynamics in meiosis, and implicate dysfunction of the zygotene cilium as an overlooked etiology of human infertility. Zebrafish models currently being generated for the human variants will identify variant-specific defects underlying infertility and illuminate mechanisms of the zygotene cilium. *Preprint: https://doi.org/10.21203/rs.3.rs-7733942/v1

113

A novel disordered protein Pinchado (pin) reveals Balbiani body RNA granule organisation and the mechanisms governing oocyte polarity

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Topic

9 Germline and Early Development

Abstract Content

The Balbiani body (Bb) is a conserved, membrane-less organelle composed of mRNAs, proteins, mitochondria, endoplasmic reticulum (ER), and Golgi complex in oocytes of many animals including zebrafish. In zebrafish, Bb plays crucial roles in germplasm localisation in oocytes and specification of the future embryonic animal-vegetal (AV) axis. How RNA granules within the Bb are organised and how the Bb contributes to oocyte polarisation is not fully understood. We identified a novel maternal factor, *pinchado (pin),* which harbours disordered regions, as a potential germplasm and Bb component. Maternal *pin* mutants produce defective eggs with multiple openings in chorion and loss of oocyte animal-vegetal polarity, with circumferential cytoplasmic distribution.

We demonstrate that *pin* mRNA is a bonafide component of the Bb. Using smFISH, we find that zebrafish Bb RNP granules are organised in nested layers, with distinct localisation patterns of germplasm RNAs in the Bb layers. In *pin* mutant oocytes, the Bb is enlarged and/or dispersed, and localisation of germplasm markers (e.g. *dazl*) is altered, suggesting compromised Bb and germplasm integrity. Proteomic analysis of early oocytes revealed no significant changes in overall Bb protein composition in *pin* mutants compared to wildtype oocytes. Transcriptomic profiling of mutant *pin* oocytes suggests differential expression of cytoskeletal regulators and ER stress markers. Consistently, *pin* mutants display altered microtubule organisation and aberrant nuclear actin accumulation.

Thus, Pin has crucial roles in **maintaining Bb integrity, germplasm RNA distribution, cytoskeletal organisation, and oocyte polarity.** Our findings identify a previously

uncharacterised organisation of RNP granules within the Bb, and provide new insights into the mechanisms governing oocyte polarity in zebrafish.

451

Elucidating how sexual fate is determined in the African turquoise killifish aging model

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Topic

9 Germline and Early Development

Abstract Content

Background: The African turquoise killifish (*Nothobranchius furzeri*) is the shortest-lived vertebrate that can be bred in captivity. With a lifespan of one year and distinct aging phenotypes, *N. furzeri* constitutes an excellent aging model. It displays a strong sexual dimorphism, leading to profound differences between sexes that affect lifespan. *N. furzeri* has an XX/XY sex-determination system with *gdf6* as a suspected sex-determining gene. We aimed to elucidate whether and how the Y-chromosomal *gdf6* (*gdf6Y*) determines sex in *N. furzeri*.

Methods: We applied CRISPR/Cas9 and transgenesis to address *gdf6* gene functions. Furthermore, we analyzed transcriptomes from males and females at different developmental stages or from heterologous cell culture systems to elucidate the timeframe of *N. furzeri's* sexual development and to identify Gdf6Y-responsive genes, respectively. To localize gene expression in gonadal cell populations, RNAscope was utilized.

Results: *Gdf6Y*'s inactivation in XY or expression in XX individuals led to a male-to-female or female-to-male sex reversal, respectively, proving its necessity and sufficiency for the male sex in *N. furzeri*. *Gdf6Y* also retained the skeletal developmental function shared with the X-chromosomal *gdf6* allele. Concerning sex determination, *gdf6Y* is expressed by somatic supporting cells of the developing testes. Female germline proliferation, meiosis, and oogenesis are the earliest measurable processes in *N. furzeri's* sexual development. However, *gdf6Y* induces the male sex in a germline-independent manner, like in mammals, and in contrast to zebrafish. Regarding *gdf6Y* downstream effectors, we identified the mRNA decay activator *zfp36l2* as a new Gdf6 signaling target.

Conclusion: We validated *gdf6Y* as the sex-determining gene in *N. furzeri* and revealed that it determines sex by signaling from and to the somatic supporting cells. Next, we want to investigate the role of *gdf6Y*'s downstream factors in sexual development, the influence of gonosomal genetic content on lifespan, and generate *N. furzeri* sex reporter lines.

CARDIOVASCULAR I

362

Divergent Immune Programs in Diseased and Regenerating Cardiac Valves

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Topic

4 Cardiovascular

Abstract Content

Background

Cardiac valves ensure unidirectional blood flow and are continuously exposed to mechanical stress, making them particularly susceptible to disease. A major contributor to cardiovascular dysfunction is soft tissue calcification, characterized by abnormal mineral deposition within cardiac tissues. Although this process is frequently observed in aging and cardiovascular disease, the cellular populations and signaling pathways that initiate and propagate calcification remain incompletely understood. Understanding how tissue homeostasis is disrupted and how this differs from regenerative responses is essential for developing new therapeutic strategies.

Methods

Using zebrafish as a model system, we combined genetic models of cardiovascular calcification with single-cell RNA sequencing, tissue analyses, and live imaging approaches. We analyzed cellular composition and transcriptional states during disease progression and compared them with a cardiac valve regeneration model to identify cellular and molecular differences between pathological and regenerative conditions.

Results

Prior to the onset of calcification, we observed increased endothelial apoptosis, immune cell infiltration, and valve thickening. These changes were accompanied by endothelial cell death and a marked expansion of valve interstitial and immune cell populations, indicating overactivation of structural and inflammatory responses that remodel the extracellular matrix and contribute to calcification. Notably, immune cells displayed distinct transcriptional profiles when comparing calcification and regeneration models, indicating that similar cell types can adopt divergent molecular programs that either promote pathological mineralization or support tissue repair.

Conclusion

Our findings highlight coordinated multicellular dynamics that precede cardiac valve calcification and reveal key differences between degenerative and regenerative programs. Ongoing work aims to selectively manipulate specific cell populations and leverage live imaging in zebrafish to visualize how these cells interact in vivo, ultimately identifying mechanisms capable of shifting diseased tissue back toward homeostasis.

357

Flow-mediated endothelial remodelling and inflammation drive developmental vascular susceptibility in *ldlr* knockout zebrafish

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Topic

4 Cardiovascular

Abstract Content

Background:

Atherosclerosis, the leading cause of cardiovascular disease, is associated with aberrant lipid metabolism, endothelial dysfunction, and chronic inflammation, yet its early manifestations and mechanisms remain incompletely understood.

Methods:

As low-density lipoprotein receptor loss of function is the most common monogenic cause of atherosclerosis, we employed low-density lipoprotein receptor knockout (*ldlr*-/-) zebrafish to investigate the developmental origins of atherosclerotic cardiovascular disease.

Results:

Single-cell RNA-sequencing under differential flow conditions in embryonic *ldlr*-/- zebrafish identified a population of disproportionately stressed endothelial cells marked by overexpression of heat shock protein 70 (*hsp70*). Hsp70 is induced in stressed endothelial cells in a flow-dependent manner in zebrafish and in a subset of human endothelial cells, and its activation is associated with disrupted remodeling angiogenesis *in vivo*. Genetic and pharmacological studies demonstrated that *hsp70* upregulation inhibits vascular apoptosis and ciliogenesis, leading to altered angiogenic remodeling. Concurrently, pro-inflammatory processes, including enhanced myelopoiesis and thrombogenicity, are amplified at early stages in *ldlr*-/-

zebrafish, which also exhibit impaired regenerative angiogenesis and heightened neutrophil recruitment post-vascular injury.

Conclusion:

Our findings reveal how abnormalities in flow-mediated endothelial remodelling and inflammation converge during embryogenesis to drive vascular susceptibility to hemodynamic and other stressors in *ldlr* loss of function.

27

Fasting reverses PLN R14del-mediated cardiomyopathy through lysosomal reactivation

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Topic

4 Cardiovascular

Abstract Content

The heart is a highly organized organ and its function depends on tightly coordinated calcium signaling, cytoskeletal integrity and intracellular protein turnover. Disruption of these processes can lead to cardiomyopathies, a diverse group of heart muscle disorders often caused by genetic variants. Variants in *PLN,* encoding phospholamban (PLN), the main inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), have been linked to both dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ACM). Among these, the PLN Arg14del (R14del) variant is the most prevalent. PLN R14del cardiomyopathy is characterized by the accumulation of large perinuclear PLN protein aggregates in cardiomyocytes of end-stage heart failure tissue. However, the mechanisms driving aggregate formation and their role in disease progression remain unresolved.

Using a humanized *plna* R14del zebrafish model and left ventricular tissue obtained from patients with end-stage cardiomyopathy carrying the PLN R14del variant, we demonstrate that previously described PLN aggregates are sarcoplasmic reticulum (SR)-derived PLN-containing vesicles that form due to impaired SERCA2a activity and increased cytosolic Ca^{2+} levels. Additionally, we show that these SR-derived vesicles localize at the microtubule organizing center (MTOC), where they are associate with lysosomes. Interestingly, we find that Ca^{2+} dysregulation in *plna* R14del hearts leads to reduced lysosomal function resulting in vesicle accumulation near the nucleus. This perinuclear accumulation induces microtubule aster formation, nuclear deformation and sarcomere disorganization. Strikingly, reactivating lysosomal function through fasting reduces SR-derived vesicle accumulation, restores microtubule integrity and rescues cellular organization in *plna* R14del zebrafish hearts.

Together, these findings identify impaired lysosomal clearance of SR-derived vesicles and the resulting microtubule disorganization as key pathological mechanisms driving PLN R14del

cardiomyopathy. Additionally, our results highlight lysosomal reactivation as a promising potential therapeutic strategy to halt or reverse PLN R14del cardiomyopathy progression.

133

Smarca4 Maintains Mitochondrial Homeostasis and Energy Metabolism during Cardiac Development

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Topic

4 Cardiovascular

Abstract Content

Background

Mitochondrial metabolism is essential for cardiac and skeletal muscle function due to the high adenosine triphosphate (ATP) demand required for sustained contractility. While mitochondrial dysfunction is a hallmark of metabolic myopathies, the epigenetic mechanisms regulating mitochondrial structure and energy metabolism during muscle development remain poorly understood.

Methods

To investigate this, we characterized a *smarca4a*-deficient zebrafish model (*smarca4a*$^{a8-/-}$) and performed integrated transcriptomic and chromatin accessibility analyses, including heart-specific RNA-seq, ATAC-seq, and single-cell RNA-seq. Mitochondrial structure and function were assessed using high-resolution confocal imaging and Seahorse-based metabolic profiling. In addition, AAV-mediated SMARCA4 knockdown was performed in human cardiomyocytes and murine myotubes to examine the conservation of the observed mechanisms.

Results

Loss of Smarca4 resulted in ventricular hypoplasia, pericardial edema, and disorganized skeletal muscle, accompanied by severe impairment of cardiac and muscular function. Multi-omics analyses revealed reduced chromatin accessibility and transcriptional suppression of genes involved in mitochondrial biogenesis and oxidative phosphorylation. Consistent with these findings, Smarca4 deficiency led to marked reductions in mitochondrial content, respiratory capacity, and ATP production. Similar mitochondrial defects were observed following SMARCA4 depletion in mammalian muscle cells.

Conclusion

These findings identify Smarca4 as a conserved chromatin remodeling factor that links nuclear gene regulation to mitochondrial energy homeostasis during vertebrate muscle development.

85

A Novel Computational Model of the Adult Zebrafish Action Potential and Intracellular Calcium Transient

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Topic

4 Cardiovascular

Abstract Content

Background:

The zebrafish is an increasingly popular experimental model for studying cardiac electrophysiology and autonomic control, due to its physiological similarity to humans and unique experimental accessibility. While computational modelling of the action potential (AP) and calcium (Ca^{2+}) transient (CaT) is advanced in mammals, adult cardiac zebrafish-specific models are lacking. We developed novel computational models of the zebrafish atrial and ventricular AP and CaT using an experimental–computational approach, and used them to investigate chamber-specific autonomic effects on electrophysiology, Ca^{2+} dynamics, and mechanics.

Methods:

Ion current formulations from the 2004 Ten Tusscher and Panfilov model were re-parametrized using zebrafish patch-clamp data. The transient outward and the plateau potassium currents (not present in the Zebrafish) were removed from the original model, and the T-type Ca^{2+} current and the acetylcholine dependent potassium current, $I_{K,ACH}$ were added. The model was calibrated with microelectrode recordings and optical mapping of the AP and CaT in isolated adult zebrafish atria and ventricles. Simulations of autonomic effects were compared to pharmacological experiments targeting sympathetic and parasympathetic receptors, and preliminary mechanical work-loop data were collected to assess autonomic-induced changes in contractile force.

Results:

The computational models reproduced the zebrafish AP, CaT, and rate-dependent responses. Pharmacological experiments showed responses largely consistent with mammalian data, though chamber-specific differences emerged: adrenergic stimulation prolonged the atrial AP and CaT but shortened the ventricular AP, whereas cholinergic stimulation caused AP shortening in the atrium and prolongation in the ventricle. Mechanical experiments showed that adrenergic stimulation increased peak force and loop area, consistent with human data, providing useful information for the next phase of model development.

Conclusions:

In conclusion, we present the first adult zebrafish-specific computational models of the atrial and ventricular AP and CaT. These models are valuable tools for future experimental-computational investigations of cardiac (patho)-physiology and pharmacological interventions using zebrafish.

449

Transient Membrane Fusion Facilitates Metabolic Switching and Proliferation in Developing Cardiomyocytes

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Topic

4 Cardiovascular

Abstract Content

Background: Cardiomyocyte (CM) proliferation is an essential step during heart development and regeneration. The proliferative potential of developing CMs is heavily dependent on their metabolic states, with higher rates of glycolytic metabolism being associated with increased proliferation. Although glycolytic activation was originally thought to be a direct consequence of hypoxia, it is increasingly recognized that active mitochondrial processes play an important role in metabolic reprogramming and cell fate determination.

Methods: In this context, we studied how mitochondrial density, health, and activity influence the metabolic profile and proliferative potential of developing CMs. To do this, we combined genetic, transcriptomic, and functional staining approaches. To measure glycolytic activation in live larval hearts, we relied on genetically encoded Peredox, a NADH:NAD+ ratiometric reporter. We also performed live imaging of beating *Tg(myl7:mitoRFP; myl7:EGFP-Has.HRAS)* hearts to observe transient fusion events. We utilized FACS along with bulk RNA sequencing approaches to explore the transcriptomic effects of glycolytic activation and transient fusion.

Results: We found that mitochondrial membrane potential and oxidation status, rather than density, are predictive of CM glycolytic activation levels. We observed, to our knowledge, for the first time, discrete homotypic, transient membrane fusion events between CMs and identified unidirectional movement of mitochondria between fused cells, suggesting the facilitation of intercellular mitochondrial traffic. RNAseq analysis revealed that transient fusion influences the expression of metabolism and proliferation-related genes, including various mitochondrial genes. We confirmed that a potential downstream effector of transient fusion can partially rescue the proliferative impairment caused by morpholino-induced CM fusion deficiency.

Conclusions: Our results support the hypothesis that fusion events in the developing heart promote mitochondrial composition changes and transcriptional reprogramming of CMs, facilitating, in turn, metabolic switching and proliferation. These findings advance our understanding of how mitochondrial dynamics regulate cardiac development and may have implications for cardiac development and regeneration.

MORPHOGENESIS & ORGANOGENESIS I

267

Dysfunction of Ciliary Transition Zone Proteins Leads to Body Curvature and Scoliosis in Zebrafish

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Proper extension of the body axis is essential for axial stability in vertebrates. In zebrafish, defects in ciliary motility cause abnormal body axis development, including embryonic ventral curvature and later scoliosis. This phenotype is largely attributed to reduced expression of the urotensin neuropeptides Urp1 and Urp2 secreted by cerebrospinal fluid–contacting neurons in the central canal. Here we show that dysfunction of ciliary transition zone (TZ) proteins, which function as gatekeepers of ciliary signaling, causes scoliosis through a distinct mechanism. Unlike classical ciliary motility mutants that exhibit ventral curvature, TZ mutants display embryonic dorsal curvature. We find that this phenotype results from ectopic expression of Urp2, in contrast to the loss of Urp2 expression observed in motility mutants. Mechanistically, TZ proteins regulate Hedgehog (Hh) signaling through the PKA–pCREB pathway to control floor plate development. Disruption of this pathway leads to ectopic activation of urotensin signaling, ultimately causing body curvature and scoliosis. Together, our findings reveal a previously unrecognized role of TZ proteins in regulating body axis morphogenesis and suggest that cilia selectively modulate specific branches of Hh signaling in Hh-secreting cells.

343

Rbm8a regulate ciliogenesis through PCP signaling pathway in zebrafish

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Cilia are essential cellular organelles that drive motility and mediate sensory signaling. Defects in cilia cause a broad spectrum of disorders affecting multiple organs, including laterality defects, limb abnormalities, neural defects, and polycystic kidney disease. Although numerous genes

required for ciliary structure and function have been identified, how RNA processing mechanisms contribute to ciliogenesis remains poorly understood.

To identify novel regulators of ciliary biology, we performed a functional genomics screen in zebrafish using pronephric cyst formation as a phenotypic readout. We identified several candidate regulators of ciliogenesis, including **RBM8A**, a core component of the **Exon Junction Complex**.

In humans, mutations in RBM8A cause **Thrombocytopenia-absent radius (TAR) syndrome**, a rare congenital disorder characterized by thrombocytopenia, forelimb anomalies, and cardiac and renal defects. Consistent with a potential role in renal development, rbm8a crispant zebrafish develop pronephric cysts. Detailed phenotypic analysis revealed reduced cilia and disrupted Hedgehog signaling in rbm8a mutants, suggesting a role for Rbm8a in ciliogenesis.

Unexpectedly, RNA-seq analysis did not reveal significant downregulation of canonical ciliary genes, indicating that Rbm8a does not regulate ciliogenesis through direct transcriptional control of ciliary components. Instead, we observed defects in planar cell polarity (PCP) within the pronephric tubules. Long-read sequencing further revealed widespread splicing defects affecting multiple PCP pathway genes in *rbm8a* mutants. Functional analyses demonstrated that disruption of several PCP genes results in pronephric cyst formation and ciliary defects in zebrafish embryos. Moreover, PCP proteins exhibit planar-polarized localization in the pronephric tubule epithelium, a pattern disrupted in *rbm8a* mutants.

Together, our findings reveal an unexpected link between RNA splicing and ciliogenesis and identify Rbm8a as a key regulator of cilia formation through the PCP pathway. These results provide a new mechanistic framework connecting exon junction complex function to ciliary biology and offer potential insights into the pathogenesis of TAR syndrome.

414

The Importance of Being Empty: The Development of Brain Ventricles in the Context of Ion Channels and Gravity

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Background: During development the brain ventricular system (BVS) provides space for newly born neurons and acts as a reservoir for circulating cerebrospinal fluid (CSF). The BVS accommodates the Reissner fiber (RF), which together with CSF-contacting neurons (CSF-Nr) plays a role of the graviceptor. The RF is a rope-like structure composed of microfilaments that originate from the midline floor plate and anterior roof plate-derived subcommissural organ (SCO). The RF extends through the BVS and the central canal to the end of the spinal cord. The RF is found in vertebrates with horizontal body posture. The RF consists of the giant matricellular protein Scospondin (Sspo) and several auxiliary polypeptides.

Methods: We used a combination of immunohistochemistry, light-sheet microscopy, and genetic and toxicological analyses to study RF development under normal gravity in different positions.

Results: RF develops through two mechanisms. First, the posterior RF forms due to secretion from the apical surface of the floor plate. It remains attached to the anterior floor plate (flexural organ), though in the hindbrain it detaches from the floor plate soon after formation. Second, the anterior RF forms in the cerebral aqueduct through SCO secretion. It attaches to the flexural organ, where it fuses with the posterior RF. Later, the SCO elongates posteriorward through mechanoelastic stretching. Analysis of mutants affecting the two subunits of the voltage-gated potassium channel Kv2.1 revealed that Kv2.1 regulates BVS and RF development. Toxicological analyses demonstrated that RF formation depends on Hedgehog and Wnt/β-catenin signaling pathways, as well as cholesterol. Maintaining the developing embryo in fixed orthograde and retrograde positions causes deformation of the RF and SCO.

Conclusions: RF and SCO development depends on multiple internal (developmental) and external (gravity) inputs.

157

SIX1/4 Orchestration of Endodermal-Neural Crest Crosstalk Patterns the Craniofacial Skeleton

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

In humans, while heterozygous mutations in *SIX1* gene cause Branchio-otic Syndrome, homozygous mutations are predominantly lethal, underscoring their absolute requirement for survival. Despite its known importance, the specific roles of this factor within the complex landscape of craniofacial tissues—including the neural crest, ectoderm, and endoderm—remain unresolved. In this study, we utilize zebrafish to characterize *six1a/4a* and *six1b/4b* mutants. While *six1a/4a* loss is non-lethal, *six1b/4b* mutants exhibit profound defects in placode-derived structures (pituitary, ear, olfactory, lateral line), as well as compromised gills and opercular flaps. Notable skeletal abnormalities include altered hyomandibular morphology and a major reduction of branchial arch cartilages. To capture the regulatory landscape prior to these gross morphological changes, we conducted single-nucleus multiome analysis (snRNA-seq + snATAC-seq) at 18 hours post fertilization. Our data show that, although early ectomesenchyme (neural crest) remains largely unaffected at the transcriptomic level, chromatin accessibility and gene expression are clearly altered in epithelial-derived tissues. Most importantly, we identified early defects in the endoderm, including disrupted planar cell polarity and misregulation of *itga5* and *notch2*. Importantly, loss-of-function of these specific genes has been previously shown to phenocopy the endodermal pouch and hyoid domain malformations observed in our mutants, suggesting they are key mediators of the six1/4 regulatory network. Mosaic rescue experiments

confirmed that *six1/4* function cell-autonomously in the epithelia and endoderm, while their influence on neural crest patterning is cell-non-autonomous, mediated by the endodermal pouches. Together, these findings clarify why loss of *six1/4* is so catastrophic, highlighting a critical orchestrating role that bridges tissue layers during craniofacial morphogenesis.

128

Dissecting control of skeletal muscle growth by exercise, nutrition and the circadian clock: what people can learn from fish about staying fit.

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Background: How various aspects of whole-body physiology combine to control muscle growth and maintenance is poorly understood but of importance in metabolic health and ageing, sport and agri/aquaculture.

Methods: Growth of the zebrafish myotome can be imaged in vivo at single cell resolution, something impossible in other genetically-tractable model vertebrates. Over recent years, we have used confocl microscopy to elucidate how muscle forms and then grows through a combination of new myofibre formation from committed stem cells and increase in size of pre-existing myofibres.

Results: In early larvae, muscle activity and nutrition are both required for optimal growth. Muscle grows twice as fast during the day than at night under the control of the molecular circadian clock. Increased autophagy at night limits growth and is controlled by the clock acting within muscle fibres. Muscle clock disruption leads to premature sarcopenia (ageing-related muscle weakening). In feeding larvae, adequate nutrition differentially regulates stem cell-dependent and -independent growth and suggests a 'shopping bag' hypothesis for cell size control. Moreover, physical force derived from muscle contraction cooperates with the clock and nutrition to promote muscle growth, mechanistically involving an interaction between TORC1 activity and redox metabolism.

Conclusion: Our findings illuminate not only how those 'reps' in the gym may drive muscle growth, but also how muscles weaken with age and what we might do about it.

448

Zebrafish Semicircular Canal Development Requires the Serine Protease Tmprss5

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Zebrafish semicircular canal (SSC) development is an example of highly complex epithelial morphogenesis, involving the fusion of opposing extracellular matrix (ECM)-filled epithelial projections to form continuous pillars of tissue. Gene regulation controls ECM deposition and tissue fusion, but despite advancements in this area, the SSC developmental gene regulatory network (GRN) remains incomplete. tp219e is an adult-viable ENU-derived SSC mutant, previously mapped to chromosome 15, which exhibits aberrant projection outgrowth in the developing ear. This phenotype has characteristics similar to mutants of the adhesion GPCR gene adgrg6, in which epithelial projections overgrow and fail to fuse, resulting in a lack of SSC formation. Adult homozygous mutants for both adgrg6 and tp219e have balance defects and abnormal swimming behaviours.

To identify the genetic lesion in tp219e mutants, we performed bulk RNA sequencing on pooled 3-day-post-fertilisation zebrafish larvae, with subsequent homozygosity mapping of single nucleotide polymorphisms alongside differential gene expression analysis between mutants and wild types. Candidate genes were compared against the open-source single cell RNA sequencing database Daniocell for inner ear-specific expression. We identified a G>A transition mutation in tmprss5, predicting a Gly>Asp substitution in the Tmprss5 protein, as the most likely causative lesion. tmprss5 is expressed at the tips of the outgrowing projections in the ear, and is up-regulated in mutant embryos. We have used CRISPR/Cas9 to generate a truncating allele of tmprss5, which phenocopies the ear defects in tp219e, and fails to complement the tp219e missense mutation. Taken together, these data confirm tmprss5 as the causative gene.

Tmprss5 is a type II transmembrane serine protease with no previously identified role in the developing inner ear. We are currently testing whether Adgrg6 is a proteolytic substrate of Tmprss5, and undertaking a detailed phenotypic characterisation of the tmprss5 -/- ear using fluorescence microscopy and gene expression analysis.

TOXICOLOGY I

93

Development of a high-content analysis workflow to screen the effect of endocrine disrupting chemicals on macrophages and enteric neurons in transgenic zebrafish embryos

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Topic

10 Toxicology

Abstract Content

Industrially manufactured chemicals are released into the environment, where their potential adverse effects on living organisms are typically uncharacterized. One particularly sensitive biological target of chemical pollutants is the endocrine system. Current EU regulations state that Endocrine Disrupting Chemicals (EDCs) must be avoided. However, while there are a range of cell-based testing systems to detect potential EDCs, there are a lack of alternative models that consider the full complexity of the endocrine system. Here, we developed an alternative zebrafish embryo-based assay to detect potential EDC effects on intestinal inflammation and enteric nervous system (ENS) development, both targets of ingested EDCs. Using Tg(mpeg1:mCherry) zebrafish embryos, we developed an imaging and analysis workflow to detect region-specific inflammation based on macrophage count and shape, derived from three dimensional (3D) images. The workflow automates anatomical region detection in transmitted light images using the AI-based nnU-Net model to distinguish structures. We then applied Voronoi-Otsu labelling of cells in regions of interest by combining pre-processing, thresholding, and watershed segmentation algorithms to automatically identify individual macrophages in 3D fluorescence images. To establish the screening assay, multiple reference chemicals and exposure windows were assessed. Exposure to 0.15% Dextran Sodium Sulfate (DSS) from 3-5 days post fertilization (dpf) increased intestinal macrophages by 10%. Bisphenol A was selected as a prototypical EDC. Exposure to 10 uM BPA from 3-5 dpf resulted in morphological changes in macrophages, including increased size and volume, which indicates an enhanced proinflammatory state. Intestinal macrophage infiltration supports normal ENS development. In line with previous

work, exposure to 1 µM mycophenolate from 2-3 dpf caused a 35% reduction in ENS cell counts in 5 dpf Tg(phox2bb:GFP) larvae. As part of the HORIZON ENDOMIX project, future work will examine whether exposure to mixtures of co-occurring EDCs identified in human cohorts alters macrophage functionality or ENS development.

103

Distinct and Reversible Neural Mechanisms Governing Anxiety-Like Behaviour Following Acute Exposure to Contaminants of Emerging Concern in Zebrafish

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Topic

10 Toxicology

Abstract Content

Contaminants of emerging concern (CECs) are increasingly detected in aquatic environments and can alter neural function and behaviour. However, chemically distinct contaminants often produce similar neurobehavioral phenotypes despite acting through different biological mechanisms. Understanding how contaminant classes perturb neural signalling and behaviour is therefore critical for interpreting toxicological outcomes and recovery processes.

Using adult zebrafish together with our behavioural analysis platform ZebraTrack, we investigated mechanisms underlying anxiety-like behaviour induced by two widely detected CECs representing distinct chemical classes: the organophosphate formulation Roundup and the organochlorine antimicrobial triclosan.

Acute exposure to Roundup (12.8 mg/L) and triclosan (0.6 mg/L), produced robust anxiety-like behaviour within 6 h, characterised by reduced locomotor activity and increased freezing behaviour.

Roundup-induced anxiety-like behaviour was associated with suppression of brain acetylcholinesterase (AChE) activity without detectable changes in reactive oxygen species. Behavioural recovery occurred only after contaminant withdrawal and coincided with restoration of AChE activity. Pharmacological inhibition of AChE using donepezil prevented behavioural recovery, demonstrating that restoration of cholinergic signalling is required for behavioural normalization.

In contrast, triclosan-induced anxiety-like behaviour was independent of cholinergic disruption and oxidative stress. Acute exposure significantly reduced serotonin levels while expression of canonical stress-related genes (BDNF, CRH, CRHR1) also remained unchanged. Behavioural recovery occurred spontaneously within 24 h and was accompanied by restoration of serotonin levels. Pretreatment with the selective serotonin reuptake inhibitor fluoxetine prevented triclosan-induced anxiety.

Together, these findings demonstrate that although distinct CECs induce similar behavioural phenotypes through different neural pathways, the mechanisms governing behavioural recovery are also pathway-specific. Defining these recovery mechanisms provides critical insight into how neural systems restore function following chemical perturbation.

164

The zebrafish Visual and Acoustic Motor Response (VAMR) assay has the potential to add value to the Developmental Neurotoxicity In Vitro Battery (DNT IVB)

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Topic

10 Toxicology

Abstract Content

Less than 150 chemicals have been evaluated for developmental neurotoxicity (DNT) using OECD rodent-based test guidelines. Although the DNT in vitro battery (DNT IVB), comprised of 17 human or rodent cell-based assays, is a proposed alternative, uncertainties limit regulatory implementation. Recent work identified three false negative compounds that are developmentally neurotoxic in rodent or human studies but negative in the DNT IVB (5,5-diphenylhydantoin, maneb, nicotine). We hypothesized that multi-behavioral phenotyping in early-life stage zebrafish can complement the DNT IVB to improve battery sensitivity. Zebrafish were acutely (40 min) or developmentally (1-5 days post-fertilization) exposed to 12 DNT positive chemicals or 0.4% DMSO. Additionally, five predicted DNT negative compounds (acetaminophen, D-mannitol, ibuprofen, saccharin, and sodium benzoate) were evaluated under both exposure paradigms. Behavior was evaluated using the Visual and Acoustic Motor Response (VAMR) assay, comprised of 26 behavioral endpoints, including visual and acoustic startle responses, and non-associative habituation learning. Hit calls suggest that inclusion of the zebrafish VAMR assay within the DNT IVB would increase battery sensitivity without compromising battery specificity. Hits in the VAMR assay were detected at 1-4 orders of magnitude lower concentrations relative to the DNT IVB. This work supports the concept that the zebrafish behavior-based VAMR assay can add value to the DNT IVB by increasing test battery sensitivity and enhancing potency detection. To build confidence in using zebrafish behavioral data for human risk assessment, an 88-chemical set of DNT-positive and DNT-negative compounds is undergoing evaluation as part of the European Partnership for the Assessment of Risks from Chemicals. This effort aims to define the molecular applicability domain of the VAMR assay and to more broadly evaluate assay performance relative to the DNT IVB.

330

Fungal screen reveals novel compounds coltripyrone A and B that reversibly arrest zebrafish embryonic development by inhibition of oxidative phosphorylation

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Topic

10 Toxicology

Abstract Content

Fungi are well-known for their production of a wide variety of medicinal compounds. Yet, the majority of fungi have not yet been assed for the production of biologically active compounds, suggesting there is a wealth of bioactive compounds still to be discovered. Previously, we generated a library of 10,207 fungal filtrates and tested their biological activity using zebrafish embryos. 56 filtrates induced a developmental delay/arrest in zebrafish embryos. From selected fungi, we purified and identified two newly described fungal compounds, coltripyrone A and B, and citreoviridin A, a known ATP-synthase inhibitor. All three compounds induced developmental arrest in zebrafish embryos, however the arrest in response to coltripyrone A and B was reversible, whereas the effect of citreoviridin A was irreversible. Metabolic flux analyses on zebrafish embryos revealed that coltripyrone A and B reversibly inhibited oxidative phosphorylation (OXPHOS), resulting in reversibly reduced adenosine triphosphate (ATP) levels. In human HepG2 cells OXPHOS was inhibited by coltripyrone A and B as well and HepG2 cells showed elevated glycolysis. Metabolomics showed that several pathways were affected including ATP-generation and the purine salvage pathway. We conclude that coltripyrone A and B are OXPHOS inhibitors with reversible effects on mammalian cells and zebrafish embryos. Elevated OXPHOS is a hallmark of various diseases including certain types of cancer, including leukemia and melanoma. Coltripyrones may be interesting lead compounds for further development as drugs against these diseases.

271

Linking Chemical and Genetic Disruption of Retinoic Acid Signaling to Developmental Phenotypes in Zebrafish Embryos Using High-Content Imaging

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Topic

10 Toxicology

Abstract Content

Regulations require developmental toxicity data for chemical registration. The zebrafish embryo model is proposed as a screening or alternative model for developmental toxicity information. As proof of principle, we focused on the retinoic acid (RA) signaling pathway, crucial in vertebrate embryonic development, and a key molecular target of developmental toxicants. Chemicals can disrupt RA homeostasis by interfering with RA synthesis and metabolism through ALDH1A and CYP26 enzyme families. However, quantitative evidence linking disruption of chemical RA signaling to specific phenotypic outcomes is lacking.

We combined automated imaging of zebrafish embryos with high-content image analysis and morphometric quantification. An automated microinjection system was used for transient CRISPR/Cas9 gene editing to investigate how chemical disruption of RA signaling translates into developmental phenotypes. Zebrafish embryos were also exposed to well-known RA pathway modulators, including the synthesis inhibitor DEAB and the metabolism inhibitor talarozole. This is one of the first examples where transient gene editing was combined with a robust morphological assessment using quantitative image assessment with a robotic imaging platform to compare chemical effects with a CRISPR/Cas9-mediated pathway disruption.

DEAB exposure induced concentration-dependent morphological alterations, including reduced or missing pectoral fins, cornea abnormalities, defects in ventral and caudal fin folds, and malformed craniofacial structures. Talarozole exposure induced similar alterations and additionally caused abnormally curved tail tips. Comparable phenotypes were also observed following CRISPR-based knockdowns. Notably, curved tail tips appeared specifically in CYP26A1 disruption, indicating a key role of this gene in tail development. Comparative analysis revealed consistent patterns between chemical and genetic perturbations, supporting a mechanistic link between RA signaling disruption and specific developmental abnormalities.

These findings demonstrate that integrating high-content zebrafish phenotyping with targeted genetic perturbation provides a powerful framework for identifying and mechanistically interpreting chemicals that interfere with RA signaling and vertebrate development, and lays the foundation for identifying pathway-specific biomarkers.

81

Comparative Neurobehavioral Toxicity of Synthetic Cannabinoid Receptor Agonists in Zebrafish

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Topic

10 Toxicology

Abstract Content

Synthetic cannabinoid receptor agonists (SCRAs) represent a significant challenge due to their high potency, rapid dissemination in illicit markets, and limited *in vivo* experimental data. Structurally related compounds may exhibit distinct pharmacodynamic and toxicological profiles, highlighting the need for sensitive models for behavioral screening and characterization of neuroactive effects. The aim of this work was to compare the acute effects of MDMB-4en-PINACA, ADB-INACA, and CHO-4'Me-5'Br-FUBOXPYRA in zebrafish using locomotor and spatial paradigms in larvae and adults.

Larvae were exposed to the compounds (10 µM) and evaluated in thigmotaxis and light-dark tests using an automated system. Total distance traveled, mean velocity, number of zone transitions, and time spent in the inner zone were continuously recorded during an 80-min protocol with light-dark cycles. Adults were exposed for 30 min (10 µM) and assessed in the light-dark, mirror, open field, and novel tank tests, with analysis of latency, time in the zone of interest, and number of transitions. The study was approved by the CEUA/UNICAMP Animal Ethics Committee.

MDMB-4en-PINACA induced persistent hypolocomotion in larvae, with significant reductions in distance traveled and velocity across most time intervals, along with a transient increase in thigmotactic behavior. In adults, it increased latency to explore the light zone, reduced the number of transitions in the light-dark and novel tank tests, and increased time spent in the area distant from the mirror, indicating reduced exploratory behavior and anxiogenic modulation. In contrast, ADB-INACA did not produce locomotor or spatial alterations in larvae. CHO-4'Me-5'Br-FUBOXPYRA also failed to induce locomotor changes, although punctual spatial variations were observed.

Although belonging to the same class, the evaluated SCRAs exhibited distinct profiles, with more pronounced locomotor depressant and exploratory alterations for MDMB-4en-PINACA. These findings reinforce zebrafish as a sensitive model for comparative neurobehavioral toxicity screening of emerging synthetic cannabinoids.

NEUROBIOLOGY

368

Early-Life Microbiota Shapes Neuro-Glia-Vascular Organisation and Hypothalamic Development in Zebrafish

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Topic

7 Neural Circuit Behavior

Abstract Content

Background

Early-life microbial colonisation is increasingly recognised as an important regulator of brain development through microbiota–brain interactions. While microbiota perturbation has been strongly linked to stress- and social-behavioural outcomes, the early developmental mechanisms through which microbial signals shape the neural circuits remain less well understood. The hypothalamus is a key neuroendocrine hub for stress and social behaviour and may represent an important site of microbiota-dependent development. Here, we used germ-free zebrafish to examine whether microbial depletion disrupts early neurodevelopment by altering neuro-glia-vascular organisation, with particular focus on hypothalamic maturation and downstream behavioural outcomes.

Methods

Using germ-free (GF) zebrafish larvae, vascular development was assessed in the endothelial reporter line kdrl:EGFP. Glial organisation and its association with developing blood vessels were examined through GFAP expression. qPCR analysis of larval heads was used to assess microbiota-mediated changes in neurodevelopmental, hypothalamic, and stress-related gene expression. Hypothalamic neuronal populations, including oxytocinergic neurons, were analysed to evaluate effects on hypothalamic maturation. Cellular proliferation was quantified using phospho-histone H3 (PH3) as a marker of mitotic activity. Functional consequences were assessed using behavioural assays including light-dark preference and acoustic startle responses.

Results

GF larvae displayed altered vascular architecture, including reduced fluorescence in the kdrl:EGFP reporter line, together with disrupted GFAP-labelled neuro-glial organisation. These changes were accompanied by microbiota-dependent differences in the

expression of neurodevelopmental and hypothalamic genes. Consistent with this, GF larvae showed altered organisation of hypothalamic oxytocinergic neurons and reduced numbers of proliferating PH3-positive cells within the hypothalamic region. At the functional level, microbial depletion was associated with altered behavioural responses.

Conclusion

Together, these findings suggest that early microbial colonisation supports coordinated neurodevelopment by shaping neuro-glia-vascular organisation and hypothalamic maturation. Our results identify the hypothalamus as a candidate developmental centre through which microbiota-dependent signals may influence the emergence of stress-related behavioural phenotypes in zebrafish.

11

Opening the gate: FishNAP in vivo screening identifies compounds that increase blood–brain barrier permeability

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Topic

3 Neurobiology

Abstract Content

The blood–brain barrier (BBB) is a restrictive property of the brain vasculature that limits molecular exchange between the circulation and the central nervous system. While essential for brain homeostasis, this restrictiveness presents a major challenge for therapeutic delivery. To identify small molecules that modulate BBB permeability in vivo, we developed FishNAP, a non-invasive zebrafish assay for quantifying BBB permeability. Using this platform, we screened 2,320 FDA-approved small molecules and identified seven compounds that reproducibly increase BBB permeability. These compounds act through multiple signaling pathways, with only two converging on the same pathway, vitamin D. These same vitamin D agonists increased BBB permeability in adult mice, further indicating a conserved role for vitamin D regulation of BBB function across vertebrates. Mechanistically, these compounds did not activate vitamin D signaling in vascular endothelial cells but instead induced signaling in surrounding astroglial and neuronal cells. In order to test whether activation in either cell type was sufficient to modulate BBB properties, we mosaically activated vitamin D signaling in either astroglia or neurons and observed increased BBB permeability with vitamin D activation in either cell type. Together, these findings identify vitamin D signaling in non-vascular cells as a conserved regulator of BBB permeability and demonstrate the utility of zebrafish-based in vivo screening approaches for uncovering mechanisms that modulate barrier function.

45

The effects of oestrogen and estrogenic endocrine-disrupting chemicals on vertebrate brain development and health

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Topic

3 Neurobiology

Abstract Content

Oestrogen, traditionally regarded as a female sex hormone, is now recognised as a key regulator of brain development in all vertebrates, regardless of sex. Disruptions in oestrogen signalling during early embryogenesis—prior to sexual differentiation—are increasingly associated with neurodevelopmental conditions such as autism spectrum disorder, reduced IQ, and altered social behaviours. A major health concern is the widespread exposure to oestrogenic endocrine-disrupting chemicals (eEDCs) which can mimic or interfere with endogenous oestrogen and have been aligned with long-term neurological impairments. Despite these risks, the molecular roles of oestrogen in early brain development remain poorly understood.

Using oestrogen biosensor zebrafish models, we identified a novel population of glial cells in the embryonic olfactory bulb—termed estrogen-responsive olfactory bulb (EROB) cells—as the first site of oestrogen activity in the developing brain. These cells interact closely with olfactory sensory neurons and are essential for organising olfactory glomeruli. Pharmacological alterations of oestrogen signalling or ablation of EROB cells disrupts glomerular development and affects both intrinsic and odour-evoked neuronal activity, leading to impairments in olfaction-mediated behaviours during larval stages.

Our findings demonstrate that oestrogen, via EROB cells, plays a critical role in shaping early olfactory circuits. Preliminary data further show that oestrogen triggers gene transcription in other brain regions, while eEDCs can function either as oestrogen mimics or antagonists, disrupting developmental gene regulation in a brain region- and chemical- specific manner. Our zebrafish model provides a powerful *in vivo* platform to explore conserved oestrogen signalling pathways and their disruption by environmental pollutants.

445

Gene regulatory programs in zebrafish enteric nervous system development

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Topic

3 Neurobiology

Abstract Content

A large proportion of the Western population suffer from unsolved gastrointestinal (GI) symptoms drastically affecting quality of life. Many GI disorders are associated with enteric nervous system (ENS) dysfunction. The neural crest-derived ENS is the intrinsic gut nervous system and consists of a complex network of neurons and glial cells. Despite the essential role of the ENS in regulating gut function, we lack a comprehensive understanding of the gene regulatory programs that control ENS differentiation. Consequently, the genetic basis of many ENS disorders and GI diseases with an ENS component remains poorly understood. Our work centers on understanding the gene regulatory programs that control neuronal and glial development in the ENS. We used a single-cell RNA-sequencing (scRNA-seq) dataset of ENS cells extracted for a whole embryo atlas spanning 1 to 7 days post fertilization to capture both non-neuronal and neuronal ENS cell types and ENS-specific ATAC-sequencing to identify putative enhancers across ENS development. Based on this data set, we present a comprehensive characterization of non-neuronal ENS cell types as well as a model of enteric neuronal fate specification. We find that enteric progenitor cell marker genes are expressed throughout non-neuronal cell clusters in a “salt and pepper” expression pattern resulting in transcriptional heterogeneity within clusters. Differentially expressed genes reveal specialized cell types such as neural stem cells and multiple glial cell populations. Using developmental trajectory analysis, we propose a new model of glial and neuronal differentiation across ENS development. Based on our ATAC-seq data, we have identified enhancers of key developmental regulators. We are testing their function using reporter constructs and CRISPR genome editing. Together, our findings provide insights into the gen regulatory programs important for ENS development, which is critical for a better understanding of ENS development, and the genetic mechanisms of GI disorders associated with ENS dysfunction.

193

Live Dynamics of Neurofilaments

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Topic

3 Neurobiology

Abstract Content

Neurofilaments (NFs) are neuronal Intermediate Filaments (IFs) that constitute the most abundant component of mature neurons. Earlier studies in mouse revealed that NFs bear essential functions in the nervous system and play critical roles in neurodegeneration. In humans, beyond the genetic involvement of *NF* genes in various neuropathies, abnormal NF aggregation has been identified as an early pathological hallmark for most neurodegenerative diseases. While NF proteins are considered pivotal actors in the maintenance of the nervous system, very little is known regarding their dynamics and signaling in vivo and what triggers neurodegeneration in disease.

To tackle the in vivo dynamics of NFs, we generated a novel transgenic zebrafish line expressing NFs labelled with a photoconvertible fluorophore, thus offering unprecedented spatiotemporal resolution to monitor NF behaviour in axons. We developed a methodology enabling the visualization of mobility, but also directionality and features of NF transport. Strikingly, we reveal that NFs are dynamic structures during the development of the nervous system. Combing experimental, mathematical and computational tools, we further validated, interpreted and quantified experimental data to propose meaningful physiological hypotheses. In addition, we combine this biological model with established tools to investigate the effect of environmental cues such as myelination on NF dynamics.

Overall, using our novel NF zebrafish line, we provide the first evidence for the dynamics of a stable cytoskeleton network in vivo. This work will set the stage to scrutinize fundamental roles of NF during development and in aging, and to uncover pathological signaling in neurodegenerative diseases.

75

The choroid plexus- cerebrospinal fluid system as a conduit for brain-wide signalling

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Topic

3 Neurobiology

Abstract Content

The brain is surrounded by cerebrospinal fluid (CSF) which fills its ventricles and subarachnoid spaces to cushion neural tissue and maintain homeostasis. Produced by the choroid plexus epithelium (ChP), the CSF contains not only water and ions but also a rich repertoire of neuromodulatory proteins linked to sleep, stress, and injury. While ChP-derived factors can travel through the CSF by diffusion or bulk flow depending on molecular size, the physical mechanisms governing their in-vivo

distribution remain poorly defined. Leveraging the optical transparency and genetic accessibility of the zebrafish, we resolved these dynamics with high spatial and temporal precision.

We first established the zebrafish ChP secretome through proteomic profiling of both the ChP and the CSF. To examine how these molecules are distributed throughout the brain, we employed a transgenic system in which fluorescently labelled proteins and extracellular vesicles (EVs) are directly secreted from the ChP into the CSF. By tracking these particles spanning a wide size range, we revealed bulk CSF transport modes that support long-distance movement of neuromodulatory cargo in live, awake animals across developmental stages. We then investigated how ChP-secreted molecules move through the ventricles, penetrate the brain parenchyma, and how this process is shaped by physiological drivers including cardiac pulsatility, locomotor activity, and environmental stressors such as osmotic challenge. In parallel, we introduced caged compounds into the CSF to test how CSF-borne signalling cues, when locally uncaged, can elicit neuronal responses, providing a direct readout of CSF-to-neuron communication.

Together, these findings recast the CSF as an active signalling conduit rather than a purely homeostatic medium. Our work highlights the ChP as a central coordinator of CSF-mediated communication and suggests that the brain exploits CSF flow dynamics to achieve distributed, tissue-wide regulation.

DISEASE MODELS II

67

Systematic Screening of Psychobiotic Bacteria in Zebrafish: Identification of *Lacticaseibacillus brevis* SBC8803 using a Refined Novel Tank Diving Test

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Topic

1 Disease Models

Abstract Content

Background: Identifying "psychobiotics" requires efficient and reproducible behavioral models suitable for large-scale screening. While the Novel Tank Diving Test (NTDT) is the gold standard for assessing anxiety in zebrafish, its reliability is often compromised by environmental noise and handling stress. We combined a systematic microbial screen with a technically refined behavioral protocol to identify potent anxiolytic paraprobiotics.

Methods: We performed a systematic behavioral screen of over 20 bacterial strains in adult male AB zebrafish. To ensure high sensitivity, we utilized an improved NTDT methodology incorporating structural and environmental standardizations to minimize external interference. Following a 4-week feeding period with heat-killed *L. brevis* SBC8803 (2.5×10^{10} cells/g feed), we conducted brain RNA-sequencing and intestinal 16S rRNA-sequencing. Integrative multi-omics analysis via the DIABLO framework identified coordinated gut–brain signatures.

Results: Among the strains tested, SBC8803 emerged as a lead candidate, significantly reducing anxiety-like behavior by shortening latency and increasing entries into the upper tank half without inducing nonspecific hyperlocomotion. Brain transcriptomic profiling revealed activation of serotonin receptor, CREB, and oxytocin signaling pathways. Although gut microbial alpha diversity was unchanged, functional prediction indicated increased palmitate biosynthesis and decreased L-tryptophan biosynthesis. Integration revealed a “vitamin B–serotonin–anti-inflammatory axis,” where enhanced microbial riboflavin (vitamin B2) metabolism coordinates with host neural regulation and suppresses neuroinflammatory signaling, evidenced by the upregulation of the NF-κB inhibitor *nfkbie*.

Conclusion: Integrating large-scale screening with a refined NTDT is a powerful strategy for psychobiotic discovery. *L. brevis* SBC8803 reshapes the gut–brain network at behavioral, microbial, and transcriptomic levels, providing a mechanistic basis for its potential as a treatment for anxiety-related conditions.

325

Characterising zebrafish osteocytes in heath and disease: a foundation for breakthrough discoveries in bone biology

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Topic

1 Disease Models

Abstract Content

Osteocytes, the 'superstars' of bone biology, are now understood to be critical orchestrators of skeletal remodelling. Their complex, neuron-like networks, embedded within the bone matrix, govern mechanotransduction and intercellular communication, processes vital for bone health. Dysregulation of these networks underlies debilitating conditions like osteoporosis and osteoarthritis, impacting millions globally. While zebrafish has emerged as a premier vertebrate model for biomedical research, it lacks a comprehensive characterisation of zebrafish osteocytes, hindering our ability to leverage the powerful genetic tools of zebrafish to investigate fundamental osteocyte biology and translate findings to human health.

Here we report the first characterisation of osteocytes during the lifespan of zebrafish, using advanced high-resolution imaging systems spanning from confocal microscopy, synchrotron micro-computed tomography, and customised Stimulated Raman Scattering (SRS) 3D confocal microscopy, and provide comparative gene expression profiling.

We demonstrate functional osteocytes in zebrafish as early as one-month post-fertilisation. Strikingly, in adults, osteocyte distribution mirrors predicted mechanical loading, with sparse populations in low-load regions. Consistent with mammalian models, zebrafish osteocytes form a dense, neuron-like network with significant variations in dendrite process length, including extended, non-branching processes. Furthermore, aged zebrafish display osteocyte morphology consistent with mammalian aging, including rounded lacunae and micropetrosis. We demonstrate that Sp7, crucial for osteocyte dendritic process formation in mammals, functions similarly in zebrafish, as loss-of-function *sp7* mutants exhibit rounded lacunae and abnormal osteocyte processes. Notably, wild-type zebrafish bones express a broad suite of mammalian osteocyte signature genes, with enrichment of differentially expressed genes involved in the TGFbeta pathway during ageing. Suggesting a high level of evolutionary conservation in osteocyte signalling in health and in disease.

Our detailed characterisation establishes zebrafish as a robust model for studying osteocyte biology, revealing conserved architecture and functional responses, particularly in dendritic process regulation, thereby offering new avenues for investigating skeletal diseases.

205

A multi-species approach toward pharmacological treatment of POLG-related mitochondrial disorders

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Topic

1 Disease Models

Abstract Content

Background

POLG-related mitochondrial disorders, caused by mutations in the catalytic (POLG) and accessory (POLG2) subunits of mitochondrial DNA polymerase γ, remain untreatable due to limited translationally relevant in vivo models. Current mouse models often show embryonic lethality or mild phenotypes, restricting mechanistic studies and preclinical testing. We have established a multi-species platform combining zebrafish *polg* and *polg2* mutants, *POLG* patients' derived primary fibroblasts cultures, and *C. elegans* polg-1-deficient strains to model human POLG dysfunction and enable rapid drug discovery.

Methods

We have systematically assessed survival, morphology, mitochondrial function, and behaviour across zebrafish developmental stages under both *polg* and *polg2* deficiency. Candidate therapeutic compounds, previously selected through yeast- and zebrafish-based screens, have been tested for their ability to restore mitochondrial and muscle function. Top-performing compounds are planned to be validated in *C. elegans* to confirm their effects on conserved POLG-related mechanisms and in patients' derived primary fibroblasts to test their ability to rescue the profound mtDNA copy number depletion observed in these patients.

Results

Zebrafish *polg* and *polg2* mutants recapitulate key patient phenotypes, including mtDNA depletion, skeletal muscle impairment, and locomotor deficits. A subset of compounds

improved mtDNA content and motor behaviour in zebrafish mutants, providing a scalable vertebrate-invertebrate platform for rapid drug repurposing, and accelerating translation of candidate therapies toward clinical evaluation. The mechanism of action of these molecules will be discussed.

Conclusion

Overall, our results support zebrafish as an effective *in vivo* model for studying the early etiopathology of POLG-related disorders. By integrating mechanistic insight with high-throughput pharmacology, our project addresses critical gaps in POLG-related disorder research, establishing a robust translational pipeline and advancing therapeutic discovery for patients with POLG-related disorders. A key future direction will be to assess the translatability of identified molecules to other mitochondrial diseases and to evaluate their potential to counteract cellular aging.

16

Dosage-sensitive 7q11.23 genes regulate the ontogeny of brain asymmetry and lateralized behaviour in zebrafish models of Williams-Beuren Syndrome

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Topic

1 Disease Models

Abstract Content

Background: Cerebral lateralisation influences cognition and social behaviour. Disruptions in this hemispheric specialisation are a hallmark of Williams-Beuren Syndrome (WBS), caused by the 7q11.23 hemideletion. While WBS is clinically defined by hypersociability and cognitive deficits associated with altered lateralisation, the developmental mechanisms linking specific genes and dosage to the disruption of brain asymmetry and laterality remain unexplored.

Methods: We investigated the expression of WBS candidates baz1b and fzd9b in wild-type (WT) embryos. We further generated mutant lines using the Et(gata2a:EGFP)pku588 background, which visualises left-right brain asymmetry in vivo. This allowed us to stratify larvae into left-asymmetric, left-symmetric, or right-asymmetric phenotypes. These groups were analysed via phenotype-specific qPCR for neurotransmitter markers and an automated YOLOv11 pipeline to quantify exploratory behaviour in an open-field test.

Results: In WT embryos, baz1b and fzd9b transcriptions correlate with critical symmetry-breaking windows. Conversely, hemizygosity for baz1b or fzd9b significantly increase the left-symmetric brain phenotype (~35% in baz1b$^{+/-}$; ~8% in fzd9b$^{+/-}$). Open-field behaviour analyses uncovered masked signatures: while unsorted populations exhibited high variability, phenotypic stratification revealed that changes in neuroanatomical asymmetry significantly modulate WBS-like traits. In baz1b mutants, structural brain left-symmetry was specifically associated with altered exploration and reduced bout

frequency. These distinct profiles in stratified baz1b larvae provide a strong rationale for extending investigations to a broader behavioural battery. Investigations into fzd9b neurochemical and functional profiles are ongoing.

Conclusions: Baz1b and fzd9b are essential components of the molecular mechanism regulating brain laterality. By integrating mutant lines into an asymmetry-reporter background, we provide a high-resolution framework for understanding how single-gene dosage imbalances drive the divergent neuroanatomical and behavioural landscapes of WBS.

78

Reverting mitochondrial dysfunction with a specific mPTP inhibitor triggers a pro-regenerative environment in the *sapje* model of DMD.

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Topic

1 Disease Models

Abstract Content

Background

Duchenne muscular dystrophy (DMD) is characterized by progressive muscle degeneration driven by Ca^{2+} dysregulation, mitochondrial dysfunction, and chronic inflammation. While mitochondrial dysfunction has traditionally been considered secondary to sarcolemma instability, emerging evidence suggests it may act as an upstream pathogenic driver that contributes to defective regeneration and persistent immune activation. We investigated whether pharmacological inhibition of the mitochondrial permeability transition pore (mPTP) could restore mitochondrial function and reshape the regenerative and inflammatory landscape in the *sapje* zebrafish model of DMD.

Methods

Sapje larvae were treated with 1µM of the specific mPTP inhibitor TR001, 1µM prednisone analogue (X1), or their combination. We assessed their effects on: i) muscle integrity (birefringence and TEM analyses); ii) motor performance (touch-evoked escape response and distance moved analyses); iii) mitochondrial bioenergetics and dynamics (TMRM assay, Seahorse analysis, and live imaging of mitochondrial biosensors); iv) fish survival (Kaplan-Meier analysis); and v) macrophage recruitment and polarization (live imaging and immunofluorescence analysis).

Results

TR001 treatment significantly improved muscle architecture, restored mitochondrial membrane potential, normalized oxidative metabolism, enhanced motor performance, and extended survival of *sapje* larvae. Importantly, mitochondrial stabilization was associated

with reduced macrophage infiltration and an early shift toward a CD206-positive pro-regenerative (M2-like) phenotype. In contrast, X1 produced only partial structural and functional improvement and failed to fully rescue mitochondrial bioenergetics. Combined treatment did not provide additive benefit, supporting a model in which mitochondrial dysfunction acts upstream of inflammatory activation.

Conclusion

Our findings identify mPTP opening as a central pathogenic node in DMD and demonstrate that targeting mitochondrial dysfunction is sufficient to promote a pro-regenerative microenvironment despite dystrophin deficiency. These results i) position mitochondrial permeability transition as a promising therapeutic target, ii) reinforce the value of zebrafish for mechanistic and translational studies in muscular dystrophy, and iii) support further development of mitochondrial-targeted therapies as potential disease-modifying strategies.

435

Epigenesis (enters the stage) in a vascular anomaly

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Topic

1 Disease Models

Abstract Content

Cerebral cavernous malformations (CCMs) are anomalies of the cerebral vasculature. Loss of the CCM proteins CCM1/KRIT1, CCM2, or CCM3/PDCD10 triggers a MAPK-Krüppel-like factor 2 (KLF2) signaling cascade, which induces a pathophysiological pattern of gene expression. The downstream target genes that are activated by KLF2 are mostly unknown.

We combined genetic approaches in zebrafish, murine, and human endothelial models using transcriptomic analyses, pharmacological inhibition strategies and high-resolution imaging to assess cardiovascular morphology and lesion formation across different CCM disease models

We show that Chromobox Protein Homolog 7 (CBX7), a component of the Polycomb Repressive Complex 1, contributes to pathophysiological KLF2 signaling during zebrafish cardiovascular development. This is the first evidence for an involvement of epigenetic regulation in any vascular anomaly disease.

CBX7/cbx7a mRNA is strongly upregulated in lesions of CCM patients, and in human, mouse, and zebrafish CCM-deficient endothelial cells. The silencing or pharmacological inhibition of CBX7/Cbx7a suppresses pathological CCM phenotypes in *ccm2* zebrafish, *CCM2*-deficient HUVECs, and in a pre-clinical murine CCM3 disease model. Whole-transcriptome datasets from zebrafish cardiovascular tissues and human endothelial cells reveal a role of CBX7/Cbx7a in the activation of KLF2 target genes, including TEK, ANGPT1, WNT9, and endoMT-associated genes.

Our findings uncover an intricate interplay in the regulation of KLF2-dependent biomechanical signaling by CBX7 in CCM. This work also provides insights for therapeutic strategies in the pathogenesis of CCM.

EPIGENETICS AND GENE REGULATON

238

Deciphering regulation and functional conservation of dormant ribosomes

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Cellular quiescence is a reversible state of dormancy where energy-intensive processes like transcription and translation are repressed. As translation is one of the most energy-demanding processes, ribosomes play a central role in quiescence: their activity is suppressed, but a stable pool is maintained to allow rapid reactivation.

We recently identified a conserved set of four factors that stabilize and repress ribosomes in zebrafish and *Xenopus* eggs, generating dormant ribosomes (Leesch et al., 2023). While the composition and function of dormant ribosomes in fish and frogs are known, how they are reactivated during development and their relevance in mammals are unclear.

To address these questions, we expressed the ribosome dormancy factor DAPL1 in mammalian cells and found that it can induce ribosome dormancy in this context as well. Additionally we found that DAPL1's stability depended on ribosome binding since mutations disrupting this interaction led to its proteasomal degradation. Harnessing DAPL1's ribosome binding-dependent stabilization, we performed FACS-based genome-wide CRISPR screens to identify factors able to release DAPL1 from the ribosome. These screens reveal previously underappreciated key roles of conserved components of the core translation machinery in the release of ribosome dormancy.

To study mammalian DAPL1's function in a physiologically relevant context, we studied its role in murine naïve T cells where DAPL1 is endogenously expressed at high levels. We found that DAPL1 associates with ribosomes in quiescent naïve T cells and is released upon T cell activation similar to dormant ribosomes during zebrafish embryonic development.

Together, our results reveal a conserved role for dormant ribosomes in supporting quiescence from fish to mammals, uncovering a novel layer of translational regulation in quiescent cells.

351

A Blast from the Past: 'Living Fossil' Fish Genomes Bridge Gene Regulation Across Long Evolutionary Distances from Zebrafish to Humans

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Identifying orthologous cis-regulatory elements (CREs) across distant vertebrate lineages remains a major challenge. Many CREs are undetectable across taxa due to sequence divergence beyond recognition, CRE turnover, and the emergence of lineage-specific regulatory elements. This disconnect is especially pronounced in widely used model systems like rodents and teleost fishes, which exhibit rapid molecular sequence evolution.

Teleosts, including key biomedical models like zebrafish, are often used to study vertebrate genome function and regulation. However, a lineage-specific teleost genome duplication (TGD) drastically reshaped their genome structure and regulatory networks. Their accelerated sequence evolution - likely driven by the TGD - has obscured many ancestral CREs, complicating efforts to trace the evolution of gene regulation across vertebrates. In contrast, holostean fishes – gars (genera *Lepisosteus* and *Atractosteus*) and bowfins (genus *Amia*) – retain some of the slowest evolving genomes among vertebrates. These "living fossils" provide a unique window into early bony vertebrate evolution. Their unduplicated genomes, conserved development, and archaic morphologies offer critical insight into deep homologies that have been masked by rapid evolution in the derived teleost and tetrapod lineages.

Leveraging haplotype-resolved genome assemblies from multiple holostean species – generated in collaboration with the Vertebrate Genomes Project – we use comparative genomics, single-cell transcriptomics, and epigenomic profiling in holosteans to reveal ancestral CREs and recover hidden gene regulatory landscapes across bony vertebrates. The evolutionary stability of holostean genomes enables the identification of conserved vertebrate CREs that would otherwise remain hidden for fast-evolving taxa.

Our work underscores the immense value of including slow-evolving 'living fossil' lineages across the Vertebrate Tree of Life to reconstruct the deep history of gene regulation and uncover the genome regulatory logic that shapes vertebrate biodiversity.

268

The role of nuclear organization in transcription regulation

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

There is accumulating evidence for a new gene expression paradigm, in which genes and transcription factors cooperate to create specialized transcription bodies. These are dynamic liquids, with quick exchange of molecules between the condensates and the nucleoplasm. While these bodies have gained a lot of interest in recent years, they are generally hard to study because they are small, abundant, and short-lived. A few years ago, we discovered that in zebrafish embryos, transcription begins in two transcription bodies that are large, isolated, and long-lived. These provide us with an excellent system to investigate how transcription bodies form and function. I will discuss our latest results.

422

Elucidating the role of the noncoding genome in heart development and diseases

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background

Congenital heart disease (CHD) encompasses structural heart defects present at birth, impacting the chambers, valves, and vessels of the heart. Genome-wide association studies (GWAS) have identified numerous single-nucleotide polymorphisms (SNPs) associated with CHD pathogenesis. However, over 90% of these SNPs are found in noncoding regions of the genome, making their functional interpretation challenging.

Methods

To elucidate the role of noncoding variants in CHD, we developed a computational pipeline to systematically identify and annotate CHD-associated SNPs. Integrating GWAS and ClinVar variants with enhancer-associated histone modification profiles from human fetal hearts across nine Carnegie stages, we identified candidate CHD enhancers within developmental regulatory landscapes relevant to cardiogenesis. To experimentally assess enhancer activity, We shortlisted five CHD-associated enhancer candidates and experimentally validated them in vivo using transgenic zebrafish reporter assays.

Results

We identified 2,056 candidate CHD enhancers, of which nearly 39% overlapped with enhancers listed in the Human Enhancer Disease Database. Motif analysis revealed enrichment for cardiac transcription factor binding sites, suggesting that many variants may alter regulatory inputs essential for cardiogenesis. Conservation analysis further demonstrated that numerous CHD enhancers are evolutionarily constrained across vertebrates, underscoring their functional importance. Strikingly, every single predicted candidate tested showed robust enhancer activity in vivo, including four in the developing heart, demonstrating exceptionally high predictive accuracy.

Conclusion

These findings provide direct evidence that human noncoding regions implicated by GWAS can act as functional heart enhancers in a vertebrate model. Ongoing work aims to expand systematic in vivo validation of additional enhancers and allelic variants. In parallel, we are generating single-cell ATAC-seq profiles in zebrafish embryos to refine the regulatory landscape of the developing heart and provide a cell-type–resolved perspective on candidate enhancers potentially affected by CHD-associated SNPs. Together, these approaches will help clarify how regulatory variation shapes heart development and contributes to congenital heart disease.

66

Beyond Mean Effects: Maternal RNA Variability as an Adaptive Response to Environmental Stress

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Epigenetic inheritance - the inheritance of genome activity states without changes in the DNA sequence – has been postulated to facilitate adaptation to changing environmental conditions and to promote population evolution and survival. We study maternally provided RNA as a crucial intergenerational information molecule that has only recently stepped into the spotlight. Maternal RNAs are protein-coding, deadenylated, and repressed RNAs that are deposited into the oocyte and are crucial for offspring development – mutations in maternal RNAs can lead to severe defects. Importantly, maternal RNA composition reflects environmental influences experienced by the mother (e.g., in Ciona or round goby).

We hypothesize that such environmental impacts not only affect the overall composition, but also the variability of individual maternal RNAs, and that both effects together impact offspring phenotypes. This would translate to stress-induced, heritable signals promoting inducible between-offspring diversification and represent a form of molecular bet-hedging that could promote population survival under stressful conditions.

To test for a diversification response, we performed single-oocyte RNAseq on zebrafish oocytes derived from control versus heat-stressed mothers. Using 12 oocytes per mother from 6 mothers per condition, we developed a range of analysis approaches that allow us to assess of within-clutch, between-mother variability and treatment effects over multiple recovery time points and two generations. We complement the oocyte results with behavioural, physiological, and gene expression readouts in larval and adult siblings of the sampled oocytes.

We find a multi-layered, heritable stress-response that combines surprisingly long-term directional effects (e.g., in heat-shock genes), mother-specific responses, and treatment-dependent effects on within-clutch variability at global and gene-specific scales.

Our results suggest that models of adaptation need to account for both diversifying and directional effects simultaneously, and variability should be routinely considered alongside mean effects when studying differential gene expression upon environmental stress.

468

Translational regulation of maternal mRNAs during early development

Laura Lorenzo Orts

Institute of Molecular Biology (IMB), Mainz, Germany

Topic

13 Epigenetics and Gene Regulation

Abstract Content

Oocytes are quiescent cells containing all components required to sustain the first embryonic divisions. In particular, the storage of maternal ribosomes and mRNAs is critical for early development, as transcription is silenced in the oocyte and resumes only later in the embryo. During my postdoctoral work in the lab of Andrea Pauli at IMP Vienna, I identified two mechanisms contributing to the storage of ribosomes and maternal mRNAs in the zebrafish egg.

Maternal mRNAs are largely deadenylated during oogenesis to prevent cap-dependent translation. While deadenylation is coupled to mRNA decay in somatic cells, poly(A) tail shortening of maternal mRNAs does not affect mRNA stability, but rather translation. We discovered an essential role for the germline-specific cap-binding protein eIF4E1b in the storage of maternal mRNAs with short poly(A) tails in P-bodies. However, how mRNAs are released from P-bodies to become translated remains a major open question and is the primary focus of my newly established group at IMB Mainz.

In this talk, I will summarize my postdoctoral work on maternal mRNA storage and highlight important questions in the study of maternal mRNA activation and translation.

HUSBANDRY

319

Longitudinal analysis of gonadal transformation dynamics in zebrafish grown individually

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Topic

21 Husbandry

Abstract Content

Background

Zebrafish gonadogenesis is initiated by primordial germ cells forming an undifferentiated gonad. Male gonads then may be formed either by going through a transient 'juvenile ovary' or progressing directly into a testis. Genetic or environmental factors can induce oocyte apoptosis of this bipotential ovary to drive testis formation. The details of this process remain poorly understood.

Methods

We established a non-invasive method to track gonadal fate in individually housed larvae/juveniles from 3 to 9 weeks post-fertilization (wpf) using the *tg(ddx4:egfp)* line. Forty-eight siblings per family were sorted into transparent micro-tanks (150–550 ml) at 2–3 wpf. Imaging was conducted weekly until 4 wpf, twice-weekly between 4-7 wpf, and once at 8 wpf. Gonadal eGFP intensity was scored on a 0–5 scale based on organ structure and signal strength, and results were validated via ImageJ.

Results

Longitudinal tracking revealed that the 'juvenile ovary' phase is highly plastic at both the individual and family levels. In male-biased families, we observed divergent trajectories with some individuals showing prolonged, bright eGFP-positive oocyte-like stages before abrupt signal loss, while others displayed only weak or transient signals. Female-biased family showed different initialization trends, with divergent timing in both the onset and intensification of the eGFP signal. Notably, transformation windows differed by several weeks between families, with some individuals transforming as late as 8 wpf. These results indicate strong genetic modulation of the timing and probability of the juvenile ovary-to-testis transformation.

Conclusion

Our data obtained from individual monitoring revealed new details of zebrafish gonad differentiation: a dynamic, family-specific process. We demonstrate the start and duration of the 'juvenile ovary' phase are more variable than indicated by previous fixed-stage

snapshots. Samples collected from this experiment make characterization of the transcriptome of specific individuals during gonadal formation and sex fate commitment possible.

464

The '1 Liter' issue: increased water volumes during routine matings have no overt beneficial impact on mating success or zebrafish well being

Andreas van Impel[1], Jessica Zwarg[1], Annesha Ghosh[2], Florian Reichmann[2], Stefan Schulte-Merker[1]

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Topic

21 Husbandry

Abstract Content

In the recent revision of Annex III of the Directive 2010/63/EU on the protection of animals used for scientific purposes, the European Parliament/Council has included the statement that '*water volumes smaller than 1 liter shall not be used for adult zebrafish*' within the 'Stocking density and environmental complexity' section. While this statement is certainly true for general housing conditions where fish should not be kept within smaller water volumes for prolonged periods, recent experiences from facilities in different European countries have shown that this statement is taken out of context and also applied by authorities when assessing requirements for non-experimental routine measures such as matings. In consequence, the use of standard 0.7-1Litre mating boxes has been prohibited in several cases. To the best of our knowledge, there is no experimental data that would suggest that water volumes of 1L per mating pair would lead to an increased stress level for the fish and that the use of larger volumes would be beneficial. Given the huge impact of this practice on the daily work in most European zebrafish facilities, we decided to assess whether fish would benefit from bigger water volumes during matings. Comparing matings carried out in 1L versus 5L mating boxes (total volume) in two independent fish facilities, we assessed parameters such as clutch size and fertilization rate and in parallel tested the impact of the mating procedure on the respective fish using the Novel Tank Test. We were unable to find significant differences across facilities that would suggest that the use of larger water volumes during mating procedures is beneficial for zebrafish wellbeing. Our results do not support a banning of smaller mating boxes from European zebrafish facilities, and it appears unfortunate that rules are implemented without a sound scientific basis and prior testing.

453

Unlocking Routine Facility Data as Welfare Indicators in Zebrafish

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Topic

21 Husbandry

Abstract Content

Ensuring high standards of animal welfare is a central priority in zebrafish research, with direct implications for both ethical responsibility and scientific reproducibility. Routine husbandry and colony management generate a wide range of data, including breeding performance, survival across life stages, age at sexual maturation, sex ratio, and general health status. While these parameters are primarily collected for operational purposes, they also represent a valuable and underutilized source of information on animal welfare.

As zebrafish facilities grow in scale and complexity, managing expanding collections of transgenic lines, the volume of accumulated data increases substantially. Despite consistent data collection practices, the potential of these datasets to inform welfare assessment and guide evidence-based interventions remains largely unexplored.

In our facility, we have established systematic and longitudinal recording of key biological and performance indicators, particularly those reflecting early-life stage outcomes and colony dynamics. These include survival and mortality rates, timing of sexual maturation, and sex distribution. By analyzing these parameters over time, it becomes possible to detect subtle deviations from expected patterns, identify line-specific vulnerabilities, and flag potential welfare concerns at an early stage.

Importantly, such routinely collected metrics can serve as practical, non-invasive proxies for animal welfare, complementing existing monitoring approaches. Leveraging these data enables a shift from reactive to proactive management, supporting timely interventions and continuous refinement of husbandry practices.

In this presentation, we will explore how facility-derived data can be reframed as welfare indicators and discuss strategies to enhance their analytical use. By unlocking the full value of existing datasets, zebrafish facilities can strengthen welfare oversight, improve colony performance, and contribute to more robust and reproducible research outcomes.

95

Refining breeding protocol and welfare monitoring for the African turquoise killifish Nothobranchius furzeri in laboratory settings

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Topic

21 Husbandry

Abstract Content

Nothobranchius furzeri, commonly known as the African turquoise killifish, is the shortest-lived vertebrate species that can be maintained in captivity. Its naturally brief lifespan has established it as a powerful vertebrate model for studying the mechanisms of aging. The growing use of this species in research facilities worldwide underscores the urgent need for standardized husbandry practices capable of supporting large-scale colony management while ensuring experimental reproducibility. The aim of our study was to develop a comprehensive, species-specific framework to optimize large-scale husbandry protocols and improve welfare in laboratory settings. After identifying important limitations in previously published protocols, we designed refined strategies for breeding, embryo management, larval rearing, anesthesia and routine experimental procedures. The protocol includes detailed descriptions of required materials, step-by-step methodologies and troubleshooting guidance. All procedures rely exclusively on commercially available equipment, intentionally avoiding custom-built devices to facilitate broad implementation and reproducibility.

We also developed age-structured welfare score sheets tailored to the biology of the species. The scoring system incorporates parameters such as body condition score, swimming activity, respiratory pattern, tank occupancy, clinical signs and food intake. Each parameter is interpreted in light of age-dependent physiological changes, allowing accurate daily welfare monitoring. This approach recognizes aging as a dynamic and multifactorial process and enables differentiation between pathological decline and physiological senescence.

The integration of standardized husbandry recommendations with structured welfare assessment tools provides a scalable and adaptable framework for managing *N. furzeri* colonies. This work supports improved reproducibility in aging research, fosters protocol harmonization across institutions, and strengthens the ethical use of this species in compliance with European regulatory standards. Overall, the proposed framework enhances both scientific validity and animal welfare in aquatic biomedical research.

EVOLUTION & ALTERNATIVE FISH MODELS I

473

An Antagonistically Pleiotropic Gene Regulates Vertebrate Growth, Maturity, and Lifespan

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

The antagonistic pleiotropy theory of aging predicts genetic trade-offs between early-life and late-life fitness. However, empirical evidence for such trade-offs in vertebrates remains scarce, particularly from causal genetic experiments. Here, combining genetic perturbation with longitudinal phenotyping in the turquoise killifish (*Nothobranchius furzeri*), we identify *vestigial-like 3* (*vgll3*), previously linked by GWAS to age at maturity in humans and male Atlantic salmon, as a gene with antagonistically pleiotropic effects. Selective disruption of vgll3 isoforms accelerates male growth and maturation in a dose-dependent manner. Transcriptomic and cellular analyses indicated increased cell division, corroborated in vivo by elevated germline and intestinal stem-cell proliferation. However, early-life maturation incurs a late-life cost, linked to altered DNA damage response. Older mutant males develop melanoma-like tumors, validated via transplantation into immunodeficient rag2 models, and exhibit a shortened lifespan. Thus, we identify *vgll3* as a key regulator of life-history variation with antagonistic effects across ages, balancing early-life fitness against late-life mortality.

346

Comparative Neurogenomics in Holostean and Teleost Fishes Informs Brain Evolution by Whole Genome Duplication

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Teleost fishes, the largest group of living vertebrates, are derived from a tetraploidization event in their crown ancestor, the teleost genome duplication (TGD). The majority of TGD duplicates (ohnologs) - about 80% - have been lost secondarily during the rediploidization of teleost genomes. However, more than 50% of genes with neuronal functions and roles in the nervous system have been retained as ohnologous pairs in extant teleost genomes. We thus hypothesize that the TGD had major significance for the evolution of teleost brain functions. We adress two major questions:

1.) Has the TGD facilitated modularization and mosaic brain evolution in teleosts?

2.) Has lineage-specific ohnolog evolution contributed to the diversification of neuronal cell types in the brains of teleosts?

To address these questions, we contrast the expression of genes in the brain of the holostean fish spotted gar (*Lepisosteus oculatus*) – a slowly-evolving outgroup to teleosts and a proxy to the pre-TGD condition – with that in representatives of all major teleost clades (zebrafish, medaka, eel, and bonytongue).

Using new genome assemblies, comparative genomics, single-cell RNA-seq, ATAC-seq, our novel computational tool ECHOmap (Evolutionary Cell HOmology map), and Hybridization Chain Reactions, we obtain a complex picture of gene regulatory sub- and neofunctionalization across cell types and teleost species after the TGD. We exemplify our results by the case of metabotropic glutamate receptors which show TGD ohnolog retention rates of over 70%.

139

Single-cell mapping of heterochrony to a species-specific phenotype

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Vertebrate embryonic development and its underlying genetic programs are highly conserved, yet the rates at which these programs proceed vary greatly between species. Such differences manifest as heterochrony – changes in the relative timing of developmental events – but the extent of such temporal shifts and their relationship to whole-organism developmental tempo remains understudied. To investigate cell-type-specific developmental timing at the molecular and cellular level, we use individual embryo sci-RNA-seq to compare zebrafish and medaka, two poikilothermic vertebrates with a ~3-fold difference in developmental rate. We assemble a time-resolved atlas of medaka from blastula to hatch, comprising >1.1 million cells from >500 embryos and create a cross-species timing resource that places embryos of both species on a shared transcriptional timescale. Using this cross-species framework of development, we identify cell lineages that display notable timing deviations from the predicted whole-embryo alignment between zebrafish and medaka. We define a new, generalisable metric, the “cell-embryo deviation”, for measuring heterochronic shifts in cell type emergence and maturation, and use it to show that many cell types display delay and acceleration that differs from the globally expected slowdown in medaka. Focusing on the notochord, we use *in vivo* imaging to validate novel timing differences predicted by our framework, and reveal that two cell types which originate from the same progenitor pool exhibit opposing temporal shifts, with one lineage being accelerated and the other delayed relative to zebrafish. Furthermore, we use our single-cell data to identify genes associated with these timing shifts, suggesting candidate mechanisms that may result in notochord-specific deviations from whole-embryo tempo. Together, our findings establish a quantitative framework for predicting and testing heterochrony across species, and suggest that evolutionary changes in developmental timing acts at the level of cell types to remodel tissue morphology and function, thereby acting as a driver of morphological variation.

146

Cellular innovations underlie the evolution of livebearing and placentation in Poeciliid fish

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Biological complexity emerges step by step from simpler forms. A fascinating example is the evolution of different reproductive modes in the fish family Poeciliidae. Unlike egg-

laying fish, all Poeciliids retain nutrient-rich eggs within the ovarian follicles, with the embryos developing inside the mother until birth (livebearing). Remarkably, several Poeciliids have additionally evolved a placenta, an organ that allows nutrient transfer from the mother to the embryo throughout development. Notably, the placenta evolved independently nine times over a relatively short period within this family, making Poeciliids an exceptional model for dissecting the evolutionary and developmental mechanisms underlying the evolution of complex organs. To investigate this, we use single-cell RNA sequencing to characterised the cellular composition of the ovary in eleven fish species (nine Poeciliids, with the turquoise killifish and zebrafish as outgroups), covering one transition from egg-laying to livebearing and five independent origins of a placenta. We found a previously uncharacterized ovarian cell type common to all Poeciliids. Imaging coupled with gene markers revealed that this novel cell type is the main component of the ovarian follicle that surrounds each developing embryo. This follicular cell emerged at the base of the family and does not appear to respond to ovulation signals, likely enabling Poeciliids to transition from egg-laying to livebearing. By tracing the evolution of ovarian cell types across the Poeciliid phylogeny, we found that the Poeciliid-specific follicle cell type was fundamental to all transitions to placentation. In each of the five placental species, the Poeciliid-specific cell diversified and deployed genetic programs for nutrient transport, enabling the evolution of a placenta. In summary, our work shows that the evolution and subsequent modification of a new cell type enabled the evolution of live birth and placentation, providing the cellular and genetic foundation for the evolution of complexity.

228

The livebearers platyfish and swordtails partially regenerate their hearts with persistent scarring

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Heart regeneration varies among vertebrates, with zebrafish serving as a reference species for efficient cardiac restoration. How this capacity diversified among teleosts is an emerging question, given the recent identification of non-regenerative cardiac repair in medaka and cavefish. Here, we investigate heart restorative capacity following cryoinjury in two livebearers, platyfish and swordtails (*Xiphophorus* species), belonging to the *Poeciliidae* family. We demonstrate that their hearts lack the vascularized compact myocardium, which is a ventricular layer implicated in the restorative response in zebrafish. After cryoinjury, both poeciliids failed to rapidly deposit fibrotic tissue that normally reinforces the damaged site. This deficiency led to striking wound protrusion reminiscent of pseudoaneurysm after myocardial infarction in humans. Although the remaining myocardium initially increased cell

proliferation, subsequently deposited collagenous scar tissue permanently sealed the interrupted ventricle, preventing complete regeneration. Transcriptomic analysis revealed several divergently regulated pathways between cryoinjured hearts of zebrafish and platyfish, particularly in immune response regulation. This was validated by delayed leukocyte infiltration and prolonged inflammation in platyfish, compared to the rapid, resolved inflammatory response in zebrafish. Our findings demonstrate that *Xiphophorus* species have evolved hearts with compromised regenerative capacity, characterized by pseudoaneurysm-like protrusion and permanent scarring. These results reveal that evolutionary traits of phylogenetic lineages can fundamentally modulate regenerative competence among teleosts, with important implications for understanding the mechanistic basis of cardiac repair.

340

A comparative study involving medaka and blind cavefish reveals a “dark” CPD photolyase function in DNA repair

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background: A complex spectrum of DNA damage is generated upon exposure to combinations of environmental stressors. Therefore, over the course of evolution, DNA repair systems must inevitably adapt to changing environments to ensure the maintenance of genome integrity. Photolyases represent a highly conserved class of enzymes which repair UV-induced covalent crosslinks between adjacent pyrimidine bases (CPD and 6-4 photoproducts) via the visible light-driven process of photoreactivation.

Methods: A comparative approach using medaka and blind cavefish and derived cell lines.

Results: In the blind cavefish Phreatichthys andruzzii which has evolved for millions of years completely isolated from UV radiation and visible light, we have documented multiple polymorphisms and loss-of-function mutations affecting both the 6-4phr and DASHphr photolyase genes while strangely, the CPDphr gene remains highly conserved. In order to investigate the possibility of a light-independent function for CPDphr, we have generated loss-of-photolyase-function medaka mutant lines as well as gain-of-photolyase-function mammalian cell lines. By studying and comparing these various in vitro and in vivo models, we reveal that CPDphr (but not 6-4phr or DASHphr), enables the light-independent repair of CPD as well as 8-OHdG, an oxidatively modified form of guanosine, with both types of DNA damage being generated under oxidative stress in the absence of UV radiation.

Conclusion: Thereby, using this comparative approach we document selective conservation of light-independent photolyase function in blind cavefish, enabling the repair of DNA damage encountered in an extreme subterranean environment.

DISEASE MODELS III

18

Alternative Lengthening of Telomeres Defines a Mesenchymal and Immunogenic Cell State in BrainTumors

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Topic

1 Disease Models

Abstract Content

Background: Diffuse gliomas (DGs) are the most common primary brain tumors and exhibit marked inter- and intratumoral heterogeneity. Dysregulation of telomere maintenance mechanisms (TMMs) occurs in over 90% of DGs. Cancer cells maintain telomeres either through telomerase reactivation (TERT) or alternative lengthening of telomeres (ALT), a homologous recombination–based pathway. ALT is used by approximately 10–15% of adult and up to 50% of pediatric DGs.

Methods: Using immunostaining for TERT and PML/TRF2, we identified extensive heterogeneity in TMM usage within individual cancer cells of the same tumor. To define transcriptional programs associated with distinct TMMs, we employed two isogenic zebrafish brain tumor models differing only in telomere maintenance mechanism.

Results: Single-cell RNA sequencing revealed that ALT+ tumor cells are enriched for mesenchymal markers, whereas TERT+ cells preferentially express proneural markers. Applying these zebrafish-derived signatures to human DG datasets confirmed the association of ALT with the mesenchymal and TERT with the proneural subtypes. Consistently, analysis of low passage glioblastoma cell lines showed that lines with a mesenchymal gene expression profile are predominantly ALT+, while proneural lines express TERT. Beyond transcriptional differences, ALT and TERT tumors exhibited distinct immune microenvironments, with increased pro-tumoral immune infiltration in ALT tumors. Given the elevated expression of the telomeric RNA TERRA in ALT tumors, we investigated its contribution to immune regulation. Targeting TERRA with antisense oligonucleotides significantly altered tumor-infiltrating immune populations.

Conclusions: Together, these findings demonstrate that ALT and TERT define conserved and distinct cellular states in diffuse gliomas, extending beyond telomere maintenance to encompass specific transcriptional programs and immune microenvironments.

96

Zebrafish-Based Functional Validation of a Novel *TPM2* Variant Underlying Familial Clubfoot

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Topic

1 Disease Models

Abstract Content

Background: Congenital talipes equinovarus (CTEV), also known as clubfoot, is a common musculoskeletal birth defect with a multifactorial etiology. While genetic mutations affecting cytoskeletal dynamics and muscle development have been implicated in congenital limb deformities, molecular mechanisms underlying isolated CTEV remain poorly understood. This study investigated the genotype-phenotype correlation of a novel *TPM2* mutation identified in a Polish family with isolated clubfoot using zebrafish model.

Methods: Aberrant splicing was assessed via qPCR. Functional validation was performed in single-cell stage zebrafish embryos overexpressing wild-type, mutant, and/or splice-variant *tpm2* mRNA. Analyses included phenotype scoring, muscle fiber architecture (phalloidin staining), neuromuscular junction (NMJ) integrity (immunofluorescence), and muscle membrane stability (Evans Blue Dye uptake).

Results: Mutant and/or splice-variant *tpm2* overexpression in zebrafish induced axial shortening, somite disorganization, and impaired muscle morphogenesis. Confocal imaging revealed disrupted filamentous actin architecture and defective myofiber alignment. NMJs displayed reduced acetylcholine receptor clustering, indicating compromised synaptic organization. Variant-expressing larvae showed significant Evans Blue Dye uptake, demonstrating decreased membrane integrity. All phenotypes were consistently more severe than the wild-type, supporting a mutation-specific pathogenic effect.

Conclusions: This novel *TPM2* variant disrupts muscle development, cytoskeletal organization, neuromuscular connectivity, and membrane stability in vivo, providing functional evidence for its pathogenic role in familial clubfoot. These findings underscore the crucial role of tropomyosin-mediated cytoskeletal regulation in musculoskeletal development and establish a translational zebrafish platform for assessing *TPM2*-associated clubfoot. This work advances understanding of the genetic architecture underlying clubfoot and the mutation's role in gene function.

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141

Modelling ER-stress in diabetes, a way to study β-cells health and identify new drug candidates

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Topic

1 Disease Models

Abstract Content

Background:

Diabetes defines different diseases manifesting an inability to control glucose levels. Despite their mechanistic differences, each type of diabetes features a reduction in functional β-cells, which exhausts the remaining β-cells with increased insulin production, inflammation and/or insulin resistance. The higher demand on each β-cell increases the risk of protein misfolding, aggregation and endoplasmic reticulum (ER)-stress. β-cells respond to the increased ER-stress by activating unfolded protein response (UPR) to promote β-cell proliferation and survival via upregulating chaperones and cytoprotective genes, while reducing protein synthesis and ER load. However, chronic UPR impacts β-cell functions and survival, leading to dedifferentiation and ultimately apoptosis.

Methods:

To mimic diabetes, we used different approaches. First, we used a hyperglycaemic zebrafish larval model through glucose treatment. Second, we generated new zebrafish strains expressing human insulin variants carrying mutations leading to insulin misfolding events and thus ER-stress in β-cells (C43G: constitutively misfolded insulin; R46E: increased misfolding events). Finally, to study ER-stress levels in these diabetes models, we developed two novel genetic ER-stress sensors based on different UPR components, the *hspa5* promoter and Ire1-dependent splicing of *xbp1* controlling nano-luciferase expression.

Results:

We successfully generated novel zebrafish strains expressing mutated forms of human insulin as well as an Ire1-dependent ER-stress sensor, without impacting overall zebrafish survival. ER-stress sensor functionality was validated using ER-stress-inducing drugs, and it can be used to monitor ER-stress triggered by insulin misfolding. The insulin-R46E expression in 5-day-old zebrafish larvae led to a 2-fold increase in signal from our β-cell specific ER-stress sensor, an effect we currently reverse with drug candidates in a pilot screen.

Conclusion:

These zebrafish systems constitute powerful screening models that we plan to use to discover new drugs with the potential to reduce β-cells ER-stress *in vivo* and decipher their mechanisms of action, with the aim of reversing diabetes progression.

358

Location-Dependent Differences in Cardiac and Skeletal Muscle Dysfunction Associated With Truncating Titin (*ttn.2*) Variants

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Topic

1 Disease Models

Abstract Content

Background: Truncating variants in the *TTN* gene (*TTN*tv), encoding the giant sarcomeric protein titin, cause a range of human cardiac and skeletal muscle disorders of varying penetrance and severity. The effects of variant location on clinical manifestations are incompletely understood.

Methods: We generated 6 zebrafish lines carrying truncating *ttn.2* variants in the Z-disk, I-band, A-band, and M-band titin regions. Expression of titin transcripts and protein levels was evaluated using quantitative polymerase chain reaction and proteomics. Phenotype analysis was performed during embryonic development and in adult hearts.

Results: Homozygous embryos from all lines except the C-terminal line, e232, showed a significant reduction of Z-disk and I-band *ttn.2* transcripts, but A-band and M-band transcript levels were reduced only in truncations distal to the *cronos* promoter. These homozygous embryos uniformly died by 7-10 dpf with marked impairment of cardiac morphology and function. Skeletal muscle motility and sarcomere organization were more disrupted in truncations distal to the *cronos* promoter compared with those proximal. In contrast, homozygous e232 embryos, which lacked only the titin kinase and M-band regions, had preserved cardiac function with incorporation of truncated Ttn.2/Cronos protein and normal sarcomere assembly, but selective degradation of fast muscle sarcomeres. All heterozygous embryos were phenotypically indistinguishable from wild type. High-frequency echocardiography in adult heterozygous fish showed reduced ventricular contraction under resting conditions in A-band mutants. Heterozygous Z-disk and I-band mutants had no significant baseline impairment but were unable to augment ventricular contraction in response to acute adrenaline exposure, indicating a lack of cardiac reserve.

Conclusions: Our data suggest that cardiac and skeletal muscle dysfunction associated with truncating *ttn.2* variants is influenced by age, variant location, and

the amount of functional titin protein. The distinctive phenotype associated with distal C-terminal truncations may reflect different requirements for C-terminal titin for maintenance of fast, slow, and cardiac muscle sarcomeres.

20

The repair of DNA-protein crosslinks: Developing new disease models and identifying molecular causes of human diseases

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Topic

1 Disease Models

Abstract Content

Background

DNA-protein crosslinks (DPCs) are the second most common DNA lesions, obstructing replication and transcription. Deficient repair of these toxic lesions is associated with the onset of cancer, neurodegeneration, and aging. Induction of DPCs is used in medicine to treat many cancers, and understanding repair at the organismal level could drive the development of new drugs and combination therapies. Additionally, the accumulation of DPCs leads to aging and neurological disorders, making it important to understand the mechanisms behind crosslink repair. In recent years, we and other groups have gained mechanistic insights into DPC repair (DPCR) factors. However, the function of repair factors at the organismal level remains largely unknown.

Methods

We use the zebrafish model and CRISPR-Cas gene editing to study the interplay of DPCR factors and sub-pathways, including proteolysis-, nuclease-, and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular levels. I will present our recent discoveries using four new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, and transgenic strains with inactive DPCR factors, tyrosyl-DNA phosphodiesterases 1 and 2 (TDP1 and TDP2).

Results

We found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality caused by the accumulation of specific DPCs, including histones, topoisomerases, and Parp1. We also show that in early development, the SPRTN protease cannot compensate for ACRC function. In addition, unpublished data about the DPCR pathway in TDP1- and TDP2-deficient zebrafish will be presented.

Conclusion

Our results provide insights into the complex DPCR pathways and their implications for human disease and cancer treatment.

33

Single-Cell Cross-Species Profiling identifies Conserved Transcriptional Networks in Early Pancreatic Tumourigenesis

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Topic

1 Disease Models

Abstract Content

Background
Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and has the worst prognosis among all cancers, largely because it is often diagnosed at advanced metastatic stages. Identifying robust markers of preinvasive disease and elucidating the molecular networks that drive progression from early lesions to invasive carcinoma are therefore critical to improve early detection.

Methods
We generated a zebrafish model in which oncogenic $KRAS^{G12D}$ is specifically expressed in pancreatic acinar cells, inducing acinar-to-ductal metaplasia that faithfully recapitulates key features of mammalian pancreatic tumourigenesis. Single-cell RNA sequencing was performed to characterize transcriptional changes occurring at early disease stages. Cross-species comparisons were conducted with published mouse and human pancreatic single-cell transcriptomic datasets to identify evolutionarily conserved genes and signalling pathways associated with pancreatic tumourigenesis. Monocle3 analysis was used to precisely reconstruct the trajectory of tumour cells from normal acinar to malignant states and active regulatory networks along this trajectory were inferred using SCENIC.

Results
Cross-species analyses revealed a striking conservation of genes upregulated during metaplasia, activating common signalling pathways and regulatory programs in zebrafish, mouse, and human. Notably, metaplastic cells reactivate a broad set of developmental genes normally expressed in multipotent pancreatic progenitors. High concordance across species is also observed when reconstructing tumour cell trajectories from acinar to cancerous states, revealing shared gene expression changes along this progression. Notably, these analyses reveal a set of cytoskeletal and migration-related genes specifically upregulated at the metaplasia–cancer transition, likely conferring invasive potential to these cells. SCENIC analysis further identified regulatory networks that become progressively activated during malignant transformation, suggesting their involvement in the acquisition of cancer-associated traits.

Conclusions
These findings demonstrate strong evolutionary conservation of molecular mechanisms

driving pancreatic cancer progression and identify key pathways and regulatory programs that represent promising targets for preventing PDAC development and progression.

NEURAL CIRCUIT BEHAVIOR

373

Powerhouses in Motion: Mitochondrial Dynamics and Neuronal Health in AKAP1-Deficient Zebrafish

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Topic

7 Neural Circuit Behavior

Abstract Content

Background
Mitochondria are essential for neuronal health, supplying ATP, buffering calcium, and regulating reactive oxygen species. Their dynamic processes—including fission, fusion, trafficking, and mitophagy—ensure proper mitochondrial quality and distribution within neurons. Disruption of these processes contributes to synaptic dysfunction, neuronal degeneration, and behavioral deficits. Akinase anchoring proteins (AKAPs) coordinate compartmentalized signaling by anchoring protein kinase A and other effectors to mitochondria and subcellular compartments, regulating neuronal function.

Methods
We investigated mitochondrial organization and neuronal function in neuro-specific AKAP1 conditional knockout zebrafish (ND1:GFP:AKAP1 cKO) and control (ND1:GFP) lines. Neurons were sorted from 5 dpf larvae for RNA sequencing, highlighting photoreceptor and neuronal impairment. Retinal mitochondrial distribution and photoreceptor integrity were assessed by immunofluorescence using Tom20 and photoreceptor-specific opsin markers. Synaptic organization and mitochondrial dynamics were examined using fluorescent reporter constructs. Behavioral activity was evaluated in larvae via the Visual Motor Response assay and in adults through locomotor paradigms including the Novel Tank Test and Light–Dark Test.

Results
JC1 assays on sorted neurons revealed impaired mitochondrial membrane potential in (ND1:GFP:AKAP1 cKO) larvae compared to controls. Reduced DRP1 phosphorylation indicated impaired mitochondrial fission. RNA-seq analyses supported neuronal and photoreceptor dysfunction (ND1:GFP:AKAP1 cKO), confirmed by confocal microscopy analyses showing reduced cone populations, altered amacrine cell distribution, and abnormal mitochondrial localization within neurons. These mitochondrial defects were associated with pronounced hyperactivity and altered locomotion in larvae, as well as increased exploratory behavior in adults. Analyses of synaptic organization and mitochondrial dynamics are ongoing, reinforcing the link between mitochondrial dysfunction and disrupted neuronal circuits.

Conclusion
Neuronal AKAP1 loss disrupts mitochondrial organization in photoreceptors and other retina l neurons, leading to measurable behavioral impairments. This zebrafish model provides a ro bust in vivo platform to study how compartmentalized signaling regulates mitochondrial dyn amics, neuronal function, and behavior.

69

Neuroimmune Guardians? How Macrophages and Microglia Shape Stress Resilience

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Topic

7 Neural Circuit Behavior

Abstract Content

Background: All organisms must maintain homeostasis, a balance that can be disrupted by physiological and psychological stressors that challenge body equilibrium. However, there is variability in the capacity to restore homeostasis, with resilient individuals displaying an adaptive response to stressors, while those with susceptibility exhibit maladaptive coping mechanisms that elevate the risk of developing stress-related disorders. The mechanisms underlying this variability remain unclear. Our recent study in zebrafish larvae indicates that stress resilience is developmentally determined and linked to differential gene expression, particularly of complement factors, components of the innate immune system. Here, we focus on macrophages (MΦs) and microglia, innate immune cells that produce complement factors, and elucidate their role in stress resilience.

Methods: We used the pan-myeloid transgenic line *mpegGal4:UASeGFP*. Resilient and susceptible phenotypes were defined at six dpf using a behavioral stress paradigm. Conditional genetic ablation of MΦs/microglia was achieved using two methods: the NTR-NFP and bacterial toxin-Kid systems. Single-cell RNA sequencing (scRNA-seq) of these cells was performed using MARS and On-Chip Multiplexing 10X Genomics platforms.

Results: Conditional genetic ablation of MΦs/microglia in zebrafish larvae increased the proportion of resilient individuals. To identify differential responses of MΦs/microglia in resilient and susceptible individuals, we isolated mpeg-GFP+ cells from these populations. Thereafter, MARS scRNA-seq was performed on mpeg-GFP+ resilient and susceptible individuals to determine cellular and tissue heterogeneity. This strategy enables precise correlation between the behavioral score of individual larvae and their specific MΦs/microglial landscapes. To further validate our results, we also performed RNA sequencing using the 10X Genomics platform.

Conclusion: We demonstrate that MΦs/microglia negatively influence stress resilience. Our ongoing analyses will highlight top differentially expressed markers and map subtype-specific differences in these immune cells between resilient and susceptible individuals. This will provide a comprehensive view of innate immune contributions to stress resilience.

106

Functional Characterization of chrna2a-Expressing Interneurons in Zebrafish Forebrain Circuits

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Topic

7 Neural Circuit Behavior

Abstract Content

Background: Cholinergic neuromodulation shapes vertebrate brain state and information processing, yet the cell-type–specific mechanisms linking cholinergic signalling to circuit dynamics remains incompletely understood. The α2 subunit of the nicotinic acetylcholine receptor (chrna2a) marks a genetically defined neuronal population whose functional role in vertebrate circuits has not been systematically examined in zebrafish. Previous studies conducted in mice identified a subpopulation of hippocampal Chrna2-expressing neurons, coined as bravery cells, implicated in anxiety gating. We identified homologous neurons in zebrafish subpallial region, in the ventral nucleus of ventral telencephalon (Vv).

Methods: To investigate the anatomical distribution, physiological properties, and functional contribution of chrna2a-expressing neurons to forebrain network activity, we generated transgenic zebrafish reporter line marking this population. To validate that zebrafish chrna2a-neurons regulate emotions similarly to mouse bravery cells we eliminated the Chrna2a protein using using translation blocking morpholino knock-down and CRISPR/Cas9 knock-out strategies. In parallel, we ablated chrna2a-expressing neurons using NTR/MTZ system. Behavioural outcomes were evaluated using the open field test. To further characterise Vv region, we combined confocal imaging of transgenic line with hybridization chain reaction (HCR) in situ labeling.

Results: Neither Chrna2a protein depletion nor targeted neuronal ablation produced significant alterations in general locomotor activity, indicating preserved motor function. However, treated larvae showed reduced entry and time spent in the inner zone of the open field compared to controls, indicating that chrna2a-expressing neurons might be involved in suppressing anxiety in zebrafish. Both transgenic imaging and HCR in situ hybridization confirmed the presence of chrna2a-neurons in Vv region of zebrafish forebrain, which serves as key hub of integrative information processing.

Conclusion: Together, our findings identify chrna2a-expressing neurons as a conserved inhibitory population that links cholinergic signaling to forebrain circuit modulation in zebrafish.

64

The Cxcl14 chemokine is a novel guidance cue for inner ear pioneer axons

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Topic

7 Neural Circuit Behavior

Abstract Content

The wiring of sensory circuits requires precise topographic organization to capture spatial information. This is particularly relevant in the inner ear, where hair cells placed in segregated sensory domains receive information from sound, rotational and linear acceleration. Here, we uncover the role of pioneer axons in setting the scaffold for statoacoustic ganglion development and show for the first timehow the first neurites target newborn hair cells by high spatiotemporal imaging. The primary role of chemokines is to promote chemotaxis, that is, the directed migration of immune cells , but recent studies have explored their roles in cell differentiation, survival, synaptic activity and axon guidance. Cxcl14 is one of the closest homologs of Cxcl12 but remains comparatively unexplored in the nervous system, largely because its cognate receptor has not yet been identified. Our owrek has identifyied the *cxcl14* chemokine as a novel cue regulating the navigation of statoacoustic pioneer axons to hair cells and second-order neurons. In *cxcl14* depleted embryos, pioneer axons exhibit navigation errors, fail to adequately target hair cells and hair cell activity is lost. Simultaneously, central projections of the lateral line, another mechanosensory organ, display fasciculation and turning errors, and aberrantly invade the inner ear domain, disrupting statoacoustic ganglion-lateral line segregation. Overexpression experiments suggest that *cxcl14* acts as an axonal chemoattractant. This study advances our understanding of how the mechanosensory circuit is built.

404

Basal ganglia output destabilizes behavioral states and induces transition for the optimal behavioral choice

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Topic

7 Neural Circuit Behavior

Abstract Content

The cortico-basal ganglia circuit is crucial for goal-directed decision-making. We focus here on a novel function of this circuit. In adult zebrafish, after learning of Go/No-go tasks in virtual reality space, pallidal output neurons integrate negative values of conditioned cue and “scenery flow prediction error” signals, which are separately encoded in the pallium (fish

cortex) and the striatum. This integrated signal is uniformly broadcast back to the pallium and destabilizes an ongoing behavioral state, facilitating neural and behavioral state transitions toward the most favorable state, in which the scenery flow prediction error is minimized. This mechanism explains why the limited number of pallidal output neurons can find the most appropriate behavior from the infinite variety of actions.

367

Zebrafish as a Platform to Decode Microbiota-Dependent Neurodevelopment and Psychobiotic Mechanisms

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Topic

3 Neurobiology

Abstract Content

The microbiota–gut–brain axis has emerged as an important pathway through which microbial signals shape brain development and behaviour. While mammalian models have been central to this field, zebrafish provide a powerful complementary system for dissecting microbiota-dependent mechanisms, owing to their developmental accessibility and experimental tractability. At University College Cork, we have established zebrafish as a tractable platform for microbiota–brain research, while also contributing to efforts to strengthen local capacity and help build an Irish zebrafish research community.

Two examples illustrate the breadth and value of this approach. Recent cross-species work demonstrates that the microbiota shapes early-life glial maturation and central nervous system myelination. Using germ-free mice and zebrafish, we identified microbiota-dependent effects on pathways linked to neuronal activity and myelination, alongside altered expression of genes involved in microglia maturation and synaptic pruning. In zebrafish larvae, microbial depletion also altered the spatial distribution of microglia and oligodendrocytes within the brain and spinal cord. These findings point to conserved microbial influences on early neural development and myelin-related processes.

A second strand of this work has established a zebrafish-based framework for psychobiotic discovery by integrating in silico, in vitro, and in vivo approaches. Using this pipeline, we identified Lactiplantibacillus plantarum APC2688 as a candidate strain capable of metabolising prebiotics such as fructooligosaccharides, galactooligosaccharides, and inositol, while producing neuroactive compounds

including GABA, tryptophan, and acetate. In zebrafish larvae, bacterial supernatants induced changes in the GABAergic genes gad1 and gabra1 and altered anxiety-like behaviour, illustrating how zebrafish can serve as an efficient in vivo platform for functionally validating microbiome-derived neuroactive candidates.

Together, these studies position zebrafish as a versatile model for decoding how microbial signals shape brain development across mechanistic and translational scales. Complementary work in our group extends this framework to embryonic development, stress-social relevant neurodevelopment, and behaviour.

TISSUE FORMATION

225

New imaging tools reveal insights into membrane and cytoskeletal remodelling during extra-embryonic yolk syncytial layer (YSL) formation

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Topic

5 Tissue Formation

Abstract Content

Extra-embryonic tissues do not form part of the final body plan but serve vital functions during embryonic development. The yolk syncytial layer (YSL) is an extra-embryonic tissue present in many fish species, with crucial roles in axis formation, morphogenesis and gastrulation. The zebrafish YSL forms when marginal blastomeres lose their cell-membranes, depositing their nuclei into the yolk cytoplasm[1]. The YSL is thought to form by failed cytokinesis of marginal blastomeres[2] and retraction of existing membranes, but how membranes and cytoskeleton are remodelled is unknown.

To investigate membrane and cytoskeletal dynamics during YSL formation, we are developing a suite of new monomeric StayGold[3] reporters that are bright, highly photo-stable and suitable for deep tissue imaging. Yolk syncytial nuclei (YSN) arise from daughters of dividing marginal blastomeres that divide in a stereotypical manner, and individual nuclei show extensive re-arrangements. At the 9th embryonic cell division, regardless of the division plane orientation, one daughter cell moves into the upper blastoderm and one remains at the margin. At the 10th division, the daughter cell at the margin divides with both nuclei incorporated into the YSL. Tracking of cytoskeletal rearrangements shows that actin moves into newly forming cleavage furrows between dividing nuclei. However, no new membranes form between dividing marginal blastomeres following the 9th division, and pre-existing membranes between blastomeres begin to retract before the 10th cell division. Our new reporters revealed highly dynamic membrane ruffles at the yolk-blastoderm interface. Membrane ruffling has been proposed to be a mechano-sensor for changes in extracellular fluid viscosity[4]. Membrane ruffling ceases during cell divisions as blastomeres round up, and subsequently, membranes of daughters at the yolk-blastoderm interface are lost as the YSL forms. Our findings suggest that changes in membrane properties and remodelling of cell membranes drive extra-embryonic yolk syncytial layer formation.

1.PMID:3972183; 2.PMID:23213468; 3.PMID: 38081970;4.PMID:37220497

90

Rab8a-positive vesicles transport Wnt8a along cytonemes in zebrafish embryogenesis

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Topic

5 Tissue Formation

Abstract Content

The Wnt signalling pathway has a conserved role in orchestrating developmental processes by regulating cell fate, proliferation, and tissue patterning. As Wnt ligands are lipid-modified and membrane-associated, a major area of research has focused on characterising the mechanism of long-range ligand dissemination to explain how Wnts establish a morphogenetic signalling gradient *in vivo*. We have previously demonstrated that Wnt ligands are transported between cells via long, actin-based filopodia known as cytonemes. Cytoneme-based transport is essential for establishing the Wnt morphogen gradient in early zebrafish embryogenesis. However, little is known about the mechanism of Wnt ligand trafficking within cytonemes.

In this study, we demonstrate that the intracellular trafficking of the canonical ligand Wnt8a is controlled by the small GTPase Rab8a. Using cultured zebrafish fibroblast cells and zebrafish embryos, we show that Wnt8a is transported with its carrier protein Wntless (Wls) in Rab8a-positive vesicles along cytonemes. When in contact with a receiving cell, the Wnt8a-loaded vesicles fuse at the cytoneme tips, facilitating ligand transfer. For the first time, we have imaged Wnt8a and Rab8a-positive vesicles in cytonemes using correlative light and electron microscopy. Furthermore, we determine an essential function of Rab8a in the dissemination of Wnt8a in the early zebrafish embryo using a CRISPR-Cas13d-based knockdown strategy. Conversely, Rab8a activation in the zebrafish embryo enhances Wnt8a transport and spreading across tissues, leading to posteriorisation of the body axis and the formation of headless embryos.

Overall, we have revealed a dedicated vesicle-based intracellular trafficking route for Wnt delivery along cytonemes. These insights challenge the classical view of long-range morphogen dissemination as an extracellular process and instead support a novel paradigm in which morphogen spread is tightly coupled to a Rab-mediated intracellular transport process.

390

Dual role of Notch signaling in sclerotome induction and migration independent of somite segmentation

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Topic

5 Tissue Formation

Abstract Content

Somites are transient, segmentally repeated blocks of paraxial mesoderm that define the vertebrate body plan. Each somite is subdivided into the dermomyotome and sclerotome, which give rise to skeletal muscle and the axial skeleton, respectively. Somite segmentation is controlled by Notch signaling, and mutations in this pathway lead to skeletal defects. However, how Notch signaling regulates somite subcompartment formation remains unclear. Our lab recently showed that the sclerotome in zebrafish has a unique bipartite organization and that sclerotome-derived cells undergo extensive migration to generate diverse fibroblast subtypes via platelet-derived growth factor receptor a (Pdgfra)-mediated chemoattraction. Inhibition of Notch signaling using either the gamma-secretase inhibitor LY-411575 or *mindbomb* mutants results in expanded sclerotome domains and increased migration of sclerotome-derived cells around the neural tube and notochord. Interestingly, embryos with somite segmentation defects, such as *tbx6* mutants or *her1; her7* double mutants, do not display these phenotypes, suggesting that Notch signaling regulates sclerotome development independently of its role in somite segmentation. Timed Notch inhibition using LY-411575 reveals that Notch signaling antagonizes sclerotome induction during somitogenesis. Expansion of the sclerotome domains upon Notch inhibition requires BMP signaling, as expression of the BMP antagonist *hsp70:noggin3* effectively abrogates this expansion. To investigate sclerotome cell migration, we analyzed the expression pattern of *pdgfab*, which encodes a ligand for Pdgfra. Notch inhibition induces precocious differentiation of supernumerary *pdgfab*-expressing motor neurons. Consistently, sclerotome cell migration upon Notch inhibition is completely blocked in either *pdgfab* or *pdgfra* mutant embryos, despite expanded sclerotome domains. Collectively, our results suggest a dual role for Notch signaling in sclerotome development: restricting the number of sclerotome cells per somite and indirectly controlling sclerotome cell migration by modulating Pdgfra ligand distribution.

172

A structural transition ensures robust formation of zebrafish skeletal muscle

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Topic

5 Tissue Formation

Abstract Content

During organ development cells undergo significant morphological and positional changes. Yet, by the end of organogenesis, internal organ structure is typically robustly defined, with cells tightly packed. It remains an open question as to how the three-dimensional (3D) internal structure of an organ emerges reliably, particularly when there are multiple cell types interacting and dynamic boundary constraints. Here, we utilise quantitative live imaging and 3D morphological measures of the developing zebrafish myotome to unravel how early muscle organisation emerges. Contrary to the textbook view of muscle fibres as cylindrical, myocytes undergo an ordered chiral twist, the direction and magnitude of which depends on their position within the myotome. Further, cells skew and rearrange, seemingly to facilitate close packing of neighbouring muscle fibres. Cell movement undergoes a rapid decline in speed once the cells span the myotome segment. We find that cell packing is altered in mutants that disrupt cell fate or cell fusion, even though the final muscle segments remain largely confluent. Biophysical perturbation reveals that the cells are mechanically plastic, able to adjust to changes in the local cellular environment and boundary constraints. Taking these results together, we propose that the early myotome undergoes a structural transition, from a fluid-like state into a frozen state, resembling glass-like behaviour. Cellular plasticity in response to varying boundary constraints may be a general mechanism for ensuring robust organ morphogenesis in dense 3D tissues.

10

Shroom3 controls vascular architecture by regulating contractile apparatus formation and constriction of zebrafish gill pillar cells

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Topic

5 Tissue Formation

Abstract Content

In developing organisms, cells undergo dynamic changes to generate the complex architecture of tissues, a process critically dependent on actin cytoskeleton dynamics. One fundamental morphogenetic mechanism is apical constriction, frequently mediated by the actin-binding protein Shroom3 in epithelial tissues through indirect activation of non-muscle myosin. In our previous work (Preußner et al., 2025), we generated a detailed vascular atlas of zebrafish gill development. This atlas identified key cellular and temporal mechanisms driving gill development and

provided the foundation to investigate an unforeseen phenotype associated with the loss of the morphogenetic regulator Shroom3.

We combined comprehensive high-resolution 3D-confocal microscopy with newly identified transgenic markers for gill cell types, tissue clearing, live imaging and electron microscopy to investigate the function of Shroom3 using a zebrafish *shroom3* mutant generated with TALEN. We further characterized the anatomy and development of gills and pillar cells. Comprehensive morphometric and quantitative characterization during gill development and analysis of *shroom3* mutants were performed using IMARIS.

Gill lamellae, the functional unit of the fish respiratory organ, consist of thin epithelial sheets overlying a vascular network formed by neural crest–derived pillar cells that surround extracellular collagen columns. These columns are enveloped by parallel actomyosin-rich apparatuses of pillar cells that long have been hypothesized to be contractile. In *shroom3* mutants, these structures fail to assemble properly, pillar cell constriction is impaired, and lamellar vascular architecture is severely disorganized. Finally, we uncovered an alteration in the composition of the haematopoietic stem cell niche in the *shroom3* mutants.

We propose a previously unrecognized role of Shroom3 in regulating actomyosin-driven constriction in the non-epithelial pillar cell, where it is required in the formation of the contractile apparatus, maintaining the integrity of the lamellar vascular space. The resulting morphological defects likely induce chronic hypoxia, triggering compensatory changes in erythropoiesis and haematopoietic stem cell composition.

249

Fgfr2 and Gpcr126 Receptors Form a Signaling AND Gate for Tissue Fusion in Zebrafish Semicircular Canal Morphogenesis

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Topic

5 Tissue Formation

Abstract Content

Tissue fusion is a fundamental event in organogenesis, coordinating cell movements as pre-existing adhesion complexes are remodeled and new adhesions form to transform tissue topology. This process underlies diverse morphogenetic events, including craniofacial

development, vascular network formation, separation of the trachea and esophagus, and the sculpting of inner ear architecture. A key question is how signaling pathways control the cellular and subcellular dynamics that drive precise tissue fusion. Using live-imaging of genome-edited zebrafish, genetics, and pharmacological perturbations, we uncovered a signaling AND gate that regulates cell behaviors that fuse epithelial tissues for semicircular canal (SCC) development in the zebrafish inner ear. First, we defined cellular and subcellular behaviors that occur as three pairs of SCC epithelial buds make contact and fuse, transforming the ear's topological genus from zero to three. Second, we characterized mutants for signaling receptors, *fgfr2* and *gpcr126*, that are necessary for SCC tissue fusion. Third, the distinct SCC fusion phenotypes suggest complementary roles for Fgfr2 and Gpcr126 in adhesion remodeling. In *fgfr2* mutants, buds anchor but cells fail to disengage from their original neighbors, indicating a role in remodeling existing adhesion complexes. In contrast, *gpcr126* morphants have buds that grow past one another, suggesting a role in forming new adhesions between opposing buds. In wild type buds, dynamic lamellipodia form at fusion sites, but these are absent in *fgfr2* or *gpcr126* mutants. Pharmacological elevation of cAMP rescues the *gpcr126* mutant defect, restores lamellipodia, and relocalizes adhesion molecules, such as claudins, to the apical membrane. Signaling reporters and genome-edited fish, including *arpc3-neongreen and claudin7b-neongreen*, reveal coordinated signaling, cytoskeletal dynamics, and adhesion remodeling during fusion that is regulated by two distinct signaling inputs. Such a framework is likely to apply more broadly to fusion events, ensuring that fusion occurs at the correct place, time, and between appropriate tissue partners.

MORPHOGENESIS AND ORGANOGENSIS II

260

An ex-vivo ovary system reveals novel regulators of early oogenesis: An mTOR–Stat3–Stathmin pathway controls oocyte centrosome and microtubule dynamics

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

In sexually reproducing organisms, adequate egg production is essential for fertility, reproduction, and embryonic development. Yet, our understanding of the fundamental regulatory mechanisms underlying the cellular and developmental programs of oogenesis is lacking. The zebrafish provides an excellent model for studying early oogenesis, however, only a limited number of regulators are known, and experimental manipulation requires demanding genetic approaches.

Here, we report a new experimental platform for studying ovarian development and the discovery of overlooked regulators of early oogenesis. First, we establish a long-term ovary culture system which enables physiological oocyte development from oogonia to primordial follicles, providing an effective *ex-vivo* platform for investigation. Next, we utilized this system for functional screening of candidates from stage-specific oocyte transcriptomic data. We identified mTOR, Stat3, and the microtubule-destabilizing protein Stathmin as novel regulators of oocyte polarity. During polarization, microtubules control localization of the polarity regulator Buc to the centrosome and its condensation into the Balbiani body, an oocyte membraneless compartment. Through a combination of genetics and pharmacological manipulations *in-vivo* and *ex-vivo*, we show loss of mTOR or Stat3 function, or overactivation of Stathmin, disrupts centrosome regulation and destabilizes microtubules, leading to dispersed Buc condensates and loss of polarity. We demonstrate mTOR acts upstream of Stat3 in oocytes, and inhibition of Stathmin in *stat3*$^{-/-}$ or mTOR-deficient ovaries rescues both cytoskeletal and polarity defects. These findings establish an unpredicted mTOR-Stat3-Stathmin pathway that controls centrosome and microtubule dynamics to drive oocyte polarity.

We propose that through cytoskeletal regulation this pathway provides oocytes with polarization competence, a key general step in morphogenesis. Furthermore, our *ex-vivo* ovary system provides a powerful platform for rapid functional studies and screening, and to elucidate intricate mechanisms of oogenesis. This promises to advance research on oogenesis and ovarian biology and guide the development of *ex-vivo* platforms in other organ systems.

412

Differential spreading behaviors of Nodal signalling molecules in the extracellular space cooperatively shape left-right asymmetry in zebrafish embryos

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Background

Left-right (LR) patterning of vertebrate embryos is mediated by diffusive proteins secreted mainly from the left-right organizer (LRO), such as TGF-β superfamily ligands Nodal and Gdf1, as well as their inhibitors Dand5 and Lefty. Although genetic studies have established their essential roles in LR asymmetry formation, precise modes of action for these proteins remain unclear due to difficulties in visualizing and manipulating their extracellular behaviors in the embryo.

Methods

To investigate their extracellular dynamics, we used novel transgenic zebrafish lines and extracellular trapping molecules, including morphotrap, which enables detection of GFP-tagged diffusing ligands. We also developed a synthetic molecule, tethered-Dand5, which traps extracellular Nodal and suppresses its signalling, allowing us to test the requirement of Nodal diffusion from the LRO in LR asymmetry formation.

Results

We found that the heterodimer of Nodal and Gdf1, the major signalling ligand in vertebrate Nodal signalling, spreads from the LRO through the extracellular space in the paraxial mesoderm (PM) to its target tissue, the lateral plate mesoderm (LPM), possibly in a free-diffusion-like manner. In sharp contrast, Dand5, a DAN-family secreted Nodal inhibitor, accumulates in a punctate manner in the extracellular spaces of the right side of the PM, where it is immobilized through interaction with heparan sulfate proteoglycans and asymmetrically suppresses Nodal signalling.

Conclusion

Our study uncovers diverse modes of action for LR-related secreted proteins in the extracellular space, providing a new framework for understanding how spatially organized Nodal signalling molecules shape embryonic LR asymmetry.

43

A density dependent jamming transition terminates convergence flows in zebrafish embryos

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Convergence-extension (CE) is a tissue-scale morphogenetic process during development, where tissues thin along one direction, while simultaneously elongating in an orthogonal direction. While numerous studies have characterized the cellular and molecular machinery that drive this process in various contexts, it remains unknown, once initiated, how convergence comes to a halt in any system. Towards this end, using zebrafish embryos as a model, we characterised bilateral convergent flows in the paraxial mesoderm using light-sheet microscopy and quantitative image analysis modules. We found that convergence flows slow down and ultimately halt via a density-dependent jamming-like transition, where the slowdown in convergence correlates with increased cell crowding, decreased extracellular space and reduced velocity fluctuations. Concomitant with a slow down in convergence, we also observed an increase in cell-cell adhesion in these tissues. Reducing adhesion enhanced convergence, ultimately leading to thinner and longer embryos, whereas increasing cell-cell adhesion led to wider and shorter embryos, indicating a vital role played by cell-cell adhesion in terminating convergence. We further show that Wnt signaling primarily provides the driving force for convergence and a signaling-related mechanism does not explain when convergence flows stop in the embryos. We thus reveal a physical mechanism that largely determines when bilateral convergence comes to a halt in the paraxial mesoderm, thereby ensuring symmetric tissue dimensions.

203

From dynamic morphogen gradients to complex tissue mechanics

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Seminal work showed that a few morphogens can trigger patterning and morphogenesis during development. While previous studies focused on the regulatory logic of morphogens specifying cell fate, less is known about how they encode cellular behaviours and mechanical forces driving morphogenesis. Recent work identified a role for the morphogen Nodal — a conserved inducer of meso- and endoderm (mesendoderm) patterning — in controlling gastrulation movements in zebrafish.
To investigate how Nodal elicits such diverse responses, we established an *in vitro* assay linking Nodal signaling dynamics and transcriptional programs to mesendoderm mechanics. Since both Nodal concentration and duration vary along the gradient *in vivo*, we combined unbiased sequencing with quantitative imaging to decouple these variables *in vitro*. While well-established Nodal targets were strongly upregulated, transcriptional changes across most of the concentration range were modest. This contrasts with the linear response of pSmad2/3, the downstream effector of Nodal signalling, suggesting that Nodal-encoded transcriptional responses saturate more rapidly than that of pSmad2/3. In contrast, cell protrusions — a proxy for motility — showed a dose-dependent response to increasing Nodal concentrations, supporting that tuning concentration alone is sufficient to generate a motility gradient. Using pulsed rather than sustained induction, we found that mesendoderm cells become irreversibly protrusive after ~2 hours of signalling, regardless of the exact Nodal concentration used. This supports a model where Nodal concentration sets the magnitude of motility, while signalling duration controls the irreversible switch to a motile state. Using time-controlled perturbations *in vivo*, we confirmed that sustained Nodal signalling (~2 hours) is indeed necessary for correct mesendoderm morphogenesis. Remarkably, despite largely normal morphogenesis, these embryos still showed patterning defects, suggesting differential signalling requirements for fate specification versus morphogenetic programs. Overall, with this systematic approach, we aim to uncover the regulatory logic by which Nodal co-encodes both fate and morphogenetic programs.

291

Paracrine and endocrine regulation of ear pressure homeostasis by spatially restricted tissue failure

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Sensory and neurological organs like the ear, eye, and brain are organized around pressurized, fluid-filled cavities essential for structure, protection, and function. Imbalances in fluid pressure underlie widespread diseases, including glaucoma, traumatic brain injuries, and hearing disorders. Ménière's disease, a hearing and balance disease that can cause deafness, arises from disrupted pressure regulation in the inner ear. Despite this burden, fluid pressure regulation remains poorly understood in the ear due to its interdisciplinary nature and its optical inaccessibility in mammals. We previously discovered a tissue-scale pressure relief valve in the ear's endolymphatic sac, explaining long-observed but unaccounted physiological behaviors. Using quantitative live imaging of genome-edited cell biological proteins, genetics, and molecular tools in zebrafish, whose optically accessible inner ears enable real-time study, we investigate the development and physiology of ear pressure homeostasis. Reasoning from first principles, we show that the endolymphatic sac functions as a tissue-scale pressure-relief valve whose rupture localizes to a single site each cycle, consistent with material failure theory. Valve formation requires Activin, which destabilizes epithelial junctions through endolysosomal turnover; blocking Activin with Follistatin stabilizes adhesion and prevents valve formation. Activin reduces *epcam* expression and promotes EMT-like transcriptional programs. In parallel, zebrafish vasopressin rapidly activates Erk, accelerating endocytosis and adhesion remodeling. Paracrine Activin defines where rupture can occur, and Erk signaling defines when it occurs; together they maintain inner ear hydraulic homeostasis by determining the pressure set-point. Elevated Activin or vasopressin, both associated with Ménière's disease, may disrupt this valve by eliminating weakly adhesive cells or altering cellular responsiveness to homeostatic signals to disrupt the pressure set point.

25

Tra2a-mediated alternative splicing in neighboring cells drives defective cell elimination to ensure tissue robustness

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Although a variety of defective cells often emerge in proliferative tissues, neighboring normal cells eliminate them via cell-cell communication to ensure tissue robustness and prevent potential diseases. This phenomenon is known as 'cell competition', and cell competition studies have focused on the molecular mechanisms operating in the defective cells. However, the gene regulatory networks and signaling pathways that act within

neighboring cells are largely unknown. Using the special transcriptomics Photo-Isoloation Chemistry (PIC) and zebrafish in vivo imaging, we identified Tra2a, a highly conserved RNA-binding protein that controls the alternative splicing of pre-mRNA, as a critical driver of defective cell elimination in neighboring cells. Tra2a expression in neighboring cells is downregulated by the spontaneous appearance of various defective cells, including Ras-mutated oncogenic cells and cells with abnormal Wnt signaling activity. Forced expression of Tra2a blocks cell competition and stimulates the defective cell accumulation, leading to developmental anomalies and primary tumors. Tra2a downregulation likely controls the expression levels of cell competition mediators by altering splicing patterns to promote defective cell elimination. We also show that Tra2a downregulation is amplified by the inclusion of the poison exon in their pre-mRNA, which causes premature translation termination and nonsense-mediated decay of the transcript, and Tra2a's poison exon inclusion can be utilized as a novel universal marker to specifically detect neighboring cells that eliminate defective cells in living animals. Thus, our study identifies alternative splicing as a cell competition conductor and reveals the novel role of alternative splicing in tissue robustness.

CELL FATE

26

Redox biology in retinal development: unraveling the role of the Nrf3/Prdx6 antioxidant axis in retinal stem cells fate and homeostasis

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Topic

19 Cell fate

Abstract Content

The ciliary marginal zone (CMZ) is a post-embryonic structure located at the periphery of the zebrafish differentiated retina that contains the stem and progenitor cells (RSCs and RPCs) spatially distributed and maintaining tissue homeostasis throughout the lifelong growth of the animal. As such, it serves as a powerful model for studying mechanisms governing retinal stem cell fate transition. We previously showed that reactive oxygen species (ROS) regulation, via the H_2O_2-quenching enzyme Catalase (*Cat*), controls the transition from proliferation to differentiation in RSPCs, demonstrating that, within a particular range, ROS act as key signaling molecules that actively regulate cell fate decision.

Through single-cell RNA sequencing of developing zebrafish retinas, we identified several redox-related genes enriched in RSPCs with unknown functions during retinogenesis. We focused on Nrf3, a Cap'n'Collar transcription factor and master regulator of antioxidant programs, and Prdx6, a peroxiredoxin involved in phospholipid repair and ROS detoxification. CRISPR/Cas9 was used to generate knockout lines for both genes, and their expression was assessed by qRT-PCR and multiplex *in situ* hybridization. Cell death and proliferation were evaluated by TUNEL and anti-PHH3 staining. Bulk and RPC-specific transcriptomic analyses were performed in both mutant backgrounds to identify downstream pathways

nrf3-/- embryos display persistent RPC proliferation accompanied by compensatory *cat* upregulation. We demonstrated that Prdx6 is a downstream effector of Nrf3 and, corroborating our scRNAseq data, *prdx6* is found strongly expressed in the CMZ in a gradient manner from RSCs to RPCs. *prdx6-/-* embryos exhibit a reduced progenitor niche, premature apoptosis in the eye, and ectopic RPC proliferation. Additionally, bulk and RPCs-specific transcriptomic analyses in both mutant backgrounds identify several pathways underlying these phenotypes.

Together, our findings position Nrf3 and Prdx6 as core components of a redox-regulated network that shapes RPCs behavior during retinogenesis and is essential for retinal homeostasis.

409

sox10-derived progenitors trans-differentiation underly neuronal regeneration in the zebrafish retina after injury.

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Topic

19 Cell fate

Abstract Content

Background:
Damage to retinal neurons and the optic nerve in humans, such as in glaucoma and age-related macular degeneration, leads to irreversible vision loss due to limited regenerative capacity. But unlike humans, zebrafish can regenerate their retina after injury, primarily via Müller glial cells (MG). Whether additional progenitor populations contribute to retinal regeneration is still unclear.

Methods:
We use a model of targeted retinal ganglion cell ablation using the nitro-reductase/metronidazole system. In this transgenic line, the bacterial nitro reductase enzyme is selectively expressed in RGCs, allowing specific ablation of the RGC layer and optic nerve. At 5pdf, larvae are treated with metronidazole, which induces selective death of nitro reductase-expressing cells, resulting in degeneration of RGCs and their axons. Following injury, we monitor the regenerative response and track the fate of regenerating cells in the retina.Transcriptomic profiling, genetic lineage tracing, photoconversion, live imaging, and pharmacological pathway inhibition are used to identify and characterize regenerative progenitor populations and to examine the roles of PDGF, WNT, and insulin signaling pathways during RGC regeneration.

Results:
A previously uncharacterized population of mesenchymal progenitor cells with neural crest–like features (*sox10/pdgfrb*$^{+}$) accumulates in the ganglion cell layer following RGC ablation and expresses proneural markers such as *ascl1a* and *olig2*. Lineage tracing demonstrates that these cells give rise to regenerated RGCs through direct transdifferentiation, independent of proliferation. Functional analyses show that PDGF and WNT signaling are required for this regenerative process. In addition, insulin mRNA is expressed in *pdgfrb*$^{+}$ progenitors during regeneration, and genetic

ablation of insulin-producing cells impairs RGC regeneration, highlighting a regulatory role for insulin signaling in regeneration.

Conclusion:
Neural crest–derived mesenchymal progenitors can regenerate retinal neurons through trans differentiation. This mechanism offers an alternative pathway for retinal repair and suggests mesenchymal progenitors as potential targets for regenerative therapies in retinal degenerative diseases.

208

Decoding the skeletal stem cell niche identity and its function in skeletal homeostasis

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Topic

19 Cell fate

Abstract Content

Skeletal stem cells (SSCs) are essential for bone development and homeostasis, yet the identity and function of their supporting niche remain poorly understood. In zebrafish, we identified a previously unrecognized population of dermal fibroblasts (DFs) located within the SSC niche, characterized by enrichment in phenylalanine/tyrosine (Phe/Tyr) metabolism - a pathway whose disruption underlies metabolic disorders in humans like phenylketonuria and alkaptonuria that manifests skeletal defects. We hypothesize that DFs form a critical metabolic niche that sustains SSC function and bone homeostasis. Using single-cell RNA-seq and ATAC-seq, we identified a 2.7 kb hpdb regulatory element - encoding a key enzyme in the Phe/Tyr cascade - that drives specific expression in DFs, in a domain spatially distinct from other skeletal cell types. To functionally interrogate this population, we developed DF-specific Cre-lineage tracing and ablation transgenic fish. Notably, ablation of DFs in adult zebrafish results in impaired caudal fin regeneration, providing preliminary functional evidence that DFs are essential for skeletal tissue repair. To test whether DFs protect skeletal tissue from toxic metabolite buildup, we generated mosaic zebrafish with metabolically impaired DFs using a model of alkaptonuria. Preliminary results suggest that defective DFs can influence surrounding bone integrity. Together, our findings indicated niche-resident DFs play a key role in supporting SSCs through metabolic regulation, offering insight into how local environments contribute to skeletal health.

173

Sequential Dll4 and Jag1a signalling act cooperatively to drive haematopoietic stem cell fate specification

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Topic

19 Cell fate

Abstract Content

Background: Hematopoietic stem and progenitor cells (HSPCs) arise during embryonic development and provide mature blood cells throughout an organism's lifespan. They originate from a specialized subset of arterial endothelial cells, the haemogenic endothelium (HE), in a process regulated by several key pathways, including Notch signalling. The current understanding is that different Notch ligands perform opposite functions in arterial endothelium: Jag1 is required for HSPC formation but not arterial identity, whereas Dll4 mediates arterial identity and acts as a negative regulator of HSPC emergence.

Methods: To investigate the roles of Dll4 and Jag1a in HSPC development, we combined genetic ablation of *dll4* and *jag1a*, single-cell transcriptomic profiling, and live imaging of HSPCs in *zebrafish*. This multi-modal approach enabled temporal and mechanistic dissection of ligand-specific contributions to HSPC emergence.

Results: Our data revealed that Dll4 is a positive regulator of HSPC emergence. Dll4 acts early, promoting the transition from pre-HE to HE by modulating the MAPK pathway to enable differentiation towards the haematopoietic stem cell fate. By contrast, Jag1a acts later, after blood circulation is established, to drive the transition of HE cells into HSPCs. This activity depends on mechanical cues arising from blood flow-induced shear stress Strikingly, endothelial-specific *jag1a* overexpression rescues the loss of HSPCs caused by chemical or genetic inhibition of blood flow.

Conclusion: These findings reveal that Dll4 and Jag1a act in a coordinated, sequential manner to drive endothelial-to-haematopoietic transition, overturning the prevailing view that they play opposing roles in HSPC emergence. This work highlights the need for future studies to determine how biomechanical and molecular cues are integrated at the single-cell level to fine-tune Notch-dependent fate decisions.

METABOLISM

147

Dermal Fibroblasts as Local Metabolic Regulators in Zebrafish Skeletal Maintenance

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Topic

8 Metabolism

Abstract Content

Adult stem cell homeostasis relies on the niche—a complex microenvironment providing biochemical and mechanical cues. Emerging evidence suggests that cell-cell communication within these niches is also driven by "metabolic signaling," where metabolites act as secondary messengers to regulate signaling pathways and epigenetic states. I aim to explore the metabolic interplay between a unique zebrafish mesenchymal cell population – dermal fibroblasts - spatially positioned in the outer layer of periosteum, directly overlying skeletal stem cells/progenitors (SSC/Ps). This metabolically highly active cell population expresses, among others, enzymes involved in Phe/Tyr degradation and gluconeogenesis, both traditionally attributed to hepatic function. We hypothesize that these pathways are coupled to generate specific niche signals. Using *in situ* hybridization and zebrafish *ex vivo* model coupled with ^{13}C stable isotope tracing, we confirmed the coupling of Phe/Tyr degradation with gluconeogenesis flux in dermal fibroblasts. Our data suggest that while SSC/Ps maintain a quiescent metabolic profile, the neighboring DFs act as a "metabolic powerhouse," potentially provisioning specific metabolites to the SSC/P population. I will discuss our latest data and upcoming *in vivo* approaches designed to test the metabolic signaling hypothesis. By utilizing targeted genetic perturbations and live imaging, we aim to demonstrate how this localized metabolic coupling directly influences SSC/P activation and bone maintenance. This work establishes a novel model for "metabolic communication" where spatial metabolic heterogeneity serves as a primary driver of tissue homeostasis.

186

Retrograde Peristalsis is Essential for Nutrient Utilization and Shapes Gut Microbiota in Zebrafish

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Topic

8 Metabolism

Abstract Content

Background: While gastrointestinal peristalsis is essential for digestion, the physiological significance of retrograde (reverse) contraction remains poorly understood in mammals as well as in teleost. In our previous report (Fujii et al., Cell Reports, 2000), *hcn4* and *hcn4l* genes encoding hyperpolarization activated channels were implicated to underly retrograde contraction.

Methods: We generated a *hcn4*/*hcn4l* double knockout line, expected to display selective disruption of reverse intestinal waves sparing anterograde motility.

Results: Using live imaging and functional assays, we showed that the loss of retrograde peristalsis accelerates movement of food along the intestinal tract and alters luminal content dynamics. This motility defect was accompanied by profound remodeling of the gut microbiota, characterized by shifts in community composition and a pronounced bias toward immune-related microbial functional pathways. Consistent with these microbial changes, double knockout larvae exhibited elevated basal expression of the innate immune signaling adaptor Myd88 at levels comparable to flagellin-stimulated wild-type larvae. Notably, flagellin exposure further increased Myd88 expression in wild-type larvae but did not enhance expression in double knockout larvae, indicating a constitutively elevated innate immune state with reduced inducibility.

Conclusion: Our findings identified retrograde peristalsis as a previously underappreciated regulator of the intestinal microenvironment that shapes host–microbe interactions and innate immune homeostasis in early development.

314

Connexin 43 propagates the synchronicity of pancreatic beta cells initiated by neural signalling

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Topic

8 Metabolism

Abstract Content

Pancreatic endocrine cell activity coupling is important for maintaining the mature pattern of hormone secretion. Gap junctions play a well-established role in coordinating beta-cell activity and insulin secretion. In mammals, *Cx36* encoded gap junctions mediate most of the activity coupling between beta cells. Although previous studies have reported the absence of beta-cell expression of the zebrafish homolog *cx35*, we detected *cx43* expression in zebrafish beta cells by 100 hours post fertilisation. In this project we aimed to investigate the how *cx43* contributes to pancreatic islet physiology.

We first confirmed the functionality of the gap junctions in isolated islets loaded with calcein-AM and observed a complete loss of fluorescence recovery after photobleaching in *cx43* mutant beta cells. Next, we conducted *in vivo* analyses of whole-islet physiology by imaging calcium dynamics using a GCaMP6s-encoded biosensor. Analysis of single-cell activity dynamics, followed by statistical analyses of islet coordination, revealed reduced beta-cell activity coupling in the absence of *cx43.* We also found increased larval glucose levels, suggesting that insulin secretion is impaired in *cx43* mutants.

As pancreatic islet innervation remained unaltered in *cx43* mutants, we investigated whether neural signals contribute to the residual beta-cell activity coupling. Upon two-photon ablation of the peri-islet neurons that innervate the islet at 110 hours post fertilisation, we observed exacerbated islet physiology defects in *cx43* mutants. This finding suggests that neural signalling complements gap-junctional activity to achieve optimal islet function.

Healthy islet function requires the coordination of hormone output from different endocrine cells, through paracrine, vascular, gap junctional, and neural communication. Our studies demonstrate that simultaneous interrogation of multiple regulatory inputs in an *in vivo* context can reveal new synergies that support optimal organ maintenance and function.

237

Advancing Batten Disease Research: A Zebrafish *In Vivo* Discovery Platform Coupled with a Whole Animal LysoIP Method

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Topic

8 Metabolism

Abstract Content

Background

Mutations in CLN3 gene lead to the juvenile form of Batten disease (BD) characterised by the accumulation of autofluorescent storage material in lysosomes. BD is a currently untreatable and fatal neurodegenerative disorder in children. Usually, the first symptoms are vision loss, followed by cognitive decline, behavioral changes, epileptic seizures, and finally premature death. Given the high conservation of the CLN3 protein sequence across species, several disease models have been established to study its molecular function and the disease

mechanisms. One of the most consistent phenotype among models is the accumulation of glycerophosphodiesters (GPDs) and reduction in bis(monoacylglyero)phosphates (BMPs).

Method

We generated a *cln3*$^{-/-}$ zebrafish mutant using CRISPR/Cas9 and characterized it across multiple larval stages with mass spectrometry–based analyses. Using this model, we established a zebrafish-based drug-validation assay to identify therapeutic compounds capable of restoring metabolic alterations. To study lysosomal content, we implemented the LysoIP-approach by generating a *tmem192*-HA transgenic line using the Tol2 transposon system, enabling rapid immunoprecipitation of intact lysosomes from 5 dpf larvae.

Results

Our analyses confirmed the BD phenotype: accumulation of GPDs, along with a consistent ~50% reduction in BMPs. Based on these metabolic phenotypes, we screened ten candidates drugs (selected based on reported activity in CLN3 *in vitro* systems or Niemann–Pick type C models) and identified three compounds that significantly rescued either BMP and/or GPD levels. Using the LysoIP approach, we further confirmed that *cln3*$^{-/-}$ lysosomes accumulate the mitochondrial ATP synthase subunit c, the major component of the storage material previously reported in patient cells.

Conclusion

Overall, *cln3* mutant zebrafish larvae recapitulate key disease-specific alterations, positioning this model as a powerful and quick *in vivo* system for therapeutic compound screening. In addition, the new lysoIP line will enable rapid progress in defining CLN3 function.

364

A quantitative in vivo CRISPR-imaging platform identifies regulators of hyperplastic and hypertrophic adipose morphology in zebrafish

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Topic

8 Metabolism

Abstract Content

Background

Adipose tissues exhibit a remarkable capacity to expand, regress, and remodel in response to energy status. The cellular mechanisms underlying adipose remodelling are central to metabolic health. Hypertrophic remodelling - characterised by the enlargement of existing adipocytes - is associated with insulin resistance, type 2 diabetes, and cardiovascular disease. In contrast, hyperplastic remodelling – in which new adipocytes are generated - is linked to improved metabolic outcomes. Despite its clinical importance, the regulation of hypertrophic and hyperplastic adipose morphology remains poorly understood.

Methods

Here, we integrate human transcriptomic data with a quantitative CRISPR-imaging platform in zebrafish to identify regulators of adipose morphology. We developed an image-based phenotyping pipeline that captures lipid droplet size, number, and spatial patterning, and applied generalised additive modelling to quantify hyperplastic versus hypertrophic morphology signatures.

Results

Using this platform, we conducted an F0 CRISPR screen targeting 25 candidate genes and identified three that induced hypertrophic morphology (*txnipa*, *mmp14b* and *foxp1b*) and an additional candidate that altered total adiposity (*kazna*). For functional validation, we generated stable loss-of-function alleles for both zebrafish foxp1 paralogues. Spatial analysis along the anterior-posterior axis revealed that *foxp1b* mutants display developmental hypertrophy but profoundly blunted adaptive responses to high-fat diet (∼68% reduction across all spatial zones), while *foxp1a* mutants show normal baseline morphology but disrupted spatial patterning of diet-induced hypertrophy.

Conclusions

Together, these findings establish a scalable CRISPR-imaging platform for in vivo genetic screening of adipose morphology, and reveal distinct roles for Foxp1 paralogues in developmental patterning and adaptive responses to dietary challenge in adipose tissue.

TOXICOLOGY II

295

Loss of the Oatp1d1 Transporter Disrupts Pigmentation and Enhances PFOS Sensitivity in Zebrafish

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Topic

10 Toxicology

Abstract Content

Oatp1d1 is a member of the OATP/Oatp superfamily of membrane transporters involved in absorption, distribution, metabolism, and excretion (ADME) processes. In zebrafish, Oatp1d1 is ubiquitously expressed, with the highest expression in the liver and brain, and has been suggested to play a role in steroid hormone homeostasis. To investigate the function of this transporter *in vivo*, we generated a CRISPR-Cas9 oatp1d1 knockout zebrafish line and analysed phenotypic and molecular changes during embryonic development and in adults. Compared with wild-type (WT) fish, oatp1d1 mutant embryos lacked yellow pigmentation during early development, as confirmed by reduced pteridine-associated fluorescence and methylene blue staining. In contrast, adult mutant fish exhibited increased numbers of xanthophores and melanophores. Crossing mutant females with WT males restored the WT phenotype, confirming that the pigmentation changes are associated with the oatp1d1 mutation. Exposure of embryos to thyroid hormones (T3 and T4) resulted in reduced yellow pigmentation in WT embryos, suggesting that Oatp1d1 may be involved in the transport of pigment precursors or in thyroid hormone-related pathways. Particles were observed in the perivitelline fluid (PVF) in embryos derived from mutant females, suggesting a maternal effect potentially related to estrogen signalling and estrogenic compounds such as 17β-estradiol, an Oatp1d1 inhibitor. We examined the expression of other organic anion transporters as well as genes involved in thyroid development and thyroid hormone transport. Moderate alterations in *oatp2b1* and *oat2a* expression were observed, indicating potential compensatory regulation within transporter networks. Finally, mutant embryos exhibited increased sensitivity to the environmental contaminant PFOS, a known xenoestrogen and Oatp1d1 substrate, with lower LC_{50} values, more severe developmental abnormalities, reduced locomotor activity, and elevated oxidative

stress compared with WT embryos. Overall, our results suggest that Oatp1d1 has previously unrecognised roles in pigmentation, vitellogenesis, and steroid hormone homeostasis, and contributes to xenobiotic sensitivity in zebrafish.

332

Establishing Zebrafish Embryo Model to Study Normal Tissue Sparing with Ultra-High Dose Rate Electron Beams

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Topic

10 Toxicology

Abstract Content

Background:
FLASH irradiation reduces normal tissue toxicity while maintaining tumor control, but optimal beam parameters and biological mechanisms remain unclear. Zebrafish (Danio rerio) embryos are a valuable in vivo model due to their developmental sensitivity and genetic similarity to humans. Within the FLASHlab@PITZ platform — a collaboration between PITZ/DESY Zeuthen, TH Wildau, Charité, and the MDC — we aim to establish zebrafish as a robust model for studying FLASH-mediated tissue-sparing effects.

Methods:

Wild-type AB zebrafish embryos at 24 hpf were irradiated with X-rays (0.04 Gy/s, 5–30 Gy) for model validation, then with LDR (0.05 Gy/s) and UHDR (10^5 Gy/s) electrons at 8–10 Gy. At 5 dpf, toxicity was assessed via survival, spinal curvature, pericardial edema, body length, and eye diameter. Alcian blue and H&E staining protocols are being established.

Results:

Initial X-ray irradiations used relatively high doses due to the greater accessibility of the X-ray source. At doses of 20–30 Gy, 100% embryo mortality was observed. Following

optimization, doses of 8 and 10 Gy were selected for comparative analysis between X-ray and electron irradiation modalities. X-ray irradiation induced clear dose-dependent toxicity, characterized by increased spinal curvature, pericardial edema, and impaired growth, with moderate to severe malformations observed at doses ≥10 Gy. LDR electron exposure resulted in similar, though slightly attenuated, toxic effects compared to X-rays. In contrast, UHDR electron irradiation significantly reduced radiation-induced toxicity. Most embryos exposed to UHDR conditions preserved normal spinal and cardiac morphology, and growth parameters remained significantly closer to control values across all tested doses.

Conclusion:

UHDR electron irradiation confers protection against radiation-induced toxicity in zebrafish embryos, consistent with the FLASH effect. These findings establish zebrafish as a sensitive in vivo model for FLASH radiobiology, supporting mechanistic and translational research within FLASHlab@PITZ.

41

Impairment of mitochondrial functions on zebrafish embryonic development, following chronic low-dose exposure to gamma ionizing radiation.

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Topic

10 Toxicology

Abstract Content

Following nuclear accidents, surrounding ecosystems are subjected to chronic low dose exposure of ionizing radiation (IR), affecting organisms at all stages of development. While the effects of IR on nuclear DNA are well recognized, their impact on mitochondria remains far less studied, despite the central roles of these organelles and the high radiosensitivity of mitochondrial DNA. Field studies conducted in Fukushima and Chernobyl have highlighted the radiosensitivity of numerous organisms and revealed significant mitochondrial alterations.

This project adopts a multi-scale approach to investigate the effects of IR from molecular alterations to organism-level outcomes such as behavior, with a focus on disruptions of mitochondrial functions, which are central to energy metabolism and cellular stress regulation. Zebrafish represent a highly suitable biological model for such approaches due to their fully sequenced genome, the transparency of their embryos and their rapid development.

Zebrafish embryos were exposed to low dose rates of gamma radiation ranging from 0.5 to 5 mGy/h for up to five days. Impacts were found at multiple biological levels with transcriptomic analyses revealing altered expression of genes involved in the oxidative phosphorylation, the oxidative stress management, the apoptosis, and the fatty acid β-

oxidation, associated with modifications in the activity of respiratory chain complexes at the functional level. At the tissue level, neuromasts exhibited a loss of mitochondrial membrane potential and increased cell death, associated with structural disorganization. Neuromasts are mechanosensory organs that play a crucial role in detecting water currents, vibrations, and movement. Behavioral analyses are currently being conducted to determine whether these cellular alterations translate into individual effects.

These findings highlight the mitochondria's vulnerability to chronic low dose rate exposure during embryonic development and provide new insights into the biological mechanisms underlying the effects of IR in a post-accidental environmental context.

88

Conserved developmental toxicity signatures of teratogenic chemicals in zebrafish embryos

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Topic

10 Toxicology

Abstract Content

Zebrafish embryos have emerged as a powerful vertebrate model for investigating chemical-induced developmental toxicity. However, the robustness of toxicological responses across genetically diverse zebrafish populations has not been fully established. To address this issue, we examined whether developmental and molecular responses to representative teratogens are conserved across commonly used laboratory strains. Embryos derived from five zebrafish strains (AB, TU, RW, WIK, and PET) were exposed to six well-characterized teratogenic compounds (valproic acid, hydroxyurea, methotrexate, acitretin, topiramate, and ibuprofen). Across all strains, exposure produced consistent dose-dependent effects, with increasing concentrations leading from morphological abnormalities to lethality. The spectrum of developmental defects induced by each compound was highly reproducible, suggesting that chemical-specific toxicity patterns are largely independent of strain background. To further characterize molecular responses, transcriptome profiling was performed following treatment with valproic acid, hydroxyurea, and warfarin. These compounds induced pronounced and concentration-dependent transcriptional alterations. Multivariate analyses revealed that variation in gene expression was primarily driven by chemical exposure level rather than genetic background. Although limited differences were observed among strains under baseline conditions or at low exposure levels, these differences had little impact on the overall transcriptional response to teratogenic stress. Our findings indicate that teratogen-induced phenotypic and transcriptional signatures are strongly conserved among zebrafish strains. This conservation suggests that developmental toxicity endpoints in zebrafish embryos are largely robust to genetic background, reinforcing the suitability of diverse zebrafish lines for chemical screening and toxicological research.

239

Thalidomide metabolite, 5-hydroxythalidomide, is teratogenic in zebrafish

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Topic

10 Toxicology

Abstract Content

The teratogen thalidomide induces limb, ear and craniofacial defects during human development. Thalidomide is hypothesised to undergo metabolism to 5-hydroxythalidomide and 5'-hydroxythalidomide enabling these products to bind to the primary targets of Cereblon (CRBN) & Spalt-like transcription factor 4 (SALL4). Thalidomide insensitivity in mice is believed to be due to multiple factors including low strength of binding to the drugs primary targets and preferential metabolism of thalidomide into 5'-hydroxythalidomide (rather than 5-hydroxythalidomide) which is the minimally teratogenic metabolite. Whilst zebrafish have been used to model thalidomide embryopathy, the literature is inconsistent with varying degrees of sensitivity reported between studies. Here we investigate whether the lack of metabolism and/or preferential metabolism to 5'-hydroxythalidomide contribute to reduced zebrafish sensitivity through characterisation of their response to 5-hydoxythalidomide.

To visualise internal structures we used transgenic (col2a1:mCherry,sp7:GFP) zebrafish to image collagen (mCherry), osteoblasts (GFP) and the associated structures. Larvae were dosed with thalidomide and 5-hydroxythalidomide at 12 hpf under static and/or semi-static exposure and observed the larvae at 3dpf. We found thalidomide had no adverse effects in zebrafish, whilst 5-hydroxythalidomide caused pectoral fin defects, vestibular defects and craniofacial defects at equivalent external concentrations. We also found the otolith demonstrated increased mCherry fluorescence, indicating increased or altered collagen deposition in larvae treated with 5-hydroxythalidomide, but not in thalidomide treated larva.

Our results demonstrate that 5-hydoxythalidomide is considerably more potent in zebrafish than its parent compound. 5-hydroxythalidomide causes zebrafish to develop defects all classically associated with thalidomide embryopathy in sensitive mammals. Furthermore, zebrafish otoliths demonstrate a particular sensitivity to 5-hydroxythalidomide. Previous mammalian studies have described gross defects in the vestibule but have not studied otoconia, the mammalian equivalent of the otolith, and as such our results may present a previously unexamined defect of thalidomide embryopathy.

198

Chemically-induced steatosis in zebrafish embryos after exposure to mixtures of cholestatic and steatotic compounds

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Topic

10 Toxicology

Abstract Content

Background

There is an increasing incidence and prevalence of fatty liver disease in the western world, with steatosis as the most prevalent variant. Mechanistic knowledge on chemical-induced steatosis has greatly evolved and has been organized into adverse outcome pathways (AOPs) describing the chain of events from first molecular interaction of a substance with a biological system to the adverse outcome and key characteristic of steatosis, intrahepatic lipid accumulation. For toxicological screening purposes, the zebrafish (*Danio rerio*) embryo provides an important whole-organism model system bridging the gap between cellular models and humans. Since humans are exposed to mixtures of substances targeting various hepatic endpoints we assessed the impact of combinations of substances on intrahepatic lipid accumulation.

Methods

We exposed zebrafish embryos to selected steatotic and cholestatic compounds, both individually and in binary mixtures from 72 or 96hpf until 120 hpf. Intrahepatic triglyceride accumulation and gene expression changes were analyzed by LipidGreen2 staining and qPCR to further elucidate chemical-induced steatosis and underlying mechanisms.

Results

Mixture experiments revealed various interaction patterns: in some cases, combined exposure resulted in dose addition on hepatic triglyceride accumulation, while in others, antagonistic or neutralizing effects were observed. This confirmed that some cholestatic compounds also drive steatotic mechanisms.

Conclusion

These results indicate that mixtures of steatotic and cholestatic compounds can produce a range of effects on the outcome of intrahepatic lipid accumulation in zebrafish embryos. Understanding interactions and their mechanisms is crucial for improved chemical mixture risk assessment.

EMERGING TECHNOLOGIES II

361

Building the reagent toolbox for the Cell Observatory

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Topic

2 Emerging Technologies

Abstract Content

Organismal development is an emergent process that depends on coordination across scales, from genomic and epigenetic regulation to cellular behavior and tissue-level dynamics. While reductionist approaches have identified molecular components, they have not been successful at teasing out mechanistic answers at scale, consequently understanding development *in toto* as an integrated process requires tools that capture biology in living organisms, in real time, at subcellular resolution. To accomplish this we are combining expertise in developmental biology, light sheet microscopy and artificial intelligence to build a foundational model for interrogating developmental dynamics in vivo.

The Cell Observatory is a multi-year effort. Our initial focus is to generate the project's first petabyte-scale 5D (spatial, temporal, chemical) datasets for training in leveraging the many strengths of zebrafish as a research model. To support these goals, we are benchmarking new fluorescent proteins and exploring several strategies to overcome positional effects in transgenesis: from optimizing ubiquitous promoters, buffering transgenes with a universal chromatin opening element derived from the human genome, and generating landing pad insertions compatible with the pIGLET system. We are also generating lines for Cre/Lox or FLP/FRT-based recombinase-mediated cassette exchange.

Using this suite of tools as well as CRISPR-based genome editing, we are generating bright, photostable lines for subcellular dynamic imaging, biosensors for cell state (e.g., cell cycle, cell signaling, etc), cell-type specific reporters, mosaic-labeling strategies, and transgenic tools to aid in 4D segmentation by cell shape. These lines, together with the accompanying training data, will be a valuable community resource as we build towards a foundation model for interrogating complex 4D data.

183

In Toto Lineage Analysis of Craniofacial Development in Zebrafish.

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Topic

2 Emerging Technologies

Abstract Content

Cranial neural crest-derived cells (CNCCs) generate diverse musculoskeletal tissues that support essential functions of the vertebrate head. While single-cell studies have captured distinct cell states, how specific cell fate decisions are reached within native tissues remain unclear. Traditional lineage tracing studies provide population rather than single cell-level information, and sequencing-based approaches using lineage barcoding lack spatial information due to cell dissociation. To comprehensively determine CNCC lineage structures in vivo, we have developed a new type of in situ “intMEMOIR” lineage barcoding system that simultaneously captures transcriptome data and lineage barcodes at single-cell resolution within native craniofacial tissues. This approach utilizes an inducible Bxb1 integrase to stochastically edit each of a 10-unit barcode array into three distinct states – unedited, reverse, deleted – that can be read out by highly multiplexed sequential fluorescence in situ hybridization (“seqFISH”). We then generated intMEMOIR transgenic zebrafish to determine in toto craniofacial lineage relationships during development. Based on previous single-cell RNA sequencing datasets, we designed a 206-gene seqFISH panel to uniquely detect 23 cell clusters and an additional 20 probes to detect the 3 states of each barcode bit, thus allowing us to simultaneously read out cell types and their clonal relationships in consecutive sections of the zebrafish head. After inducing barcoding at 10, 20, or 72 hpf and performing seqFISH at 1 month of age, lineage coupling and neighborhood analyses reveal progressive fate restriction of CNCCs over developmental time. We find cartilage to be one of the earliest segregating lineages. In addition, we uncover distinct perichondrium and periosteum subtypes biased toward either bone or tendon and ligament fates, as well as joint-restricted multipotent progenitors. In ongoing work, we are using intMEMOIR to assess how distinct types of craniofacial injuries, which lead to robust regeneration in zebrafish, induce lineage plasticity to restore the correct missing cell types.

419

Deep Learning-Driven 3D Tissue Prediction Frees Spectral Bandwidth and Automates Spatial Mapping in Zebrafish Embryo

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Topic

2 Emerging Technologies

Abstract Content

Background: Multiplexed fluorescence imaging is essential for zebrafish research, but spectral overlap limits standard microscopes to 3-5 channels. Dedicating channels to structural reference markers restricts the visualization of the expression of target genes. Extracting spatial context from a single ubiquitous channel (e.g., DAPI or phalloidin) is an unmet need to free up spectral bandwidth.

Methods: We developed a 3D U-Net pipeline that predicts multiple tissue architectures directly from one ubiquitous reporter in zebrafish embryos. Trained on multiplexed ground-truth datasets with tissue-specific markers hidden, the model learns to deduce complex tissue shapes solely from the ubiquitous signal. We benchmarked training strategies, including dataset size and compensatory conditions, to maximize accuracy across different imaging setups.

Results: Our tool automatically segments six embryonic tissues (enveloping layer, neural ectoderm, presomitic mesoderm, axial mesoderm, endoderm, dorsal forerunner cells) in 3D using only global phalloidin staining. This annotates tissues that are difficult to label manually (e.g., notochord) and those requiring specific markers (e.g., endoderm). The approach works effectively in wild-type embryos, as well as those with defective Nodal or planar cell polarity (PCP) signaling. By replacing structural reporters with deep learning predictions, we increase multiplexing capacity. For example, predicting the spinal cord and notochord from a nuclear stain frees all five commercial HCR channels to visualize five ventral neural progenitor domains (p3, pMN, p2, p1, p0) simultaneously. Additionally, this segmentation enables continuous, tissue-relative coordinate mapping. We demonstrate this by quantifying continuous positions of neural progenitors relative to the Shh-secreting notochord and floor plate as the Shh gradient shifts from medio-lateral to ventro-dorsal embryonic axes in time-lapse movies.

Conclusion: Extracting spatial information from a single channel streamlines high-throughput phenotyping and expands multiplexing limits. We deployed the entire pipeline on Google Colab, allowing researchers to easily train custom models on their own data without specialized computational infrastructure.

153

Developing single-cell retrospective transcriptomics to reveal the transcriptomic trajectories of individual neural stem and progenitor cells *in vivo*

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Topic

2 Emerging Technologies

Abstract Content

In the adult vertebrate brain, neural stem cells (NSCs) predominantly reside in quiescence (qNSCs). Quiescence is a dynamic and reversible state regulated by intrinsic cellular programs and interactions with neighboring cells, allowing qNSCs to maintain quiescence for extended periods. Recent findings indicate that qNSCs exhibit transcriptomic heterogeneity (Morizet et al, 2024; Llorens-Bobadilla et al, 2015), suggesting differences in the depth and / or regulation of quiescence that individual qNSCs may transit through during their quiescence phase. However, the transcriptomic trajectories of individual qNSCs over time remain unknown. Notably, because NSC trajectories are asynchronous and the system remains overall in equilibrium, temporal series cannot be used to infer single cell behavior. To reveal transcriptomic trajectories and their temporal regulation *in vivo* at the individual cell level, we are establishing an innovative method, ReMeMber-Seq. ReMeMber-Seq is a 'molecular time-recorder' that reconstructs past transcriptional states while jointly profiling present transcriptomic and methylome landscapes at single cell resolution. It is based on a transient pulse of Dcm methylation targeted to active genes (via a fusion with RNA PolII (Boers et al, 2023)) at a chosen time, followed by scNMT-seq upon a chase time. Using multimodal cell-omics approaches calling methylation marks at Dcm sites and RNAseq in single cells, we can then determine and link the past and present transcriptomic states of each cell. As a proof of concept, we use ReMeMber-seq to map neurogenesis progression in the zebrafish embryo. This method can a priori be broadly applied to study low-turnover stem and progenitor cells across all tissues.

452

Accelerating Zebrafish Research Using AI Agents: Open-Source Infrastructure and Tools Now Available for Any Lab

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Topic

2 Emerging Technologies

Abstract Content

Large language models (LLMs) offer transformative potential for accelerating zebrafish research, but their deployment is constrained by confident hallucination including mis or fabricated citations, and inaccurate experimental outcomes.

To address this at the Center for Rare Disease at UT Austin, we are using agentic AI platforms to enable any research lab to deploy custom AI scientists with domain-specific skills. Using this infrastructure, we have deployed the Validation as a System (VaaS) pipeline, a multi-layer agentic workflow achieving near net-zero hallucination in biomedical knowledge curation. VaaS was used to rapidly construct an initial 324-gene Rare Disease Database (RDD), with each entry requiring verified PubMed identifiers, correct clinical trial status, and accurate therapeutic data across disease categories central to zebrafish modeling. Prospective benchmarking across 757 controlled runs confirmed unguided LLMs produce 95.9% wrong-topic citation rates; VaaS reduced this to near zero at less than $1 per gene review. The RDD is publicly accessible via RareLens, a searchable open-source database viewer.

These AI agentic systems enable the rapid development of open-source tools for even the smallest research group. These include CloningBay (plasmid visualization and construct design for genome editing workflows), AI-assisted pipelines for RNAseq interpretation and molecular imaging analysis, and we are developing workflows for genetic mapping and spatial transcriptomics integration.

Critically, these tools are not laboratory-specific. Built on open-source AI agent infrastructure (i.e. OpenClaw, Hermes), they democratize access to AI-accelerated research — enabling any zebrafish lab, regardless of computational resources, to deploy custom AI scientists, automate routine analyses, and accelerate discovery. We envision a community model in which shared tools, shared databases, and AI agentic support lowers barriers to collaboration and amplify the collective productivity of the zebrafish research ecosystem.

EVOLUTION & ALTERNATIVE FISH MODELS II

345

The medaka mutant *hirame* reveals YAP mechano-homeostasis: A mechanical feedback regulating vertebrate 3D body formation

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background

Vertebrates have a unique 3D body shape with correct tissue and organ shape and alignment. Although a century ago D'Arcy Thompson postulated that terrestrial animal body shapes are conditioned by gravity (On growth and form, 1914), how mechano-morphogenetic processes coordinate to develop a body shape capable of withstanding gravitational forces remained elusive.

Methods

We identified a medaka fish mutant, *hirame* (*hir*), which is sensitive to deformation by gravity resulting in a flattened body caused by mutation of YAP (Porazinski et al., Nature 2015).

Results

Analysis of *hir* mutants showed that 3D organ/body is formed by 3D tissue formation and their alignment. These processes are mediated by actomyosin-mediated cell/tissue tension, optimised by the YAP target gene, ARHGAP18 via F-actin polymerization.

Our findings indicate that YAP functions not only as a mechano-transducer but also as a mechano-effector, orchestrating tissue shape and alignment to ensure correct 3D body architecture. Since YAP is required for the reciprocal mechanical control between cells and ECM, which regulates stem cell proliferation and differentiation for tissue homeostasis, we have proposed the concept of **YAP-mediated mechano-homeostasis** (Asaoka & Furutani-Seiki, *Curr Opin Cell Biol*, 2017). However, dysregulation of YAP dynamics can lead to cancer, impaired wound healing. These insights advance our understanding of mechano-chemical control of tissue homeostasis and have important implications for functional organoid engineering, cancer therapy, and regenerative medicine.

Conclusion

We proposed that Zebrafish and Medaka play complementary role for uncovering vertebrate genome functions (Furutani-Seiki & Wittbrodt 2005), based on the two large-scale mutagenesis screens in the two species, (Haffter *et al.,*Development 1995, Furutani-Seiki Mech Dev. *et al.,*

2004). We will discuss the two species complement in the case of YAP/TAZ analysis, and the perspectives of the use of the two species.

376

Strain-specific mitochondrial alterations during brain aging in the African turquoise killifish

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

The African turquoise killifish (*Nothobranchius furzeri*) has emerged as a valuable vertebrate model for studying aging due to its naturally short lifespan and pronounced age-related phenotypes. Laboratory strains display marked lifespan differences, providing an opportunity to investigate mitochondrial alterations associated with aging and longevity, as well as potential differences in mitochondrial epigenetic landscapes.

In this study, mitochondrial abundance and functional status were further assessed through measurements of oxidative stress markers, mitochondrial enzyme activity, mitochondrial DNA (mtDNA) copy number, and gene expression analyses. To assess mtDNA methylation across aging, we enriched mtDNA using exonuclease digestion and analyzed 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), and N6-methyladenine (6mA) levels using Oxford Nanopore sequencing. We focused our attention to whole brain of two *N. furzeri* strains with different lifespans: the short-lived GRZ strain and the longer-lived MZCS strain.

Nanopore sequence results revealed very low levels of mtDNA methylation for 6mA, 5hmC, and 5mC compared to nuclear DNA. Mitochondrial abundance remained relatively stable across ages and between strains. However, a comparison between GRZ and MZCS revealed distinct mitochondrial responses to aging. Several indicators suggested that GRZ develop oxidative stress earlier and to a greater extent than MZCS. Oxidative stress markers increased with age, including elevated malondialdehyde (MDA) levels. This increase was accompanied by a significant reduction in mtDNA copy number in GRZ, while remaining relatively stable in MZCS. The decline in mtDNA abundance in GRZ was associated with reduced expression of mitochondrial DNA replication machinery genes. In parallel, mitochondrial-encoded respiratory chain genes showed stronger downregulation in GRZ than in MZCS.

Together, these observations suggest that the short-lived GRZ strain experiences an earlier and more pronounced accumulation of mitochondrial stress, potentially affecting mtDNA maintenance and mitochondrial gene expression. Comparing these two strains provides a useful way to investigate how mitochondrial dysfunction may emerge and progress during vertebrate aging.

214

Extreme intermittent fasting: cellular mechanisms of starvation tolerance in cavefish

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Access to nutrition is fundamental for life, yet in many environments food availability is unpredictable or chronically limited, imposing strong selective pressure on metabolic and physiological systems. The Mexican cavefish *Astyanax mexicanus*, which evolved repeatedly in nutrient-poor cave ecosystems, provides a powerful evolutionary model to investigate how vertebrates adapt to extreme starvation.

Previous work suggested that cavefish display increased starvation resistance compared to their surface-dwelling relatives. Here we show that this capacity is far more extreme than previously appreciated. In controlled experiments, cavefish survived up to 20 months without food, losing less body weight than surface fish after only two months of starvation.

One hallmark of this tolerance is the preservation of tissue integrity during extended fasting. While surface fish rapidly develop intestinal disruption and compromised barrier function during starvation, cavefish maintain epithelial organization even after prolonged food deprivation. Single-cell RNA-seq maps of intestinal tissues from fed and starved fish revealed enhanced activity of absorptive epithelial cell types and coordinated regulation of immune populations that together stabilize intestinal homeostasis during nutritional stress.

These observations raise the question of whether starvation tolerance reflects systemic physiological adaptations or intrinsic cellular resilience. To address this, we established liver and gut derived cell lines from both cavefish and surface fish populations. When subjected to serum deprivation, cavefish liver cells survived up to fivefold longer than surface fish cells, revealing a strong cell-autonomous resistance to nutrient limitation. RNA-seq analysis under starvation conditions identified signaling pathways associated with metabolic adaptation and stress tolerance.

Together, these results suggest that extreme starvation resistance in cavefish arises from coordinated adaptations across tissues and cell types. The development of tractable in vitro systems now provides a platform to functionally dissect the genetic and molecular pathways that enable vertebrates to survive prolonged nutrient deprivation.

458

Goal-directed behavior in the shell-dwelling cichlid *Lamprologus ocellatus*.

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Goal-directed behavior relies on the flexible organization of action sequences to achieve specific outcomes. This process depends on knowledge of the causal structure of the environment (a world model), which allows organisms to evaluate outcome value and select appropriate actions (Pezzulo et al., Trends Cogn. Sci., 2014). We use the shell-dwelling cichlid *Lamprologus ocellatus* (LO), a species from Lake Tanganyika that breeds exclusively in nests constructed from abandoned snail shells, as a model to study these processes. Nest building in LO involves a sequence of stereotyped behavioral motifs, including placing the shell vertically into the substrate and covering it with sand (Grätsch et al., Curr. Biol., 2026). Because reproduction depends on access to a species-typical nest, nest-building behavior is highly robust and reproducible, making it a powerful model system for investigating the neuroethological underpinnings of goal-directed behavior in a lab setting.

In this study, we challenge this behavioral program by testing its flexibility in response to environmental manipulations, including changes in shell geometry, substrate properties, and nest state. To examine the fish's knowledge of the causal structure of the environment, we present the fish with challenges that prevent immediate nest construction: shells may be obstructed, placed on solid substrate, or positioned on a track, requiring the fish to remove the obstacle or maneuver the shell to sandy substrate before nest building can proceed. In our previous neuroanatomical studies, we identified glutamatergic and GABAergic neurons in the dorsolateral telencephalon, a hippocampus homologue, that are active during nest building. We now build on these findings by using third-generation in situ hybridization chain reaction and DiI-based neuronal tracing to investigate which brain regions contribute when fish solve experimentally imposed challenges.

Together, this approach links robust behavior with experimental manipulation to study the neural and cognitive mechanisms underlying goal-directed behavior.

265

Social context modulates cell proliferation in zebrafish and fighting fish: oxytocin and social system effects

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background: Social and solitary species are expected to respond differently to shifts in their social environment according to their species-specific needs. Social isolation, for instance, may constitute a stressor for highly social species but not for solitary ones. One consequence of social stress occurs at the level of brain cell proliferation, a process regulated by both extrinsic and intrinsic factors. In zebrafish, a highly social species, social isolation negatively impacts cell proliferation, an effect that can be rescued by exposure to a social stimulus (a mixed-sex shoal). One possible mediator of this response is oxytocin, a nonapeptide well known for its role in regulating social behaviour and, in mammals, for modulating cell proliferation in the hippocampus. This study investigated whether oxytocin mediates the effects of shifts in the social environment on cell proliferation within brain nuclei of the social decision-making network (SDMN), a conserved circuit that regulates social behaviour. This effect was investigated in two species with contrasting social systems, the highly social zebrafish and the territorial and solitary Siamese fighting fish.

Methods: The impacts of oxytocin on brain cell proliferation after shifts in the social environment were investigated in zebrafish by comparing a mutant line lacking the oxytocin receptor with wild-type fish. In the Siamese fighting fish a pharmacological approach was used, comparing fish that received intraperitoneal injections of an oxytocin receptor antagonist (L-368,899) with controls.

Results: In both species, distinct areas of the SDMN exhibited differential responses to the social environment and to oxytocin modulation. The direction and magnitude of these effects vary by brain region and differ between zebrafish and fighting fish, suggesting species-specific modulation of cell proliferation.

Conclusion: These findings highlight that cell proliferation in the brain and its modulation by oxytocin are strongly regulated by species-specific and context-dependent factors.

251

Developmental transcriptomics and chromosome-level assembly of the Arabian killifish as a novel biomedical model species

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background: We have developed the Arabian killifish (*Aphanius dispar*) as an alternative embryo model for studying human diseases such as fungal infections and cancer progression due to the thermotolerance of the species at human body temperature and fever temperature. Engineered mutant lines with defects in pigmentation produce transparent embryos suitable for live imaging and display an extended period of independent feeding (13 days post-fertilisation).

Methods: Using Illumina RNA-seq, we performed gene expression profiling of Arabian killifish embryos across 9 developmental time points; from the maternal stage to the larval pre-hatching stage. A contiguous reference genome was also generated with the combination of Nanopore long-read, Omni-C long-range, and Illumina short-reads.

Results: Embryonic development in *A. dispar* progresses more slowly than in Zebrafish at the cleavage to gastrula stage, with equivalent stages occurring several hours later. At the onset of zygotic genome activation, the transcriptome showed the conserved up-regulation of many development-related regulatory genes including Zinc finger-associated genes, consistent with Zebrafish and other teleost species. We also generated a 1.45 Gb high-quality reference assembly with an N50 score of 3.25 Mb and 24 chromosome-level scaffolds. Busco gene completeness analysis revealed gene completeness scores of 99% and 98.1% for the Actinopterygii and Cyprinodontiformes lineages, respectively. The mitochondrial genome is 81.55% similar to that of *Aphanius iberus* (the Spanish toothcarp), with minor structural modifications. *A. dispar* exhibits highly conserved synteny with *Fundulus heteroclitus* within the Cyprinodontoidei clade.

Conclusion: Together, these findings provide valuable transcriptomic and genomic resources for further genetic manipulation and gene regulation studies. The high-resolution genome data and associated transcriptome would also serve as a valuable resource for comparative genomics within the teleost community.

INFECTION & IMMUNITY

184

Differential subversion of host immune signalling by tuberculous and non-tuberculous mycobacteria

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Topic

15 Infection and Immunity

Abstract Content

Background: *Mycobacterium abscessus*, a rapidly growing nontuberculous mycobacterium (NTM), has surpassed *Mycobacterium tuberculosis* (Mtb) in many countries that have controlled Mtb transmission. Unlike Mtb, whose stereotypical tuberculous granuloma formation largely depends on the ESX-1 secretion system, *M. abscessus* lacks ESX-1 system, and the host factors driving *M. abscessus* granuloma formation are poorly understood.

Methods and results: Zebrafish has been widely used to study mycobacterial infections due to their cellular and genetic similarities to the human immune system and their ability to form human-like granulomas. In this study, granulomas were dissected from adult zebrafish infected with wild-type *Mycobacterium marinum*, ESX-1 mutant *M. marinum*, and *M. abscessus*. RNA sequencing analysis uncovered a distinctive transcriptional identity for *M. abscessus* granulomas compared to the more similar *M. marinum* granulomas. Analysis of immune gene expression identified interleukin-17 (IL-17) as a differential marker enriched in *M. abscessus* granulomas.

Depletion of *il17* in zebrafish embryos resulted in increased *M. abscessus* burden but not *M. marinum* burden, indicating *il17* is protective against *M. abscessus*. Given the importance of STAT3 in IL-17 production and susceptibility to NTM, we investigated the role of STAT3 during *M. abscessus* infection. Consistent with the *il17* results, genetic or chemical inhibition of STAT3 increased *M. abscessus* burden without significantly affecting *M. marinum*. IL17 mRNA overexpression rescued the increased *M. abscessus* burden in *stat3* mutant embryos. However, *stat3* knockdown upregulated *il17, il6, il10 and tnfa* expression during *M. abscessus* infection, suggesting STAT3 restrains excessive inflammation in response to Mabs infection, from which *M. abscessus* could benefit for its growth.

Conclusion: In summary, these findings characterized divergent granulomatous immune responses involved in controlling *M. abscessus* infection compared to

tuberculous mycobacteria. Moving forward, our study implicates STAT3 and IL-17 as potential nodes of susceptibility to *M. abscessus* infection in otherwise immunocompetent patients.

229

Hormonal NOD-Sense: Estrogen's Role in Gut Equilibrium

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Topic

15 Infection and Immunity

Abstract Content

Background

Loss-of-function mutations in the innate immune receptor NOD2 are the strongest genetic risk factor for Crohn's disease, yet we still do not know how NOD2 maintains intestinal homeostasis. Zebrafish have emerged as a powerful model for studying intestinal inflammation and NOD2-dependent host-microbe interactions. Understanding how NOD2 regulates epithelial barrier integrity, immune balance, and tissue renewal is critical for explaining why disruption of this pathway predisposes individuals to chronic inflammatory disease.

Methods

To investigate the role of NOD2 in intestinal homeostasis, I used a CRISPR-generated *nod2* mutant zebrafish model. I combined single-cell RNA sequencing, microbial analysis, and *in vivo* functional assays to examine intestinal epithelial and immune dynamics under both homeostatic and colitogenic conditions.

Results

Loss of NOD2 caused substantial shifts in intestinal epithelial composition and functional states, indicating an important role for NOD2 in maintaining epithelial balance prior to the onset of overt inflammatory disease. Single-cell transcriptomic analysis revealed widespread dysregulation of epithelial growth and differentiation programs in *nod2* mutants, along with enrichment of estrogen-responsive transcriptional signatures across epithelial and immune compartments. Functionally, estrogen exposure phenocopied key *nod2* mutant defects, while pharmacological inhibition of estrogen signaling restored epithelial renewal, rebalanced secretory cell populations, and improved survival following colitogenic challenge.

Conclusion

These findings reveal a previously unrecognized NOD2-estrogen signaling axis regulating intestinal homeostasis. My results demonstrate that hormonal signaling can act as a critical modifier of NOD2-dependent epithelial and immune responses and highlight an unexpected interaction between innate immune sensing and endocrine regulation of gut physiology.

350

Spatio-temporal characterization of a native zebrafish virus and its interactions with innate immunity

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Topic

15 Infection and Immunity

Abstract Content

Background

Zebrafish are ideal for whole-organism dynamic studies, enabling a systems level approach to characterizing viral infection and the immune response. Previous work in our group identified a picornavirus infecting the zebrafish gut, termed Zebrafish Picornavirus (ZfPV), which is a naturally occurring virus infecting both wild and lab-reared zebrafish populations globally. Because ZfPV persists in zebrafish populations over long time periods, this virus provides a unique opportunity to study the interactions of a co-evolved virus-host pair.

Methods

To characterize the spatio-temporal dynamics of the innate immune response to the virus, we use a fish line expressing an isg15:GFP promoter fusion. To characterize the virus itself we developed a fish line expressing a ZfPV-protease-specific transcriptional reporter. We imaged whole larvae over a period of 2 days, from 5-7 dpf, or 18-64 hours post virus exposure. To corroborate our spatial data, we also performed Hybridization Chain Reaction (HCR) based RNA FISH to identify cells expressing ZfPV RNA and several innate immune signaling and response genes at several timepoints post infection.

Results

Through our isg15 reporter imaging, we observed high levels of heterogeneity in viral infection and isg15 response across individuals. Generally, we observed the immune response starting in the foregut, spreading across the rest of the gut, and in some, but not all cases, spreading to gills, skin, and liver. By segmenting organs of interest, we were able to identify specific patterns of response in the gut, liver, and skin of the infected larvae. HCR confirmed these patterns, showing infected cells and interferon producing cells primarily localized to the foregut, while interferon responsive cells were spread throughout the entire gut.

Conclusions

We observed virus infection and innate immune response emerging in real time in whole fish, demonstrating heterogeneity but also broad patterns in the individual larval response to virus exposure.

442

Receptor-Specific Regulation of LC3-Associated Phagocytosis During *Staphylococcus aureus* Infection

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Topic

15 Infection and Immunity

Abstract Content

Background
Staphylococcus aureus is a major human pathogen increasingly recognised for its ability to survive in host cells by subverting phagocytic defences. Antimicrobial autophagy, including LC3-associated phagocytosis (LAP), is an important host response, but its role in *S. aureus* infection remains unclear. Our previous work using larval zebrafish showed that LAP is protective in *Streptococcus pneumoniae* and *Salmonella Typhimurium* infections, whereas in *S. aureus*–infected macrophages LAP appears less critical and is induced in fewer than 40% of infected cells.

Methods
Using the larval zebrafish model, we investigated the role of phagocytic receptors in LAP induction in phagocytes during *S. aureus* infection. We focused on mannose receptor 1 (Mrc1b/CD206) and integrin β2 (Itgb2/CD18) using ligand blocking (mannan) and CRISPR/Cas9-mediated gene targeting. LAP activity was assessed via Lc3 association and NOX2 dependence, alongside bacterial clearance and survival analysis. Human monocyte-derived macrophages are also currently used to assess conservation of these findings in humans.

Results
Blocking mannose receptor with mannan significantly increased Lc3 associations with *S. aureus* in macrophages and enhanced bacterial clearance in vivo. This response was NOX2-dependent, confirming LAP involvement. CRISPR/Cas9 targeting of zebrafish *mrc1b* similarly increased LAPosome formation, supporting an inhibitory role for mannose receptor. In human macrophages, mannan pretreatment increased ROS production following infection suggesting that similar receptor-mediated modulation of LAP may also occur in humans. In contrast, knockdown of *itgb2* reduced Lc3 associations with internalised bacteria and increased susceptibility to infection, identifying it as a positive regulator of LAP.

Conclusion
Ongoing work explores *S. aureus* cell wall components as modulators of receptor engagement in LAP. Together, our findings reveal receptor-specific regulation of LAP and suggest mechanisms by which *S. aureus* evades autophagic control, highlighting potential targets for host-directed therapies against multidrug-resistant infections.

19

Telomerase reverse transcriptase is required for resistance to mycobacterial infection

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Topic

15 Infection and Immunity

Abstract Content

Background

Age is an important risk factor for infections such as tuberculosis (TB). Telomerase is expressed in immune cells yet leukocyte telomere length declines during ageing suggesting an age-dependent loss of telomerase activity in the immune system. Leukocyte telomere length has been correlated with worse outcomes in TB patients, however the mechanisms linking telomere biology to TB susceptibility and response to therapy are unexplored.

Methods

Here we use the zebrafish-*Mycobacterium marinum* model to investigate the role of telomerase in TB resistance.

Results

We find depletion and inhibition of Tert, the catalytic subunit of telomerase, increases bacterial burden in zebrafish embryos. The Tert depletion infection susceptibility phenotype could not be rescued by p53 or STING depletion suggesting a DNA damage-independent role for Tert in controlling mycobacterial infection. Consistent with a previously described role for Tert in developmental hematopoiesis, we find Tert is necessary for demand-driven emergency myelopoiesis to support containment of extended mycobacterial infection.

Conclusion

Our findings establish a previously undescribed role for host telomerase in supporting infection demand-driven hematopoiesis to control infection.

457

ID2-TCF3-RARγ axis in orchestrating eosinophil maturation

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Topic

15 Infection and Immunity

Abstract Content

Eosinophils are a group of granulocytes well known for their ability to protect the host from parasites and regulate immune function. While diverse biological roles of eosinophils have been increasingly identified, the developmental pattern and regulation of the eosinophil lineage remain largely unknown. In our previous study, we delineated the spatiotemporal developmental pattern of eosinophils and characterized their lineage features using single-cell RNA-Seq in zebrafish. By further elucidating the transcriptional regulation of eosinophilopoiesis, we uncovered the critical role of the *Cebp1-Cebpβ* transcriptional axis in eosinophil lineage commitment and differentiation. Our data revealed that the transcription factor *ID2* is highly expressed in the eosinophil population, yet its *in vivo* function in eosinophil development remained unclear. Here, we demonstrate that *Id2* plays a conserved role in eosinophil maturation in both zebrafish and mouse models. Moreover, we show that ID2 binds to the promoter of the retinoic acid receptor gene, thereby modulating retinoic acid signaling to suppress eosinophil apoptosis. Collectively, our study indicates that ID2 regulates eosinophil lineage maturation through the retinoic acid pathway, providing novel insights into the transcriptional mechanisms governing eosinophilopoiesis.

STEM CELLS AND REGENERATION

273

Adaptive immunity and inflammatory programs governing cardiac regeneration in adult zebrafish

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Topic

14 Stem Cells and Regeneration

Abstract Content

Successful cardiac regeneration in adult zebrafish relies on a finely tuned immune response, including a self-limiting inflammatory wave that progressively alters with age. However, the adaptive immune programs involved in the regenerative process, and their crosstalk with inflammation, remain poorly understood. Our work shows the involvement of T cells and the requirement of MHC class II-dependent antigen presentation for cardiac regeneration, including cardiomyocyte dedifferentiation and proliferation. Notably, T cell-depleted zebrafish exhibit impaired cardiac regeneration and exacerbated inflammation, highlighting the requirement for global T cell programs for effective regenerative immune responses. Adoptive cell transfer experiments reveal that regulatory T cells partially restore regenerative programs in these animals, but not fully. These data suggest contributions from additional T cell subsets and point to an unrecognized T cell heterogeneity underlying regenerative outcomes, which may further shift with aging and inflammaging. To resolve this heterogeneity, and dissect distinct T cell responses to cardiac injury, we are mapping T cell diversity and clonotype dynamics through advanced sequencing approaches.

T cell responses and the inflammatory environment are highly intertwined. We identified granulin family proteins as potent regulators capable of tuning the inflammatory milieu, providing a means to experimentally manipulate the context in which T cell programs operate. In addition, we established a high-resolution *ex vivo* imaging platform to assess inflammatory cell behaviour in the adult zebrafish heart, providing a relevant functional readout for testing immunomodulatory strategies.

Altogether, our work aims to uncover the inflammatory-adaptive immune circuits that determine regenerative capacity, toward identifying strategies to sustain regeneration and limit tissue damage across life stages.

382

Cardiac-immune microniches programme macrophage states in the regenerating heart

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Topic

14 Stem Cells and Regeneration

Abstract Content

Adult zebrafish regenerate their hearts after injury, a process that requires macrophages, yet how local tissue microenvironments instruct macrophage states and function remains unclear. Here we combine single cell RNA sequencing with Visium and high-resolution MERFISH spatial transcriptomics to map the cardio-immune landscape of homeostatic and regenerating zebrafish hearts. We identify a mpeg1.1^{+} compartment comprising macrophages, dendritic, B and NK-like cells, and show that injury establishes a macrophage-centred immune environment with transcriptional programmes spanning resident surveillance, damage sensing, inflammation, antigen presentation, resolution and metabolic support. Communication-aware spatial modelling reveals that these states are not randomly distributed but organised into discrete structural-immune microniches across the injury region, each defined by stereotyped cellular compositions and ligand-receptor circuits. Within a fibroblast-macrophage microniche, we uncover an il34-csf1ra-egr1 axis in which col12a1a^{+} il34^{+} fibroblasts promote an egr1 pro-regenerative macrophage state that couples fibrosis, vascular integrity and epicardial signalling. We show that disruption of this axis by csf1ra loss of function reduces macrophage-, endothelial- and epicardial-rich microniches, amplifying fibroblast-driven domains that shift macrophages towards stress and long-sustained inflammatory programmes, thereby biasing early injury response towards a pro-fibrotic state. Our work establishes spatially defined cardio-immune microniches as key organisers of macrophage function and regenerative outcome, providing a mechanistic framework and actionable targets for reprogramming cardiac repair.

454

Definitive hematopoiesis and endocardial lineages establish distinct local immune reservoirs for cardiac regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

The developmental origins of tissue-resident immune cells heavily dictate their functional trajectories during tissue repair. While the endocardium is known to generate hematopoietic progenitors during embryonic development, its long-term contribution to adult cardiac immune populations and how this compares to definitive hematopoiesis during regeneration remains highly unresolved. Here, we identify a reservoir of AGM-derived hematopoietic stem and progenitor cell (HSPC)-like cells residing in the adult zebrafish heart alongside endocardium-derived, lineage-restricted populations.

To definitively map lineage contributions during homeostasis and regeneration, we utilized long-term spatiotemporal lineage tracing, combining tamoxifen-induced labeling of the definitive hematopoietic lineage with heart-restricted cyclofen uncaging to track endocardial-derived cells. Following cardiac injury, single-cell RNA sequencing was integrated with whole kidney marrow (WKM) datasets to map lineage trajectories across compartments. Under homeostatic conditions, the vast majority of cardiac immune cells derive from definitive hematopoiesis. Conversely, following injury, the endocardial lineage rapidly expands its contribution, though this is tightly restricted to resident macrophage populations with negligible input to erythroid or lymphoid lineages.

Crucially, functional in vivo transplantation assays demonstrated that the adult heart–resident HSPC-like cells retain long-term, multilineage repopulation potential. *In silico* integration confirmed these cells are transcriptionally analogous to resident HSPCs in the WKM. Furthermore, we identified cmyb:EGFP+ cells localized within adipocyte-associated regions of the adult myocardium, pointing to a specialized local microenvironment required for stem cell maintenance.

Together, these findings fundamentally revise our understanding of cardiac immune ontogeny. We establish that adult cardiac regeneration is driven by two distinct local immune reservoirs: a previously unrecognized, functional HSPC niche that maintains multilineage potential, and the injury-induced reactivation of developmental endocardial programs to supply specialized macrophages.

137

Leveraging cellular niches to identify factors stimulating pancreatic beta-cell maturation/regeneration using zebrafish knock-in lines

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background: Zebrafish have significant plasticity and regenerative capacity of insulin-producing β-cells, and to fully explore and leverage these features precise gene-editing approaches are needed.

Methods: Here, we use a modified knock-in strategy to introduce dsDNA-donors using CRISPR-Cas9, PCR amplification to yield dsDNA with 5' end protection as the knock-in template, and gRNA targeting sites upstream of the STOP codon in the last exon, circumventing the need of a gRNA targets spanning over the STOP codon region of the targeted locus. Overexpression of a panel of secreted peptides/proteins in the new knock-in lines constitutes a new screening platform. We ablate beta-cells with the nitroreductase/metronidazole system as a diabetic zebrafish model, and subsequently translate findings using human stem cell differentiation cultures.

Results: We successfully used this modified knock-in strategy to generate zebrafish lines, with dual cell labelling and lineage tracing functions, targeting the *hand2* and *nkx6.2* loci. Using these lines, we found that *hand2*$^{+}$ fibroblasts are heterogeneous in liver and pancreas and can form an important niche factor lining the luminal ducts. In the pancreatic islet, we found that *nkx6.2* labels the functional proportion of β-cells, and that the knock-in line can be used for instant visualization of β-cell maturity. We used this property to screen for secreted peptides/proteins that can accelerate maturation of beta-cells and identified a peptide that stimulated both the *nkx6.2*-reporter and beta-cell maturation. This effect appears conserved in differentiation of human stem cell cultures, which generated more functional beta-cells, a feature that may be useful to improve cell therapies for diabetes.

Conclusion: Our modified 3' end knock-in method is widely applicable to various tissues and models, and the generated zebrafish lines are powerful tools for delineating the pancreatic ductal cell niche, plasticity, and β-cell heterogeneity; findings with importance for pancreatic development and regeneration in diabetes.

176

Ovarian modules in zebrafish and humans: A new morphogenetic framework for oocyte production from germline stem cells

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Topic

14 Stem Cells and Regeneration

Abstract Content

Oocyte production from early progenitors in the fetal ovary determines the ovarian reserve and impacts lifelong female reproduction, yet its developmental regulation remains elusive. Early progenitors or germline stem cells (GSCs) are not maintained in adult mammalian ovaries, resulting in the finite ovarian reserve and hindering investigation. Zebrafish maintain ovarian GSCs throughout life, providing a unique opportunity for their study. However, fundamental knowledge of vertebrate ovarian GSCs and how they are regulated to produce oocytes in the developmental context of the ovary is lacking.

Here, we uncover a previously unrecognized ovarian developmental microenvironment, the ovarian module, in developing zebrafish and human ovaries. We show zebrafish ovarian modules comprise the morphogenetic unit of oocyte production, harboring GSCs at the young tip, which generate oocyte precursors and early differentiating oocytes in a spatially linear manner. We define module morphology, resolve its developmental dynamics, recapitulate its single-cell transcriptomic architecture, and identify Notch signaling as a regulator of GSCs. We map Notch pathway components in modules, identifying GSCs as sender cells and their progeny as receiver cells. Functional perturbations in ovaries *ex-vivo* demonstrate Notch activity is required to suppress GSC fate and promote differentiation. We further show that ovarian modules persist in adult zebrafish ovaries, highlighting a similar framework for oocyte production from GSCs during homeostasis and in supporting regenerative capacity. Finally, we demonstrate that modules are conserved in human fetal ovaries, where POU5F1/OCT4+ germ cells, differentiating oocytes, and Notch components are similarly organized. These findings support the module as a conserved strategy for oocyte production, and open new avenues for uncovering the missing factor(s) required for GSC maintenance in women.

Our work proposes new organizing principles for oogenesis in development and regeneration and establishes new paradigms in female GSC biology and towards women's reproductive regenerative medicine.

*Please see preprint: https://doi.org/10.21203/rs.3.rs-7325126/v1.

GERMLINE AND EARLY DEVELOPMENT II

313

The Balbiani Body forms through microtubule-controlled Buc condensation in early oogenesis

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Topic

9 Germline and Early Development

Abstract Content

Widely in animals and plants gamete polarization lays foundations for embryonic development. Vertebrate oocyte polarity is crucial for embryonic axes formation and germline specification. In oocyte polarization, essential embryonic-regulatory RNA-protein granules are delivered to the oocyte vegetal pole by a membraneless organelle called the Balbiani body (Bb). The Bb is essential for oogenesis, embryonic development and reproduction, and is conserved from insects to humans. However, despite discovery in 1845, mechanisms of Bb formation remain elusive.

Here, we discovered mechanisms of Bb formation in zebrafish by developmental molecular condensation. We first provide super-resolution microscopy, live-imaging, biochemical, and genetic evidence in developing ovaries, to conclude that the Bb forms by molecular condensation driven by phase-separation of Buc, an essential intrinsically disordered protein with a prion-like domain. Live-imaging, molecular, and FRAP experiments *in-vivo*, demonstrate that the forming Bb develops from liquid-like viscoelastic condensates with dynamic Buc mobility to a sloid-like stable compartment. Thus, Buc-dependent developmental viscoelastic changes shape Bb formation. Further, we deciphered multi-step regulation by microtubules in controlling Bb condensation: first, by directed dynein-mediated trafficking of early condensing Buc granules, then by scaffolding condensed granules likely via molecular crowding, and finally by caging the mature Bb to prevent over-growth and maintain shape. These regulatory steps ensure the formation of a single intact Bb condensate, which is essential for oocyte polarization. We further identified a repertory of novel Buc-interacting regulators, including kinases, a microtubule–RNA binding protein, and a disordered coiled-coil protein that control hierarchical steps in Buc condensation and Bb formation. Floxed and epitope tagged alleles are being generated for these regulators by precise genome-editing to elucidate the integrative mechanisms underlying oocyte polarity.

Our work resolves a long-standing question in vertebrate oocyte polarity, advances mechanistic understanding of Bb formation, and sheds new light on physiological condensate biology and female reproduction.

109

Release of Balbiani body components during zebrafish oogenesis

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Topic

9 Germline and Early Development

Abstract Content

Successful embryogenesis depends on the oocyte's ability to accumulate and store maternal RNAs, proteins, and organelles. During oogenesis, a major site where maternal determinants are accumulated within the oocyte is the Balbiani body (Bb), a conserved, solid, membraneless condensate. After fertilization, the Bb is no longer detectable and its constituents are redistributed in the egg. However, when and how Bb releases its contents to support embryonic development remains unclear.

Here we show that Bb disassembles during oogenesis in two separable steps: early in oogenesis the Bb fragments and spreads along the vegetal cortex, and later during oocyte maturation the Bb fragments dissolve and release their constituents into the ooplasm. We find that Bb fragment dissolution is independent of prior fragmentation at the cortex but requires cell-cycle progression and CDK1 activity. Phosphoproteomic analysis reveals that multiple Bb protein components, including the Bb organizer Bucky ball (Buc), undergo phosphorylation changes during oocyte maturation in a CDK1 activity-dependent manner. Together, these findings implicate cell-cycle–regulated phosphorylation of Bb components as a candidate mechanism for Bb fragment dissolution, which will be further investigated in future.

Understanding the mechanism that underlies Balbiani body fragment dissolution is essential for understanding how oocytes safeguard but at the same time precisely regulate the release of maternal resources to support early embryonic development.

416

Robust cytoplasmic partitioning in early embryogenesis

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Topic

9 Germline and Early Development

Abstract Content

Early development relies on robustly reorganizing the cytoplasm of fertilized eggs into individualized cells. This process is orchestrated by microtubule structures that traverse the embryo, partitioning the cytoplasm into physically distinct compartments. Despite the robustness of embryonic development, my work revealed an intrinsic instability in cytoplasmic partitioning driven by the microtubule cytoskeleton. In the talk, I will show that embryos circumvent this instability through two distinct mechanisms: either by matching the cell cycle duration to the time needed for the instability to unfold or by limiting microtubule nucleation. These regulatory mechanisms give rise to two strategies to fill the cytoplasm, which I experimentally demonstrate in zebrafish and Drosophila embryos. These results indicate that the temporal control of microtubule dynamics could have driven the evolutionary emergence of species-specific mechanisms for cytoplasmic organization.

154

Maternal Huluwa regulates postfertilization microtubule array organization for asymmetrical transport of dorsal determinants in zebrafish

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Topic

9 Germline and Early Development

Abstract Content

The dorsal organizer, essential for vertebrate embryonic axis formation, is induced by microtubule-mediated transport of maternal determinants. Maternal Huluwa (Hwa) has been identified as an essential organizer inducer in zebrafish and frogs, functioning at midblastula stages to activate β-catenin signaling in the preorganizer. It remains unknown if maternal Hwa functions at or before fertilization. Here, we report that maternal Hwa protein is critical for organizing the vegetal parallel microtubule array immediately after fertilization in zebrafish. Hwa protein and mRNA are enriched at the vegetal pole and facilitate microtubule network formation, enabling asymmetrical transport of dorsal determinants. Loss of maternal Hwa disrupts this microtubule architecture and abrogates mRNA transport, revealing a self-reinforcing mechanism where Hwa regulates its own asymmetrical distribution. Our findings establish a dual-phase model of dorsal specification: Hwa initially governs symmetry breaking through postfertilization microtubule organization and later on activates β-catenin signaling at blastula stages. This work provides fundamental insights into how the key maternal factor regulates the organizer and body axis formation at different developmental stages.

334

Control over Dead end 1 and Nanos 3 translation and transcription: its role in germ-cell fate maintenance.

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Topic

9 Germline and Early Development

Abstract Content

Germ granules are phase-separated RNA- and protein-containing condensates and are a common feature of germ cells in various organisms. Indeed, the granules contain transcripts such as Dead end 1 (Dnd1) and Nanos 3 that encode proteins whose functions are critical for germ line specification and fate maintenance. In zebrafish, we found that Dnd1 and Nanos3 form a complex that plays a novel role in activating translation by linking germ-line transcripts to the translation initiation machinery in the periphery of the granule. The activity of the complex, as well as the translational status of germ cells, ensures germ granule patterning with translationally suppressed transcripts positioned in the core of a granule that is devoid of functional ribosomes. At the same time, translationally active mRNAs are located in the periphery of the organelle where they can interact with the ribosomes. Knockdown of Dnd1 or Nanos3, or global translational inhibition, results in localization of germ line mRNAs to the granule core and culminates in loss of germ cell fate. Our findings position Dnd1-Nanos3 complex as the central regulator of germ cell fate and emphasize the importance of germ granule patterning and translational control for proper germ line development.

99

Daily rhythmicity and nocturnal spermatogenesis in the zebrafish testis

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Topic

9 Germline and Early Development

Abstract Content

Background: The vertebrate testis has long been regarded as lacking strong cellular daily rhythmicity. **Methods**: We employ time-series single-cell RNA sequencing to construct a temporal atlas of the adult zebrafish testis. **Results**: We identify new cell-type-specific markers and novel testicular subtypes, and reveal that Sertoli cells,

spermatogonia, and spermatids exhibit robust oscillations in key circadian clock genes. In contrast, Leydig cells and spermatocytes primarily exhibit rhythmic expression of key functional genes. We show that intercellular communication networks also exhibit daily rhythmicity. Pseudotime trajectory analysis demonstrates a striking nocturnal preference for germ cell progression. Disrupting circadian input via constant light reduced germ cell numbers and impaired fertility. Genetic ablation of *clock1a* arrests spermatogonial differentiation, whereas mutation of the rhythmic spermatocyte gene *hmgb1a* significantly reduces the number of meiotic cells. **Conclusion**: our findings redefine the testis as a highly rhythmic organ in which a precisely timed, nocturnally coordinated cellular symphony drives spermatogenesis.

CARDIOVASCULAR II

335

Cardiovascular calcification in α-klotho mutants is triggered by phosphate-mediated endothelial apoptosis

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Topic

4 Cardiovascular

Abstract Content

Background

Cardiovascular calcification (CVC) is a prevalent disease highly associated with hyperphosphatemia, leading to mineral deposition and progressive dysfunction of arteries and cardiac valves. *α-Klotho* is a key regulator of phosphate homeostasis, and its deficiency in mice and zebrafish results in vascular calcification resembling that observed in chronic kidney disease. However, CVC is multifactorial, and the initiating triggers of calcification, particularly in cardiac valves, remain poorly understood. Using *α-klotho* mutants, we investigated how hyperphosphatemia disrupts endothelial integrity to initiate vascular and valvular calcification.

Methods

We generated a zebrafish *α-klotho* mutant as a model to study the mechanisms driving CVC. To characterize early cellular and molecular events underlying CVC initiation, we performed single-cell RNA sequencing together with detailed tissue characterization. Cardiac function was assessed by echocardiography. To determine the specific role of hyperphosphatemia in CVC, we used adult zebrafish heart explants.

Results

Elevated serum phosphate levels were observed in *α-klotho* mutants prior to CVC, followed by progressive calcification of the outflow tract and cardiac valve. Echocardiography in *α-klotho* hearts revealed retrograde blood flow, indicating impaired valvular function. Single-cell RNA sequencing of hearts at early stages of calcification showed a reduction in endothelial cells (EC) numbers, accompanied by an expansion of immune-cell populations. Tissue analyses confirmed EC apoptosis preceding calcification and barrier disruption, which

coincided with increased immune cell infiltration. Vascular and valvular calcification initiated on the areas adjacent to the endothelium with overall extracellular matrix remodeling, including increased collagen and reduced hyaluronic acid in affected regions. Exposure of wildtype zebrafish hearts to hyperphosphatemic conditions reproduced the CVC phenotypes observed in *α-klotho,* which was further accelerated by selective EC ablation.

Conclusion

This work establishes a zebrafish model of phosphate-driven CVC and demonstrates phosphate-driven EC apoptosis as the triggering factor of matrix calcification.

356

Wif1-mediated fine-tuning of Wnt signalling drives vascular refinement in the Zebrafish myelencephalic choroid plexus

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Topic

4 Cardiovascular

Abstract Content

Background

The vascular network of the central nervous system (CNS) is specialised to meet the needs of distinct neural regions. Most cerebral vessels form the restrictive blood-brain barrier, whereas choroid plexus (ChP) vessels develop into a specialised fenestrated network within the blood cerebrospinal fluid barrier, which is essential for cerebrospinal fluid homeostasis. While early CNS angiogenesis is well characterised, the coordinated mechanisms that refine primitive vascular plexuses into mature ChP vasculature remain poorly understood. Wnt/β-catenin signalling is a key regulator of CNS vascularisation and is active during ChP development, but its role in shaping ChP vascular architecture remains unclear.

Methods

We used zebrafish vascular and neural reporter lines for high-resolution *in vivo* confocal imaging to map assembly of the myelencephalic ChP (mChP) vasculature. We analysed *wif1*$^{-/-}$ mutants and used pharmacological modulation of Wnt signalling to define requirements for vascular refinement. Immunostaining, qPCR, and *in situ* hybridisation were used to assess proliferative, inflammatory and neural markers in the developing mChP niche.

Results

Loss of *wif1* caused a specific defect in mChP vascular maturation. Normally, the primitive plexus resolves from a multi-luminal, “mulberry-like” network into a refined vascular bed with a single lumen. In *wif1* mutants, this transition was

arrested, producing persistent disorganised vascular bundles resembling cavernous malformations, consistent with impaired pruning and anastomosis. Expression and pharmacological analyses suggest that local compensatory Wnt inhibitors restrict the phenotype to the mChP and that tightly controlled Wnt activity is required for these morphogenetic events.

Conclusions

Precise tuning of Wnt/β-catenin signalling is essential for vascular refinement in the mChP. We propose that Wnt overactivation in *wif1* mutants disrupts pruning and anastomosis, preventing normal resolution of primordial vascular clusters and revealing a mechanism by which signalling homeostasis shapes specialised neurovascular development.

287

Sphingosine-1 phosphate signaling regulates blood-brain barrier tightness through IQGAP1 phosphorylation

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Topic

4 Cardiovascular

Abstract Content

The capillary network of the central nervous system (CNS) forms the blood-brain barrier (BBB) which protects the brain from blood-borne pathogens and toxins while maintaining a stable environment for optimal neuronal functioning. Sphingosine-1 phosphate (S1p) signaling is known to contribute to the maintenance of the BBB in mature CNS vasculature. Nevertheless, S1p can act through several receptors that can produce opposing effects on cellular adhesion and the downstream effectors controlling vascular tightness remain incompletely understood.

Using a human endothelial *in vitro* BBB model, we investigated the effects of S1p signaling on barrier properties by measuring trans-endothelial electrical resistance. S1p stimulation induced a rapid, transient weakening of the cellular barrier mediated by S1PR2 activation, followed by a more sustained enhancement of cell adhesion downstream of S1PR1 signaling. A phospho-proteomic screen identified the scaffolding protein IQGAP1 as significantly phosphorylated within five minutes of S1p stimulation. IQGAP1 can regulate cortical actin assembly through interaction with activated RAC1/CDC42. However, phosphorylation of IQGAP1 seems to prevent binding of RAC1/CDC42 and instead to mediate binding to β-catenin at adherens junctions, thereby displacing α-catenin. Therefore, phosphorylation of IQGAP1 will lead to reduced cytoskeletal anchorage and consequently low adhesive strength. Indeed, we found that phosphorylation of IQGAP1 downstream of short-term S1p stimulation reduced interaction with RAC1 which coincided with decreased barrier

strength. We could confirm our data *in vivo*, as knock-down of the ortholog Iqgap2 impaired hindbrain angiogenesis phenocopying Rac1 overactivation.

We identified IQGAPs as novel modulators of S1p signaling and therefore propose that IQGAPs dynamically regulate cellular adhesion and act as main switch downstream of S1p stimulation by mediating β-catenin versus RAC1 binding downstream of S1PR2 versus S1PR1.

160

Regulation of growth factor responsiveness in quiescent endothelium

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Topic

4 Cardiovascular

Abstract Content

The lymphatic vasculature is crucial for the homeostasis of tissue fluids, the transportation of immune cells and the absorption of dietary lipids in the intestines.

Lymphatic development is dependent on two distinct pathways. During early development, VEGFC/VEGFR3 is the main driver of specification, sprouting and migration of lymphatic endothelial cells (LECs). In parallel, the ANG/TIE pathway controls lymphatic development, maturation and regulates VEGFR3 expression on LECs.

During development, LECs are highly susceptible to VEGFC levels, while in the mature vasculature the cells achieve a state of quiescence, where further growth of lymphatic vessels is dampened in most tissues, although VEGFC is still expressed. The regulation of this process is still poorly understood.

Here, we identify Itga9 as a key regulator for mature LEC quiescence. Itga9 is a receptor that shows binding sites for lymphatic factors VEGFC, VEGFD as well as for SVEP1, a co-ligand of the ANG2/TIE axis, and antagonizes growth factor signaling.

The loss of *itga9* in zebrafish RNA-null mutants showed lymphatic hyperplasia in scales and lymphangiectasia in the facial lymphatics of adult fish, while lymphatic development in embryos remained unaffected. This age-specific phenotype suggests a role of *itga9* in the regulation of lymphatic quiescent of mature vessels. Further, the hyperproliferation phenotype of *itga9* adult mutants could be suppressed in heterozygous *vegfc* or *ang1* mutants, thus underlining the role of Itga9 on Vegfc responsiveness.

These results, for the first time identify ITGA9 as a regulator of VEGFC/SVEP1/ANG2 dependent signaling in quiescent lymphatic endothelium.

333

Defining the molecular mechanisms downstream of Vegfc-Vegfr3 signaling regulating endothelial cell sprouting

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Topic

4 Cardiovascular

Abstract Content

Background
Endothelial cell (EC) sprouting and migration are essential processes for establishing functional vascular networks in both the blood and lymphatic systems. In the zebrafish trunk, secondary sprouts emerge from the posterior cardinal vein (PCV) in response to vascular endothelial growth factor C (Vegfc)–vascular endothelial growth factor receptor 3 (Vegfr3) signalling. These sprouts generate two endothelial populations: lymphatic vessels and veins. Previous studies have shown that lymphatic progenitor cells initiate expression of *prox1a* prior to leaving the PCV. However, some progenitors appear to downregulate *prox1a* during sprouting and instead contribute to venous intersegmental vessels, suggesting cellular heterogeneity within secondary sprouts. This study aims to characterise the molecular and cellular diversity present during secondary sprout formation.

Methods
To investigate sprout dynamics, we performed live imaging of developing zebrafish embryos to track endothelial cell behaviour during secondary sprouting. Single-cell RNA sequencing was used to define the molecular signature of cells within the secondary sprouts. Because the transcriptomic analysis revealed enrichment of mechanosensitive Ca^{2+} channels, we conducted live Ca^{2+} imaging to assess calcium activity during sprout migration. Additionally, ERK signalling was pharmacologically inhibited prior to sprouting to determine whether Ca^{2+} dynamics depend on Vegfc–Vegfr3 downstream signalling.

Results
Live imaging revealed that lymphatic sprouts migrate using two distinct modes of movement. Transcriptomic profiling identified a molecular signature in secondary sprout cells enriched for mechanosensing Ca^{2+} channels. Consistent with this finding, live Ca^{2+} imaging showed active Ca^{2+} firing during sprout migration, which gradually decreased as migration progressed. Inhibition of ERK signalling before sprouting significantly reduced Ca^{2+} activity, suggesting that Ca^{2+} dynamics may act downstream of Vegfc–Vegfr3 signalling.

Conclusion
Our findings reveal a level of heterogeneity in the secondary sprouts that might contribute to inducing differential EC behaviour.

POSTER

7

Creating and characterizing zebrafish mutants impaired in the turnover of genomic uracil

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Uracil (U) in DNA arises either from erroneous DNA polymerase activity, when dUTP is incorporated instead of dTTP, or from spontaneous cytosine deamination. As the latter results in mutagenic U:G mismatches in the double helix, cells have evolved error repair mechanisms (such as base excision repair – BER) to ensure that such mismatches are repaired promptly, and the freely available dUTP pool is kept at relatively low steady-state levels, to prevent uracil incorporation into the DNA. Recent data suggests that impairment of the enzymes that guard the cells from permanent uracil accumulation, such as the uracil–DNA glycosylases Ung and Smug1 (involved in BER), or that of the dUTPase (Dut), can have profound morphological consequences on the development and survival of animals. Using zebrafish (Danio rerio) as a model we explore the dynamics of uracil incorporation into the DNA during development, and show that prior to the activation of the zygotic genome (MZT) uracil levels are significantly increased and only later reduced. This suggests the presence active regulatory mechanism that is only active post-MZT. We apply a combination of loss- and gain-of function techniques to explore the roles of Unga, Smug1 and Dut in early developmental and genomic maintenance.

8

The microbiome modulates compound-specific toxicity of environmental chemicals

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Topic

10 Toxicology

Abstract Content

Background

Humans are continuously exposed to harmful environmental chemicals, with the gastrointestinal tract being a major route of exposure. The gut microbiome is a critical component of the digestive system and a known target of many environmental chemicals. While chemical-induced disruptions to microbiome composition and diversity are increasingly recognized, there is limited insight into how those changes lead to functional consequences. In our study, we aimed to investigate how the gut microbiome modulates host susceptibility to toxicity and how microbiome depletion influences chemical toxicokinetics.

Methods

We investigated the role of chemical exposure on the microbiome in zebrafish embryos using an integrated multi-omics approach. A broad panel of environmental chemicals—including bisphenols, perfluorinated substances, halogenated pesticides, polychlorinated biphenyls, and phthalates—was screened for toxicity and microbiome alterations using metagenomics. Based on these results, bisphenol F (BPF), perfluorooctanesulfonic acid (PFOS), and boscalid (BOS) were selected for in-depth comparison of transcriptomic and metabolomic responses in conventionally raised wild-type (WT) and germ-free (GF) zebrafish embryos.

Results

All tested chemicals altered microbiome composition and abundance to various degrees, with PFOS inducing the strongest effects. PFOS exposure resulted in the highest number of differentially expressed genes, with pronounced disruption of immune and stress-related pathways in GF embryos. In contrast, BOS and BPF elicited more compound specific metabolic responses that were modulated by the microbiota. Gene network analysis revealed microbial regulation of immune and metabolic programs, while metabolomic profiling identified PFOS-specific perturbations in L-tryptophan metabolism and associated microbial metabolites, exclusively in WT embryos.

Conclusion

Our study demonstrates that the gut microbiome plays a critical role in shaping host molecular responses to environmental chemicals and can markedly influence toxicity outcomes. Our results highlight PFOS as a compound with strong microbiome-dependent effects and underscore the importance of incorporating microbiome functionality into chemical risk assessment frameworks.

9

Zebrafish models of NR6A1 and ADGB variants in rare renal disorders using CRISPR editing techniques

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Topic

1 Disease Models

Abstract Content

Rare inherited kidney diseases frequently remain undiagnosed due to the lack of functional validation of candidate genetic variants. Zebrafish provides a powerful in vivo system to model patient-specific mutations and establish causal genotype–phenotype relationships across renal and extra-renal tissues. Using genome-editing approaches combining CRISPR/Cas9 and CRISPR prime editing, we generated zebrafish models harbouring patient-derived variants identified by exome sequencing in two genes of interest, NR6A1 and ADGB.

NR6A1 encodes an orphan nuclear receptor involved in transcriptional regulation during embryonic development. Two distinct missense variants were identified in female patients with Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome associated with renal, skeletal, and genital system anomalies. These variants, affecting the DNA-binding and ligand-binding domains of the protein, were precisely introduced into the zebrafish *nr6a1a* gene using CRISPR prime editing. The resulting models were generated to determine whether the patient-specific mutations recapitulate the developmental defects previously observed in *nr6a1a* null mutants, which closely resemble the phenotypes reported in patients.

ADGB encodes androglobin, a protein known to be essential for male fertility through its role in sperm flagellum formation and expressed in ciliated cells. A homozygous frameshift mutation in *ADGB* was identified in a patient presenting congenital anomalies of the kidney and urinary tract (CAKUT) and early-onset deafness. To assess the pathogenicity of this variant, we generated both a zebrafish adgb null mutant and a frameshift-specific mutant. Phenotypic characterisation of the resulting stable lines is currently ongoing, following the observation of glomerular cysts in injected embryos.

Altogether, this work highlights the relevance of zebrafish combined with precise genome-editing technologies to functionally validate patient-specific variants, establish robust genotype–phenotype correlations, and improve our understanding of the molecular mechanisms underlying rare renal diseases.

12

Effects of Anaesthetics on Brain Activity in Zebrafish Larvae

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Topic

7 Neural Circuit Behavior

Abstract Content

Background

Fast, effective and reliable anesthesia is essential in invasive zebrafish experiments in regards to animal welfare and acquisition of reliable data. The most often used substance to induce anesthesia in zebrafish is tricaine (MS-222). When properly prepared and dosed, tricaine causes rapid loss of mobility, balance and reaction to touch. These signs are interpreted as a stage of deep anesthesia although its effects on the central nervous system have not been convincingly shown. Therefore, it is necessary to confirm tricaines effects on the brain activity and to rule out that observed behaviors are not only due to paralyzed but conscious fish to guarantee proper anesthesia.

Methods

We used zebrafish larvae [Tg(elavl3:H2B-GCaMP6s)] at 4 days post fertilization (dpf), expressing a calcium indicator (GCaMP6s) in all neurons, that allows monitoring and quantifying the neuronal activity over time after 168 mg/L tricaine treatment via confocal microscopy. In addition, cessation of spontaneous movement was observed in larvae after treatment.

Results

Tricaine treatment caused a rapid loss of neuronal activity in the forebrain regions of pallium and habenula. In contrast, only mild effects were seen in the midbrain and hindbrain regions.

Conclusion

In conclusion, the different larval brain areas showed differences in the sensitivity to tricaine treatment. The effects on the central nervous system are indicative of tricaine's anesthetic function and are consistent with behavioral observations of inactivity and unresponsiveness to touch.

13

Zebrafish facility report on artificial plants as structural enrichment

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Topic

21 Husbandry

Abstract Content

Background

Zebrafish husbandry conditions need constant improvement to guarantee optimal animal welfare and research data quality. Currently zebrafish are mostly kept in barren tanks. Structural enrichment in the form of artificial plants could be used to provide a more diverse environment and allow the fish to show more behaviors. Thus, it might have the potential to improve their well-being. It was shown, that structural enrichment can reduce stress and improve cognitive abilities, survival rate and fertility in zebrafish. Still, concerns remain regarding its long-term benefits and possible adverse effects like a shift of the sex ratio or an increased pathogen burden.

Methods

To investigate the long-term effects of structural enrichment, we placed artificial plants in the husbandry tanks. For a one-year period we evaluated for all six different zebrafish lines in our husbandry system if benefits of structural enrichment would outweigh its risks by comparing the enriched tanks to our standard conditions.

Results

We saw a 4 % increase of the survival rate during upbringing for enriched zebrafish, albeit not being statistically significant due to high variation. We saw no significant difference in terms of sex ratio, fertility or pathogen burden. When analyzing zebrafish behavior in their 8 L home tanks, we saw no attraction to areas close to the plants but rather a preference for the open water in the middle of the tank.

Conclusion

In summary, we found that introducing structural enrichment to our zebrafish facility carried low cost, had no detrimental effects for the animals but also positive effects were difficult to determine. We like to encourage other animal facilities to evaluate enrichment measures to ensure a broader database and discussion on long-term benefits of structural enrichment in zebrafish husbandry systems.

14

Development of an Innovative High-Throughput Multi-Drug Screening Assay

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Topic

8 Metabolism

Abstract Content

Background

Zebrafish xenotransplantation models are increasingly used as drug-screening platforms in precision oncology, offering high replicate numbers and rapid data acquisition. However, quantifying and automating drug-response measurements remains challenging, and increasing experimental workload. Here, we propose a rapid and precise quantification method that enables testing of a larger number of treatment conditions.

Methods

Five cancer cell lines were used to validate the protocol: HCT116 (colorectal carcinoma), MDA-MB-231, MCF7, SKBR (breast cancer), and UPCI:SCC154 (head and neck carcinoma). Cells were labeled with two non-toxic fluorescent dyes (Green and Orange CellTracker) and injected into the yolk sac of AB or Tg(fli1:EGFP) zebrafish embryos. Two cell quantities were injected, with one being three times higher than the other. Uninjected larvae served as fluorescence controls. Xenografted larvae were placed in 96-well plates. At 24 hpi, embryos were pooled in 1.5 mL tubes and treated with a high-collagenase solution and trypsin. Digestion was performed by mechanical and enzymatic digestion. Single-cell suspensions were analyzed using a multimode microplate reader.

Results

The two injected cell amounts successfully simulated distinct drug-response effects. Both fluorescent dyes generated measurable signals in AB embryos. Autofluorescence from uninjected larvae was minimal and subtracted from experimental values. Fluorescence intensity corresponded to the number of injected cells, with the higher dose producing a threefold increase. Only experiments using green-labeled cells in the Tg(fli1:EGFP) line deviated from this pattern, likely due to interference with the endogenous EGFP signal.

Conclusion

This study establishes a fast, scalable method for quantifying xenografted tumor cells in zebrafish larvae using fluorescent labeling and bulk fluorescence measurement. The protocol reliably discriminates different cell loads across multiple cancer cell lines, with minimal and correctable autofluorescence. Overall, this approach provides a rapid, reproducible, and high-throughput alternative to imaging-based quantification, enhancing the utility of zebrafish xenografts for drug-response studies in precision oncology.

15

Identification of metabolic and genetic factors governing systemic aging by inter-strain comparison of an ultra-short-lived vertebrate

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Topic

8 Metabolism

Abstract Content

Understanding the regulatory mechanisms of vertebrate systemic aging has been hindered by the inherent complexity of aging and the long lifespans of conventional animal models. To overcome these problems, we focused on the African turquoise killifish (*Nothobranchius furzeri*), an ultra-short-lived vertebrate model. Here, by leveraging the distinct advantage of two *N. furzeri* strains exhibiting significantly different lifespans, we identified a liver tryptophan metabolite and a gut ceramidase as novel regulators of systemic aging. First, comparative metabolomic analyses of two strains of *N. furzeri* revealed that the levels of the tryptophan metabolite are higher in longer-lived strain and decrease with age. Increasing the metabolite levels through genetic and pharmacological approaches preserved youthful features, particularly in the muscle, skin, and liver. Mechanistically, this metabolite mitigates age-associated decline in mitochondrial function and reduces oxidative stress. Because the levels of the metabolite in the liver are also higher in long-lived model mice, the liver metabolite may also contribute to youth in mammals. In addition, comparative genomics of two strains of *N. furzeri* revealed a correlation of reduced activity of the gut ceramidase with a longer lifespan. We demonstrated that both ceramidase knockout and ceramide species supplementation extended lifespan and blocked systemic aging phenotypes. Knockout of the ceramidase improved intestinal microbiota quality, and microbiota transplantation blocked aging phenotypes, suggesting that ceramides regulate aging by modulating the microbiota. Together, these findings illuminate previously unrecognized metabolic and genetic axes that govern vertebrate longevity, offering promising targets for systemic anti-aging interventions across species.

17

BMP–Smad1/9 signaling plays a critical role in regulating zebrafish PGC proliferation

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Topic

9 Germline and Early Development

Abstract Content

The germ cell fate in zebrafish is determined by germ plasm, whereas mammalian germ cell fate is induced by bone morphogenetic protein (BMP) signaling. It remains elusive whether BMP signaling is implicated in zebrafish germ cell development. Here, we demonstrate that BMP–Smad1/9 signaling plays a critical role in zebrafish primordial germ cell (PGC) maintenance rather than fate determination. BMP inhibition or *smad1/9* knockdown reduces PGC numbers. Furthermore, we generated PGC-specific *smad1/9* knockouts using a transgenic approach with .

22

A Fluorescence Integrated Zebrafish Larvae Screening Platform for Whole Body Imaging

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Topic

2 Emerging Technologies

Abstract Content

Traditional techniques of preparing zebrafish larvae for imaging involve mounting them in agarose and manually rotating them to the desired viewing angle for microscopy. This process can be time consuming and tedious. Consistency is often dependent on the scientists' dexterity and focus. The throughput rate then limits the type and scale of possible experiments. The VAST BioImager is a high throughput screening platform capable of automatically handling and rotating larvae to desired positions and angles at a rate of 1 larva every 1.0 - 1.5 min at its fastest. A limitation of this system is the lack of fluorescence imaging. Typically, this is dealt with by combining the VAST BioImager with a fluorescent microscope. However, there are difficulties latent in integrating these two systems together such as microscope platform and software compatibility, and hardware constraints which can restrict activating or changing fluorescence filters to be a manual process rather than an automatic one.

Improving on the previous design of the VAST BioImager, zebrafish larvae can now be imaged with automated 3 color fluorescence plus brightfield and a 2.5x lens, enabling an all-in-one whole body imaging platform. This type of platform can be used to screen large numbers of zebrafish larvae as well as to evaluate any fluorescence properties across the entire length of the body. Rapid, repeatable rotations enable high throughput generation of optical projection tomography datasets (3D volumes of regions of interest). This whole-body imaging platform can then be used in a number of ways such as assaying development in an environmental contaminant screen or tracking cancer xenograft recession after exposure to different drugs.

23

Revisiting heat-induced masculinization in zebrafish: heat-responsive families and expressed sex markers from adult fins

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Topic

21 Husbandry

Abstract Content

Exposure to elevated temperature affects the essential abilities, such as survival and reproduction of fishes. During the early development of zebrafish, there is a sensitive window between 18-35 dpf, when increased temperature can result in more male offspring . The proportion of 'neo-males' (genetic females with male phenotype) is family-specific and their performance is nearly indistinguishable from genetic males. For the study of this phenomenon, the ability to separate the sexes is essential. Currently, most labs use sexually dimorphic traits (e.g., body shape, color and anal papilla) of live specimens or visual/histological analysis of gonads of sacrificed individuals.

We revisited heat-induced masculinization and searched for treatment conditions that most reliably identify heat-sensitive families. We used a transgenic reporter line *tg{ddx4::egfp}* (formerly *vas::egfp*) that allowed us to separate the two sexes at an early stage. We optimized the conditions by testing various factors, such as density and water exchange frequency. Altogether, we tested 5 methods and ended up using a treatment between 21 and 35 dpf at 36 ^{o}C. We examined a total of 6,800 individuals in 85 batches from 14 families, seven of which were identified as heat-responsive with a sex ratio shift of 15-48% towards males. In addition to the optimization of heat treatment, we also searched for expressed sex markers in adult fins by RNAseq. Out of over 387 differentially expressed genes, we selected a total of 15 potential markers, from which we identified one female and 11 male markers.

Our optimized approach advances studies on heat-induced masculinization, improves understanding of zebrafish reproduction and may aid development of models in other teleosts vulnerable to the effects global climate change. For researchers working with other cyprinid teleost species, expressed sex markers might offer a new opportunity to identify males and females.

24

IFN-γ-Dependent Macrophage Reprogramming Coordinates Inflammatory Resolution and Matrix Remodeling in Heart Regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

Heart regeneration requires coordinated immune activation, timely inflammatory resolution, and dynamic extracellular matrix (ECM) remodeling in addition to cardiomyocyte (CM) proliferation. However, the cytokine signals that instruct immune cell functions during cardiac repair remain incompletely understood. Here, we identify interferon-gamma (IFN-γ) as a critical regulator of macrophage plasticity in zebrafish heart regeneration. IFN-γ signaling components are dynamically activated following cardiac injury, with early induction of *ifng1* and temporally coordinated receptor expression. Genetic ablation of *ifng1* impairs myocardial regeneration, resulting in reduced CM proliferation and persistent fibrotic scarring. Temporal transcriptional profiling reveals sustained inflammatory signatures, impaired efferocytosis, and abolished reparative programs, accompanied by aberrant immune cell dynamics and retention of injury-derived debris in mutant hearts. Transcriptomic analysis of cardiac macrophages further reveals that IFN-γ deficiency disrupts the transition from an inflammatory state to a reparative, ECM-remodeling phenotype, leading to reduced collagen denaturation and diminished CM protrusion at the injury border zone. Inducible- and macrophage-specific blockade of IFN-γ signaling phenocopies defects in global knockout, establishing a cell-autonomous requirement for IFN-γ in coordinating regenerative immune function. Collectively, our findings define an IFN-γ-dependent macrophage reprogramming axis that couples inflammatory resolution to ECM remodeling in heart regeneration, elucidating how cytokine signaling actively instructs tissue repair.

29

A zebrafish model of Xia-Gibbs syndrome: investigating transcriptomic, morphological, and behavioral alterations associated with *ahdc1* mutation

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Topic

1 Disease Models

Abstract Content

Introduction

Xia-Gibbs syndrome (XGS) is a rare neurodevelopmental disorder caused by *de novo AHDC1* variants, whose molecular mechanisms remain poorly understood. We established a zebrafish mutant line (*ahdc1*$_{dsao1}$) harboring a CRISPR-Cas9-induced 4 bp insertion. We aimed to investigate the impact of *ahdc1* mutation on zebrafish development, gene expression, and behavior to elucidate underlying pathogenic mechanisms.

Methodology

Using wild-type, heterozygous, and homozygous mutant larvae, we evaluated morphology during early embryogenesis by time-lapse imaging (96h). Gene expression was assessed by poly(A) RNA-Seq at two developmental time-points (30 and 48 hours post-fertilization - hpf), complemented by RT-qPCR for *ahdc1* including adult brains. Light-dark transition assays coupled with automated locomotor quantification (Zebrabox system) measured locomotor activity and visual-driven photosensitivity.

Results

Mutant larvae exhibited a transient delay in body and retinal pigmentation at early developmental stages (30-40 hpf), paralleling reduced *ahdc1* expression levels detected by RT-qPCR, followed by subsequent normalization, indicating a critical developmental window. Transcriptomic analysis of heterozygous mutants versus wild-type at 30 and 48 hpf revealed temporal dysregulation of G protein subunits, repression of ligand-gated ion channels, and upregulation of specific opsins, mitochondrial energy metabolism pathways, and ribosomal structural components.

Conclusion

This integrative *ahdc1* zebrafish modelling unveils transient delayed pigmentation, transcriptional changes, and locomotor dysregulation with photosensitivity. These findings link *AHDC1* impairment to disrupted G protein signaling, synaptic transmission, visual processing, and energy metabolism — providing insights into disease mechanisms relevant to XGS.

32

DEPDC5 regulates GABAergic synapse maintenance via NAPB-dependent SNARE recycling independent of mTOR signaling

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Topic

3 Neurobiology

Abstract Content

Epilepsy affects over 70 million people worldwide, and nearly one-third of patients remain refractory to current anti-seizure medications, highlighting the urgent need to identify novel disease mechanisms and therapeutic targets. Loss-of-function mutations in *DEPDC5* (Dishevelled, Egl-10 and Pleckstrin (DEP)-domain containing 5) account for 12-37% cases of focal seizures. Although *DEPDC5* is known as a regulator of mTOR signaling, many pathogenic variants occur in conserved domains with unknown mTOR-independent functions.

For the present work, we have used following methods: immunohistochemistry, confocal imaging, RNA sequencing, CRISPR-Cas9 based gene editing. We will further use qpcr, western blot, FM dye based assays.

Using zebrafish model, our lab previously demonstrated a reduced number of GABAergic synapses in *depdc5* mutants at 6 dpf, indicating a previously unrecognized mTOR-independent role of *depdc5* in inhibitory synapse development. In my postdoctoral work, I observed a transient increase in GABAergic synapse number at 4–5 dpf, revealing stage-dependent dysregulation characterized by early compensatory increase in synapse formation followed by impaired maintenance. RNA-sequencing identified significant downregulation of *napb* (N-ethylmaleimide-sensitive factor (nsf) attachment protein beta), a key NSF-recruiting factor required for SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) complex disassembly and synaptic vesicle recycling. We hypothesize that reduced NAPB impairs SNARE disassembly, leading to accumulation of inactive cis-SNARE complexes, defective vesicle recycling, reduced GABA release, and consequent loss of inhibitory synapses.

We are currently testing this mechanism by analyzing synaptic vesicle dynamics following *napb* downregulation in *depdc5* mutants. In parallel, we are generating zebrafish lines carrying targeted in-frame deletions of a few conserved amino acids within Depdc5 domains to identify regions mediating this non-canonical function.

This work is expected to uncover defective synaptic vesicle recycling as a novel, mTOR-independent mechanism underlying impaired inhibitory synapse development in epilepsy and may reveal novel molecular targets for development of new therapeutics for epilepsy patients.

34

Assessing the Regenerative Efficacy and Biosafety of a Novel PVA-Borax Hydrogel for Skin Wound Healing in Zebrafish

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background: Zebrafish have emerged as a powerful in vivo model for studying tissue regeneration and screening biomaterials. This study utilizes a highly controlled zebrafish evaluation platform to assess a novel, eco-friendly Polyvinyl Alcohol (PVA) and Borax hydrogel designed to accelerate skin wound healing and modulate regenerative microenvironments via water immersion delivery.

Methods: Precise full-thickness skin wounds were induced in adult zebrafish using a custom programmable CO2 laser ablation system. Wound closure kinetics under PVA, Borax, or PVA-Borax hydrogel

treatments were continuously monitored. Underlying regenerative mechanisms were investigated via qRT-PCR. Biosafety was comprehensively evaluated through embryonic toxicity assays and a novel single-camera 3D locomotion tracking system to assess adult neuromotor function.

Results: The PVA-Borax hydrogel synergistically accelerated wound closure milestones compared to controls, a finding further validated in a tilapia model. Mechanistically, qRT-PCR revealed complementary roles driving tissue regeneration: PVA significantly attenuated excessive inflammation (downregulating il1b and tnfa), while Borax actively promoted angiogenesis (vegf) and extracellular matrix remodeling (col1a2, tgfb, and col4a1). Biosafety assessments demonstrated high biocompatibility with 100% embryonic survival and normal morphological development. Crucially, 3D locomotion tracking showed that the

hydrogel preserved normal adult exploratory behavior and neuromotor function, mitigating the stress-like alterations observed in single-component treatments.

Conclusion: The PVA-Borax hydrogel provides a safe and synergistic environment for balanced immune modulation and accelerated tissue regeneration. Furthermore, integrating laser-induced ablation and 3D behavioral tracking establishes a highly effective, non-invasive preclinical platform for evaluating the regenerative functionality and neurotoxicity of novel biomaterials.

35

In vivo optical mapping of cardiac action potentials and calcium transients in a novel zebrafish model for cardiac arrhythmia.

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Topic

4 Cardiovascular

Abstract Content

Background:

The zebrafish heart closely resembles human cardiac electrophysiology in electrocardiogram and action potential (AP) morphology. The optical transparency of zebrafish larvae enables in vivo visualization of cardiac voltage and calcium dynamics using genetically encoded indicators. Here, we developed a dual-reporter zebrafish model combining a next-generation voltage sensor (Ace2N-mNeon) with a spectrally compatible calcium indicator (R-GECO) to enable high-throughput, whole-heart AP characterization in vivo.

Methods:

Ace2N-mNeon and R-GECO were expressed under control of a myocardial specific promoter via the Tol2kit system. The effect of sensor expression on normal physiology was assessed by patch clamp experiments. Fluorescence recordings were obtained with a Leica SP8 DLS microscope and a custom-built light sheet microscope. Our zebrafish sensor line was validated by experiments with quinidine drug exposure, as well as a novel model for long QT syndrome (LQTS) with a knock-in mutation in the *cacna1c* gene.

Results:

Sensor expression did not significantly alter cardiac electrophysiology. The tg(*myl7:Ace2N-mNeon/R-GECO*) line demonstrated alterations in the heart rate, depolarization and repolarization induced by quinidine exposure. We observed a significantly prolonged ventricular repolarization in the LQTS knock-in line. Three-dimensional optical maps of AP characteristics across the entire zebrafish heart exhibited the physiological characteristics of the zebrafish cardiac electrophysiology, with activation initiated at the atrial venous pole and propagated towards the ventricular outflow tract, prolonged repolarization in the ventricles compared to the atria and slowed depolarization in the atrio-ventricular canal.

Conclusion:

By expressing Ace2N-mNeon and R-GECO in the zebrafish heart, we were able to accurately detect drug- and mutation induced alterations in the cardiac AP, as well as AP

characteristics across the entire heart, without compromising normal cardiac electrophysiology. Our transgenic line will provide a promising assay for future cardiac arrhythmia studies.

36

CRISPR mc4r zebrafish KO line establishes a scalable model for studying hyperphagia and metabolic regulation.

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Topic

8 Metabolism

Abstract Content

Background. The melanocortin 4 receptor (MC4R) is a highly conserved G protein–coupled receptor that serves as a key regulator of appetite and energy homeostasis. In humans, MC4R mutations represent the most common cause of monogenic obesity. Moreover, murine loss-of-function models exhibit hyperphagia, increased adiposity, and reduced energy expenditure. Although the melanocortin pathway is evolutionarily conserved, the systemic role of mc4r in zebrafish has not been fully characterized.

Methods. To address this, we generated a CRISPR-Cas9-induced mc4r loss-of-function zebrafish line and performed phenotypic analyses across developmental stages. Larval food intake was quantitatively assessed at 8 days post fertilization (dpf) using fluorescence labelled food, followed by quantification in a FLUOstar Omega microplate reader. In this same reader, adiposity at 5 dpf was measured after whole-organism Nile-red lipid staining. At 5 months adult stage, body weight and length were recorded. Whole-organism metabolic function was assessed at 2 dpf using Seahorse extracellular flux analysis to determine oxygen consumption rate (OCR).

Results. Mc4r-deficient larvae exhibited significantly increased food intake at 8 dpf. Elevated adiposity was detectable prior to dietary intervention at 5 dpf; at 5 months, both male and female mutants displayed increased body weight and length compared to wild-type siblings. Furthermore, whole-organism metabolic profiling revealed reduced OCR, consistent with impaired oxidative metabolism and decreased energy expenditure.

Conclusion. Collectively, these findings demonstrate that mc4r disruption in zebrafish reproduces core phenotypic features of mammalian MC4R deficiency, including hyperphagia, early-onset adiposity, somatic overgrowth, and altered metabolic function. This genetically defined mc4r mutant line establishes a model to dissect MC4R-mediated mechanisms of energy regulation and to evaluate strategies for modulating metabolic outcomes.

37

Understanding the role of *rfc1* loss of function in the context of CANVAS pathogenicity using the zebrafish model

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Topic

3 Neurobiology

Abstract Content

The Cerebellar Ataxia Neuropathy Vestibular Areflexia Syndrome (CANVAS) is a rare adult-onset ataxia. In 2019, a biallelic intronic repeat expansion $(AAGGG)_{>400}$ in the Replication Factor C1 (*RFC1*) gene was identified as a cause of CANVAS. However, the mechanisms by which this intronic repeat expansion in this gene causes this neurological disorder are unknown. Recent papers identified frameshift mutations in *RFC1*, suggesting that a loss-of-function could drive pathogenicity.

Since knocking-out RFC1 in mammals leads to embryonic lethality, we generated a zebrafish loss-of-function model of *rfc1* using CRISPR/Cas9. We found that the cerebellar development of the *rfc1*$^{-/-}$ larvae is severely affected, with disorganized Purkinje cell organization and a severe reduction in the number of granule cells. Using scRNAseq, we demonstrated that *rfc1* is expressed in early neural progenitors and that its loss-of-function leads to defects in DNA replication processes. Ultimately, defective *RFC1* signalling leads to massive apoptosis among this cell population, affecting subsequent neurogenesis, particularly of cerebellar neurons. This work is the first to investigate the function of *RFC1 in vivo*.

By showing its role in cerebellar development, this suggests that CANVAS could be, at least partially, caused by *RFC1* loss-of-function mechanisms. However, CANVAS is a late-onset disease, so we wonder if *RFC1* could also play a role in a mature cerebellum…Thus, we will focus on two main areas: studying the consequences of late loss of *RFC1* expression in a mouse model and understanding the role of expansions using patient-derived cells to correlate our observations with a more clinical perspective.

38

Noise-induced hearing loss in zebrafish model: Characterization of tonotopy and sex-based differences

JUNE CHOI

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Topic

1 Disease Models

Abstract Content

Backgroud: Many studies have demonstrated the characteristics of noise-induced hearing loss in human and non-human vertebrate models, including frequency-specific noise-induced hearing loss and sex-biased differences.

Methods: Despite the increasing popularity of zebrafish as a model for NIHL, a better understanding of this model is needed to determine sex differences in NIHL. To study the features of zebrafish as they relate to an NIHL model, we tested various phenotypes after frequency-specific noise stimulation. The degree of damage to hair cells and hearing loss were investigated after exposing zebrafish to 200 Hz and 1 kHz continuous waves and broadband white noise with a bandwidth from 50 Hz to 1 kHz.

Results: After exposure to all frequencies, the larvae showed lateral line hair cell damage, which is superficially located. In adult zebrafish, the threshold of auditory-evoked potential signals is elevated. Moreover, the number of hair cells remarkably decreased in the rostral region of the saccule, after exposure to 1 kHz and white noise, whereas zebrafish exposed to 200 Hz noise showed a decrease in hair cells in the caudal region. Moreover, male zebrafish were found to be more vulnerable to noise than female zebrafish, as is the case in humans and other mammals. Cortisol levels also increased in the noise-exposed male group, as compared to the noise-exposed female and control male groups. However, there was no difference in cortisol levels when the noise-exposed female group was compared to the control female group.

Conclusion: Our study demonstrates not only that noise-induced hearing loss is frequency-dependent but also that the degree of hearing loss is affected by sex in zebrafish, emphasizing the need to consider sex in NIHL studies. This study was published in Hearing Research.

39

Analysis of Rheotaxis Behavioral Changes in Lateral Line Damaged Zebrafish Larvae for Drug Screening Applications

JUNE CHOI

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Topic

17 Chemical Biology and Drug Discovery

Abstract Content

Backgound: Aquatic animals have rheotaxis that maintains a balance in response to water flow.

They sense water flow through hair cells in lateral line, thereby leading to behavior changes relevant to damages on hair cells, the primary sensory receptor cells within auditory and vestibular systems.

This article aims at characterization of rheotactic behaviors identifying lateral line states via an analysis platform that simultaneously examines multiple zebrafish larvae.

Methods: Zebrafish are efficient animal models for high-throughput drug screening with human-like hair cells along the lateral line.

Their rheotactic behaviors could be assays for hair-cell-targeted drug screening. However, knowledge and tools for rheotaxis analysis along the extent of hair-cell damage have not been fully investigated.

Results: To this end, we developed an automated framework that incorporated animal test hardware equipment and real-time analysis software for monitoring aquatic behaviors of multiple larvae.

Through this framework, a commensurable measure for one-dimensional characterization of rheotactic behaviors was consolidated so that its linear changes could be associated with the population of hair cells remaining intact.

Conclusion:These findings satisfied requests for an automated analysis platform to conduct large-scale screening and a biomarker that discriminate the seriousness of hair cell damage to screen candidates having significant effects in otoprotective drug discovery. This study has been published in Expert Systems With Applications.

40

Long-Range Coupling of Posterior Cell Addition and Anterior Vacuolation Provides Robustness in Notochord Elongation.

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Robust tissue growth control requires long-range communication between the rate of progenitor addition and tissue expansion. However, the regulatory mechanisms that couple these two processes are unknown. In zebrafish, notochord morphogenesis is a principal driver of axis extension through the combined actions of posterior progenitor addition and anterior vacuolation. To elucidate how progenitor dynamics and vacuoledriven cell expansion interact to shape notochord development, we first generated a mathematical model that links progenitor addition rate to the progressive expansion of cells from anterior-to-posterior to simulate vacuolation rate. Comparing this with empirical measurements, we find that progenitor incorporation together with vacuolation, produces a linear gradient in nearest neighbour distance. We next explored the role of YAP/TAZ in regulating the rate of progenitor addition in mutants for YAP/TAZ inhibitor vgll4b. We find that vgll4b expression and YAP activity are enriched in posterior midline progenitors. Loss of vgll4b elevates YAP signaling, enhances progenitor addition, restricts vacuole expansion, and—after a transient buOering phase—compromises A-P axis elongation. These results support a long-range feedback mechanism linking progenitor recruitment to vacuolation, enabling the notochord to balance cellular input with volumetric expansion, thereby maintaining tissue proportions.

42

MITOCHONDRIAL DYNAMICS FUNCTION IN THE CONTROL OF RETINAL STEM CELL FATE DIFFERENTIATION

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Topic

19 Cell fate

Abstract Content

Mitochondria are dynamic organelles that regulate metabolism, cell signaling and the intracellular redox balance, essential processes for stem cell maintenance and differentiation. We previously showed that during retinogenesis, redox signaling controls retinal stem and progenitor cell (RSC/RPC) fate, with H_2O_2 as the principal signaling mediator. The alteration of H_2O_2 degradation severely affected RSC and RPC fate during zebrafish retinal development. Building on this, we now investigate how mitochondrial fusion and fission known as mitochondrial dynamics and mitochondrial ROS (mROS) levels regulate RSC and RPC outcomes. As model paradigm, we use the ciliary marginal zone (CMZ), the zebrafish retinal stem cell niche located at the periphery of the differentiated retina and that comprises RSC, RPCs and committed precursors spatially distributed. First, by serial electron microscopy analyses, we assessed mitochondrial morphologies in the different CMZ populations. Single-cell RNA seq (scRNAseq) analyses was employed to identify redox-associated genes as well as key components of the fusion/fission machinery differentially expressed in these cells. Finally, through loss- and gain- of function approaches, we evaluated how the disruption of mROS regulation and mitochondrial morphology in a cell-specific manner affects RSC differentiation. Ultrastructural analyses revealed distinct mitochondrial morphologies in the course of RSC differentiation, indicating dynamic remodeling of mitochondrial morphology during fate transition. Our scRNAseq analyses identified the mitochondrial antioxidant encoding gene *peroxiredoxin 3* (*prdx3*) as well as key regulators of the fusion/fission machinery differentially expressed in RSC and RPCs. Disruption of *prdx3* by CRISPR/Cas9 induced ectopic cell death both in the CMZ and differentiated retina as well as an increased proliferation compared to controls. Collectively, these findings support a model in which mitochondrial dynamics and mROS signaling regulate retinal stem and progenitor cell fate, positioning mitochondria as central integrators of redox signaling and developmental programs during retinogenesis.

44

The Balbiani Body is formed by microtubule-controlled molecular condensation of Buc in early oogenesis

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Topic

9 Germline and Early Development

Abstract Content

Widely in animal and plants gamete polarization lays the foundations for embryonic development. Vertebrate oocyte polarity has been observed for two centuries and is crucial for embryonic axes formation and germline specification, but how it is generated is unknown. In oocyte polarization, essential embryonic-regulatory RNA-protein (RNP) granules must be delivered to the oocyte vegetal pole by a membraneless organelle called the Balbiani body (Bb). The Bb is essential for oogenesis, embryonic development and reproduction, and is conserved from insects to humans. However, despite its discovery in 1845, mechanisms of Bb formation remain unknown.

Here, we discovered mechanisms of Bb formation in zebrafish by developmental molecular condensation. We provide super-resolution microscopy, live-imaging, biochemical, and genetic evidence in developing ovaries in vivo to conclude that the Bb forms by molecular condensation driven by phase-separation of Buc, an essential intrinsically disordered protein with a prion-like domain. Live-imaging, molecular, and FRAP experiments in vivo demonstrate that the forming Bb develops from liquid-like viscoelastic condensates with dynamic Buc mobility to a solid-like stable compartment. Thus, we uncovered Buc-dependent developmental viscoelastic changes that shape Bb formation.

Further, we deciphered multi-step regulation by microtubules controlling Bb condensation: first, by dynein-mediated trafficking of early Buc granules; then by scaffolding condensed granules via molecular crowding; and finally by caging the mature Bb to prevent over-growth and maintain shape. These steps ensure formation of a single intact Bb condensate essential for oocyte polarization. Cellular control over phase-separation by microtubules is likely widely conserved.

We further identified novel Buc-interacting regulators, including kinases, microtubule-RNA binding proteins, and largely disordered coiled-coil proteins that may control distinct steps in Buc condensation and Bb formation. Altogether, our findings shed new light on physiological condensate biology, female reproduction, and resolve the long-standing question of vertebrate oocyte polarity origins.

46

ITGBL1 Cooperates with Integrin αVβ1 to Activate TGF-β1 Signaling and Drive Fibrosis-Associated iCCA Progression in Zebrafish

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Topic

1 Disease Models

Abstract Content

Background:
Liver fibrosis represents a critical transitional stage linking metabolic liver disease to irreversible cirrhosis and intrahepatic cholangiocarcinoma (iCCA). Transforming growth factor-β1 (TGF-β1) is a central driver of hepatic stellate cell (HSC) activation; however, the molecular mechanisms governing its activation within the fibrotic microenvironment remain incompletely defined. Integrin αV-mediated mechanical release of latent TGF-β1 has been implicated in fibrosis. Integrin beta-like protein 1 (ITGBL1), an extracellular matrix-associated protein, has been reported to modulate TGF-β/SMAD signaling, but its cooperative interaction with integrin subunits during fibrosis progression has not been mechanistically elucidated.

Methods:
We utilized a liver-specific ITGBL1-overexpressing zebrafish model to investigate early fibrotic events and later malignant remodeling. Pharmacological inhibition of ITGAV was performed using Cilengitide and IN-1. CRISPR/Cas9-mediated liver-specific knockouts of itgav and itgb1 were introduced to dissect the functional contribution of the proposed ITGBL1–αVβ1 complex. Histological evaluation (H&E, Sirius Red), molecular analyses of fibrogenic markers, and co-immunoprecipitation assays were performed to assess pathway activation and protein interactions.

Results:
ITGBL1 overexpression induced early HSC activation, progressive collagen accumulation, and subsequent bile duct hyperplasia with iCCA-like characteristics. Inhibition or genetic ablation of itgav significantly attenuated fibrotic remodeling and reduced SMAD signaling activation. Disruption of itgb1 further suppressed extracellular matrix deposition, supporting a cooperative role of the αVβ1 complex. Co-immunoprecipitation confirmed interaction between ITGBL1 and integrin components, suggesting a structural platform facilitating latent TGF-β1 activation.

Conclusion:
Our findings identify ITGBL1 as a key microenvironmental modulator that cooperates with integrin αVβ1 to promote TGF-β1 activation and fibrosis-driven iCCA progression. Targeting the ITGBL1–αVβ1 axis may represent a promising therapeutic strategy to interrupt fibrosis-associated liver carcinogenesis.

47

ITGBL1 Promotes Liver Fibrosis and Intrahepatic Cholangiocarcinoma Progression via ITGAV-Mediated TGF-β1 Activation in Zebrafish

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Topic

1 Disease Models

Abstract Content

Background: Intrahepatic cholangiocarcinoma (iCCA) is a highly malignant liver cancer characterized by dense fibrous stroma. While TGF-β1 is a master regulator of hepatic stellate cell (HSC) activation and fibrosis, the specific mechanisms governing its release in the iCCA microenvironment remain unclear. Integrin beta-like protein 1 (ITGBL1) has been implicated in fibrogenesis, yet its interaction with integrin subunits like ITGAV to drive iCCA remains to be fully elucidated.

Methods: We established a liver-specific ITGBL1-overexpressing zebrafish model using a Doxycycline-inducible (Tet-on) system. To investigate the underlying mechanism, we utilized CRISPR/Cas9 to generate liver-specific knockouts of *itgav* and *ltbp1*. Fibrosis and tumor progression were evaluated through H&E, Sirius Red, and Masson's trichrome staining. Molecular changes were assessed via RT-qPCR for aHSC/fibrosis markers and Western blot for p-SMAD3 and α-SMA protein levels.

Results: Overexpression of ITGBL1 significantly induced HSC activation, extensive collagen deposition, and the formation of iCCA-like glandular structures by 6 months post-fertilization. Treatment with the ITGAV inhibitor Cilengitide, or genetic knockout of *itgav* and *ltbp1*, effectively attenuated ITGBL1-induced fibrosis and suppressed the upregulation of α-SMA and p-SMAD3. These findings indicate that ITGBL1 facilitates the release of active TGF-β1 through an interaction with ITGAV.

Conclusion: Our study demonstrates that ITGBL1 acts as a crucial oncogenic driver in the iCCA microenvironment by modulating the ITGAV/TGF-β1 axis. Targeting the ITGBL1-ITGAV interaction presents a promising therapeutic strategy for inhibiting fibrosis-related progression in liver cancer.

49

Mammalian crest cells enhance cartilage regeneration in zebrafish by stimulating endogenous Nrg1/ErbB signaling

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background: Mammalian cartilage repair is challenging due to its limited regenerative capacity, which often leads to irreversible joint disorders. To overcome this limitation, highly regenerative models such as zebrafish are increasingly used to uncover key mechanisms of cartilage regeneration. We demonstrated that zebrafish larvae can fully regenerate Meckel's cartilage (MC) after injury through the Nrg1/ErbB signaling pathway, providing a powerful system for identifying chondro-regenerative mechanisms and therapeutic targets. Stem cell-based approaches are at the forefront of regenerative medicine, highlighting neural crest cells as promising candidates for cartilage repair because of their inherent regenerative and chondrogenic potential. Using the zebrafish model, we evaluated the chondro-regenerative potential of murine NCCs (mNCCs) and human induced NCCs (hiNCCs) derived from pluripotent stem cells.

Methods and Results: MC injuries were performed in 3 days post fertilization (dpf) *Tg(col2a1:mCherry)* larvae, in which chondrocytes are labeled. One hour after injury, mNCCs or hiNCCs were microinjected locally into the damaged jaw, and regeneration was monitored by confocal imaging. At 48 and 72 hpi, larvae injected with hiNCCs and mNCCs, respectively, exhibited significantly longer regenerated MC areas compared to non-treated larvae, demonstrating the chondro-regenerative potential of both cell types. To investigate the underlying mechanisms, we focused on NRG1–ErbB signaling. We quantified the expression of *nrg1* splice variants and the *erbb* receptors. NCC-injected larvae exhibited an upregulation of endogenous *nrg1* splice variants, the receptors *erbb2* and *erbb3b*, and key chondrogenic genes at 4 and 24 hpi. Then, to assess the role of ErbB signaling in NCC-mediated enhancement of cartilage regeneration, we treated injured larvae with a pan-ErbB inhibitor. Inhibitor-treated zebrafish showed a marked reduction in cartilage regeneration regardless of NCC injection.

Conclusion: Our results demonstrate that mammalian NCCs enhance cartilage regeneration in zebrafish through the activation of the endogenous Nrg1/ErbB pathway, unveiling a novel cartilage regenerative cell product.

51

Primary tuberculous granulomas provide a niche for opportunistic superinfecting mycobacteria

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Topic

15 Infection and Immunity

Abstract Content

Prior tuberculosis infection is a susceptibility factor for subsequent mycobacterial infection and this effect can be modelled in zebrafish with a primary *Mycobacterium marinum* infection followed by a secondary *M. abscessus* infection. As tuberculous granulomas are a long-lasting histological hallmark of tuberculosis and are known to be inhabited by superinfecting *Mycobacterium tuberculosis*, we hypothesised tuberculous granulomas may provide a niche for the growth of the opportunistic pathogen *Mycobacterium abscessus*. Using optically transparent zebrafish embryos we find preferential growth of secondary *M. abscessus* infection inside primary *M. marinum* granulomas. We find granuloma-resident secondary *M. abscessus* is protected from macrophage-mediated immune control. Primary *M. marinum* granulomas caused by ESX1-deficient strains are not efficiently colonised by *M. abscessus* and maturation of wild type *M. marinum* granulomas correlates with efficient colonisation by *M. abscessus*. While a minority of colonisation is driven by *M. abscessus*-infected myeloid cells migrating into primary granulomas, the majority of colonisation is independent of myeloid cells suggesting a direct affinity between *M. abscessus* and factors at the primary granuloma. Our data provide insight into a potentially clinically important host niche for non-tuberculous mycobacterial growth.

53

Spatiotemporal emergence of somatosensory neuron diversity in zebrafish

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Topic

3 Neurobiology

Abstract Content

How cellular factors combine in space and time to generate the diverse neuron types of a functional circuit in a robust way remains unclear. The fish primary somatosensory system of Rohon-Beard (RB) neurons develops first and is thought to undergo programmed cell death and be then replaced by the DRG. Our live imaging and deep single-cell transcriptomics across development show that contrary to a ~150-year-old assumption, RB neurons do not disappear and are heterogeneous at 3 levels: their transcriptome, their axial distribution, and interindividual pattern. Spatiotemporal analysis of RB subtype emergence reveals that it follows a staggered-layer logic, with a 'pan-RB program' preceding a stochastic 'hybrid neurotrophin receptor' state that resolves into mutually-exclusive subclasses, followed by specialized functional subtypes. Combining *in toto* cell lineage reconstruction and a custom whole-mount spatial transcriptomics —in the same animal- we show RB neurons possess extremely simple cell lineages and link them to dozens of transcription factors and somatosensory genes in space. We also define the regulatory mechanism by transcription factors underlying the stochastic, yet robust emergence of the observed neuronal subtypes across time. We also show this mechanism is likely conserved across Anamniotes. Our work demonstrates that zebrafish RB neurons are an excellent model to study the molecular mechanisms underlying the development of neuron diversity at the cellular level.

54

A translational zebrafish model for preclinical compound testing and humanized allele analysis in Pseudoxanthoma elasticum

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Topic

1 Disease Models

Abstract Content

Pseudoxanthoma elasticum (PXE) is a rare, monogenic disorder characterized by progressive ectopic calcification of elastic fibers in connective tissues. Clinical manifestations typically emerge in early adulthood and predominantly affect the skin, vascular walls, and Bruch's membrane of the eye. PXE is caused by biallelic loss-of-function mutations in the ABCC6 gene, which encodes a transmembrane ATP-binding cassette transporter. ABCC6 plays a central role in physiological mineralization by regulating systemic ATP levels. Its deficiency disrupts the inorganic phosphate-pyrophosphate balance, promoting hydroxyapatite deposition in tissues normally resistant to calcification. Zebrafish has emerged as a robust vertebrate model for PXE, as abcc6a loss-of-function mutants recapitulate key aspects of the mineralization phenotype. Notably, homozygous mutants display early-onset larval skeletal alterations, providing a rapid and tractable platform for in vivo drug screening.

To establish a standardized quantitative screening assay, we optimized an Alizarin Red S-based fluorescent imaging protocol for whole-mount larvae. Structured illumination microscopy improved resolution and minimized out of focus signal, enabling the detection of early mineralization defects, including anterior vertebral fusions. For objective and reproducible quantification, we developed a Python-based image analysis pipeline incorporating geometric straightening of the spinal axis via piecewise affine transformation. This enabled distortion-free generation of one-dimensional fluorescence intensity profiles along the anterior-posterior axis and simultaneous assessment of mineralization intensity and spatial patterning. The resulting framework provides a reproducible, automation-compatible platform suitable for medium- to high-throughput compound screening. Our primary objective is to utilize this validated assay system to evaluate potential therapeutic candidates, with particular focus on synthetic pyrophosphate analogues aimed at restoring mineralization homeostasis. In parallel, we are developing a humanized zebrafish model through targeted genomic integration of human *ABCC6* coding sequences into the endogenous *abcc6a* locus. This strategy will enable allele-specific functional analyses under physiological regulatory control, thereby facilitating the investigation of pathogenic human variants and their molecular mechanisms.

56

Characterization of a novel heart enhancer within the zebrafish *isl1a* locus

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Topic

4 Cardiovascular

Abstract Content

BackgroundHeart formation relies on precise spatiotemporal gene expression controlled by cis-regulatory elements such as enhancers. In zebrafish, cardiac progenitor cells arise during gastrulation and segregate into the first and second heart fields, whose development depends on complex transcriptional networks. In this study, we characterized a regulatory element located within the first intron of the *isl1a* gene, referred to as I3.

Results
Transient reporter assay revealed that the I3 enhancer could drive reporter expression in the heart. In silico analysis identified multiple transcription factor binding sites within I3 sequence, including motifs for MEF2C, a transcription factor with a well-established role in cardiac development. We hypothesized that MEF2C binding is required for the enhancer's cardiac activity. To test this, the full-length I3 enhancer was cloned into a reporter vector containing an E1b minimal promoter, and MEF2C binding sites were selectively deleted. Removal of MEF2C binding sites decreased reporter expression in the heart, indicating that MEF2C is essential for I3 enhancer function. To further dissect its regulatory architecture, separate fragments of the I3 enhancer were each cloned into the same vector and examined for reporter expression.

Conclusion
This ongoing experiment aims to identify the minimal and functionally most relevant region of the I3 enhancer. Together, these results provide insight into the modular organization of enhancers and contribute to a refined understanding of cis-regulatory control during early cardiac development.

57

Elucidating the function of Mediator Kinase Module subunits Med13a and Med13b in zebrafish embryonic development.

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background

The mediator complex plays a central role in eukaryotic transcription regulation, acting as a bridge between enhancer-bound transcription factors and the core transcription machinery at the promoter. MED13 and MED13L are key components of the mediator kinase module, which modulates the transcriptional regulatory outcome of the complex by dynamically associating with the core complex. In humans, pathogenic variants in MED13 and MED13L has been implicated in rare congenital disorders characterized by overlapping symptoms, including craniofacial defects, intellectual disability, muscle hypotonia, and behavioural abnormalities. Despite their clinical relevance, the developmental functions of these genes remain incompletely understood. The zebrafish (*Danio rerio*) *med13a* and *med13b* possess 64-80% sequence similarity to their human counterparts.

Results

Here, we show that morpholino-mediated knockdown of *med13a* resulted in spinal cord curvature, impaired spatiotemporal patterning of cranial neural crest cell population, and global developmental delay. High-resolution imaging further revealed defects in muscle cytoskeleton organization and impaired motor neuron axonogenesis. Importantly, these phenotypes were rescued in a dose-dependent manner using zebrafish *med13a* mRNA. In parallel, loss of *med13b* function led to prominent cardiac and brain abnormalities. Together, our findings establish zebrafish as a viable model to study MED13/MED13L-associated disorders.

Conclusion

Our data suggest both overlapping and gene-specific functions of Med13a and Med13b in neural crest patterning, neuromuscular organization, and cardiac morphogenesis. Ongoing work focuses on generating stable mutant lines and defining the transcriptional networks controlled by the Mediator kinase module during embryogenesis.

62

BMP–Smad1/9 signaling plays a critical role in regulating zebrafish PGC proliferation

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Topic

9 Germline and Early Development

Abstract Content

The germ cell fate in zebrafish is determined by germ plasm, whereas mammalian germ cell fate is induced by bone morphogenetic protein (BMP) signaling. It remains elusive whether BMP signaling is implicated in zebrafish germ cell development. Here, we demonstrate that BMP–Smad1/9 signaling plays a critical role in zebrafish primordial germ cell (PGC) maintenance rather than fate determination. BMP inhibition or *smad1/9* knockdown reduces PGC numbers. Furthermore, we generated PGC-specific *smad1*/*9* knockouts using a transgenic approach with PGC-specifically expressed Cas9 and ubiquitously expressed guide RNAs. Smad1/9 deficiency in PGCs leads to impaired PGC proliferation and increased apoptosis, consequently reducing PGC numbers. Transcriptome analysis revealed unchanged PGC-specific gene expression, but a marked upregulation of DNA damage response-related genes, which is validated by ectopic ATR–pChk1 activation in PGCs and PGC restoring by ATR inhibition. Collectively, these findings underscore conserved but functionally distinct roles of BMP signaling in vertebrate PGC development.

65

Conserved DYRK2 signalling is required for spontaneous axonal regeneration after complete spinal cord injury

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Topic

14 Stem Cells and Regeneration

Abstract Content

Failure of axonal regeneration after spinal cord injury (SCI) underlies permanent functional deficits in mammals, whereas regenerating vertebrates such as lampreys and zebrafish spontaneously restore descending axonal connections. Identifying conserved intrinsic regulators of this regenerative competence is essential for developing pro-repair strategies. We previously showed that activation of GABAB receptors with baclofen enhances axon regeneration in lamprey descending neurons. Transcriptomic profiling of baclofen-treated brainstems revealed *dyrk2*, encoding the Dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2), as one of the most significantly upregulated genes. Here, we demonstrate that DYRK2 is a conserved and neuron-intrinsic driver of spontaneous axonal regeneration after complete SCI.

Quantitative PCR confirmed baclofen-induced upregulation of *dyrk2* in the injured larval lamprey brainstem. Immunofluorescence revealed DYRK2 protein expression in identifiable descending neurons in both control and injured lampreys. Pharmacological inhibition of DYRK2 with LDN-192960 after complete SCI significantly reduced regeneration of individually identifiable giant descending neurons and cholecystokinergic descending axons at 10 weeks post-lesion, without impairing gross locomotor performance. Targeted knockdown using a translation-blocking morpholino applied at the lesion site decreased DYRK2 protein levels in

descending neurons and recapitulated the regenerative deficit, demonstrating a cell-autonomous requirement.

To test evolutionary conservation, we performed complete SCI in larval zebrafish. DYRK2 inhibition with LDN-192960 significantly reduced axonal bridge formation, whereas selective DYRK1A/1B inhibitors had no effect, supporting pathway specificity. CRISPR/Cas9-mediated disruption of *dyrk2* generated high-efficiency crispants that also exhibited impaired axonal bridging without overt developmental abnormalities.

Together, pharmacological and genetic evidence across two phylogenetically distant vertebrates establishes DYRK2 as a conserved regulator of spontaneous axonal regeneration. These findings position DYRK2-dependent signalling as a core component of regenerative competence and identify a tractable molecular target for promoting axonal repair in non-regenerating nervous systems.

68

Toxicological Assessment and Neuromodulatory Effects of a Green Extra-Virgin Olive Oil Extract in a Zebrafish PTZ-Induced Seizure Model

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Topic

7 Neural Circuit Behavior

Abstract Content

Extra-virgin olive oil (EVOO) is rich in bioactive secoiridoids, particularly oleocanthal (OLC) and oleacein (OLE), known for their neuroprotective and anti-inflammatory properties. However, their therapeutic potential in neurological disorders such as epilepsy remains largely unexplored. A novel OLC/OLE-enriched EVOO extract has been developed using a sustainable natural deep eutectic solvent (NaDES) extraction method, yet its toxicological and pharmacological profiles remain undefined.

This study aimed to assess the acute toxicity of the extract in the zebrafish *in vivo* model and evaluate its neuromodulatory effects in an experimental epilepsy model.

Zebrafish embryos were exposed to seven concentrations (0.1–50 mg/L of polyphenols) following OECD guideline No. 236 to determine toxicological endpoints. Three sublethal concentrations (0.1, 0.25, 0.5 mg/L) were selected for neuromodulatory assessment using a validated pentylenetetrazol (PTZ)-induced seizure model. Larvae were pretreated for 96 h, then exposed to 2 g/L PTZ for 30 min. Locomotor activity was monitored using a light/dark assay (ZebraBox, ViewPoint), and transcriptional responses of genes involved in neuronal activity and plasticity (*fosab, bdnf, serta, oxtra, oxtrb, nos1*) were quantified by real-time PCR.

The 96-h LC50 of the extract was 3.7 mg/L (95% CI: 2.845–4.851). PTZ induced robust seizure-like hyperactivity, particularly during light phases, confirming enhanced neuronal excitability. Pre-treatment with the EVOO extract significantly and dose-dependently attenuated locomotor hyperactivity across multiple analyses. PTZ also triggered marked upregulation of immediate early genes (*bdnf, fosab*) and neuromodulatory markers (*serta, oxtra, oxtrb, nos1*), all downregulated in a concentration-dependent manner by the extract.

The coordinated behavioural and transcriptional normalization highlights a coherent neuromodulatory effect, suggesting that the NaDES-derived EVOO extract mitigates hyperexcitability through multiple activity-dependent pathways. These results support its potential as a neuromodulatory candidate and demonstrate zebrafish as an effective platform for integrated neurotoxicity and neuropharmacological screening of novel bioactive formulations.

72

Investigating the extra-nuclear function of SNRNP70 during neuronal development.

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Topic

3 Neurobiology

Abstract Content

SNRNP70 is an essential component of the major spliceosome and its nuclear role during pre-mRNA splicing has been extensively studied. Notably, SNRNP70 has also been found to localise to cytoplasmic regions of neurons including axons and synapses. Previous work from our lab has shown that the extra-nuclear portion of SNRNP70 plays a crucial role in regulating motor axonal growth and neuromuscular synaptogenesis through regulation of the local transcriptome. Despite this, the precise extra-nuclear roles of SNRNP70 in neurons remain elusive.

Using molecular genetic techniques in zebrafish, we have developed innovative transgenic tools, which enable the imaging and visualisation of the intricate relations between SNRNP70 and its axonal protein partners in vivo. To investigate the extra-nuclear function of SNRNP70 we conducted an unbiased proteomic analysis aimed at identifying the protein interacting partners of SNRNP70 specifically in the cytoplasm. Our mass spectrometry findings revealed that extra-nuclear SNRNP70 unexpectedly associates with various RNA binding proteins, core spliceosome components and translation associated proteins. We further demonstrated that SNRNP70 directly binds to these protein interactors within axons through proximity ligation assays, confirming our results.

We hypothesise that the extra-nuclear pool of SNRNP70 plays a critical role in axonal development through various mechanisms, including RNA trafficking, stability and degradation, translational repression, and local processing. Our future research will focus on investigating these interactions further to clarify how extra-nuclear the role of extra-nuclear SNRNP70 during neuronal development and neurodegeneration.

73

Redox mechanisms of retinal stem cell development

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Topic

14 Stem Cells and Regeneration

Abstract Content

Signaling by Redox molecules, and H_2O_2 in particular, is emerging as fundamental determinant of stem cell behavior. Rather than mere by-products of metabolism, reactive oxygen species (ROS) where shown to act as instructive signaling molecules that govern self-renewal, quiescence, differentiation, and stress adaptation by coordinating extrinsic cues and cellular responses to intrinsic programs.

Using the transgenic *Tg*(*ubi:hyper*) sensor line, we previously revealed the endogenous dynamics of H_2O_2, a major ROS metabolite and second messenger for cell metabolism, during zebrafish retinogenesis and in the post-embryonnic stem cell niche of the zebrafish retina, the ciliary marginal zone (CMZ). We demonstrated that H_2O_2 levels are tightly regulated during retinal development and orchestrate the proliferation to differentiation switch of retinal stem and progenitor cells (RSCs and RPCs) during retinal development and in the CMZ. We showed that this regulation notably occurs at the final cell division of in committed RPCs through the degradation step of H_2O_2 by the scavenger Catalase. These findings identified for the first time a physiological role for redox signaling during vertebrate retinogenesis *in vivo*.

Through single-cell transcriptomic analyses of zebrafish retinal cells, we identified several additional redox-related genes differentially expressed in RSCs and RPCs and are exploring their functions in this context. Based on fine gain and loss of function approaches as well as serial electronic microscopy ultrastructure analyses, our current results identified several new redox-mediated mechanisms, from transcriptional control of antioxidant activity to organelle-specific redox level control underlying the transition fate of RSC and CMZ homeostasis. Expanding our investigations to human retinogenesis using human iPSC-derived retinal organoids and human fetal samples, we are assessing the conservation and specie -specific mechanisms relating to redox function in retinal stem cell development.

76

The Pro- and Anti-convulsant Effects of Malaysian Tropical Fruit Pulps: Novel Insights from a PTZ-Induced Zebrafish Larvae Seizure Model

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Topic

1 Disease Models

Abstract Content

Background

Epilepsy affects over 50 million people globally, with approximately one-third of patients still remaining drug-resistant despite the dozens of antiseizure medication currently available. This significant therapeutic gap necessitates the exploration of dietary interventions to improve seizure control for people with epilepsy (PWE). While fruits are rich in bioactive flavonoids with known antioxidant and anti-inflammatory properties, tropical species remain largely underexplored. The aim of this study is to evaluate the pro- and anti-convulsant activities of Malaysian fruits; durian, mangosteen, and rambutan pulps.

Methods

Edible pulps were extracted via maceration using methanol and water to simulate human consumption. These extracts were evaluated using a zebrafish larvae pentylenetetrazol (PTZ)-induced seizure model. Seizure pathology was further elucidated through quantitative gene expression analysis targeting neuroinflammation, neuronal activation, and GABA-glutamate homeostasis markers.

Results

The findings revealed that the durian methanol extracts exhibited pro-convulsant activity by exacerbating PTZ-induced hyperactivity and increasing neuroinflammatory and GABA-glutamate homeostasis marker levels. Conversely, durian water extracts showed anti-convulsant activity by reducing neuronal hyperactivation. Mangosteen methanol extracts also demonstrated anti-convulsant potential, likely linked to alpha-mangostin. Interestingly, rambutan water extracts suggested a potential neurotoxic effect following seizure insult, evidenced by the exacerbation of neuroinflammatory genes.

Conclusion

This study demonstrates that tropical fruits contain bioactive compounds that can either synergize with or antagonize seizure therapy. While durian and mangosteen showed promise for neuroprotection, the pro-convulsive and pro-inflammatory risks associated with certain durian and rambutan extracts necessitate caution. These results highlight the potential for utilizing phytochemicals as adjunctive therapy but underscore the need for more robust evidence and ASM-interaction studies before definitive dietary guidelines for PWE can be established. Nevertheless, this research has identified tropical Malaysian fruits that may work synergistically with current therapies or, conversely, pose antagonistic risks.

Ultimately, these findings offer a pathway toward personalized adjunctive nutritional therapy for PWE.

77

A G protein-coupled receptor kinase 4-Stat3-NHE3-axis controls kidney patterning and function

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

G protein-coupled receptor kinase 4 (GRK4) belongs to a family of serine-threonine kinases, which had originally been identified based on their ability to phosphorylate ligand-activated G protein-coupled receptors (GPCRs). In contrast to the other six GRKs, GRK4 remains underinvestigated as it was believed to be expressed in a limited number of tissues. Moreover, the GRK4 knockout mouse is viable so that GRK4 was considered less important than other GRKs. Interestingly, though, genetic variants of GRK4 associated with salt-sensitive hypertension have challenged the conclusion of GRK4 having only little impact on physiology. Therefore, our lab developed Grk4 loss-of-function models in zebrafish, which revealed that Grk4 prevents glomerular cyst development, facilitates normal cilium formation and ensures normal kidney function. Although we uncovered the underlying mechanism, we were unable to fully rescue the observed phenotypes. Therefore, we searched for additional signaling cascades affected by Grk4 loss-of-function.

To further investigate the role of GRK4 in kidney development and function we analyzed kidney patterning using in situ hybridization. The glomerular area, proximal straight tubule and distal early tubule show an increase in length at the expense of other pronephric segments. Additionally, we detected elevated expression of *nhe3a* almost along the whole pronephric tubule. Treatment with NHE3 inhibitors in Grk4 LOF zebrafish rescued the alterations in kidney patterning, rescued cyst formation and normalized pronephric cilium length. We identified abnormal STAT3 activation as the molecular cause for increased *nhe3a* transcription. Consistently, inhibition of STAT3 using a dominant negative version corrected the majority of phenotypes implying that Grk4 controls *nhe3a* expression and consequently kidney function through Stat3.

79

Estradiol modulates neuronal network hyperexcitability in select NDD risk genes

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Topic

3 Neurobiology

Abstract Content

Background: The biological basis for the male sex bias in autism spectrum disorder (ASD) is poorly understood. Differential exposure to sex hormones during neurodevelopment has been proposed as a potential modulator of risk. We hypothesize that early exposure to the principal biologically active estrogen, 17β-estradiol, confers a protective effect against mutations in ASD and neurodevelopmental disorder (NDD) genes.

Methods: We conduct a dual-system multi-modal screen of 36 functionally diverse, large-effect ASD/NDD genes in larval zebrafish and human induced pluripotent stem cell (hiPSC) models exposed to estradiol. We assess changes in visuomotor and arousal behavior in 31 zebrafish mutants of ASD/NDD genes and identify mutants susceptible to estradiol rescue. In addition, we use a pooled ECCITE-seq CRISPR screen to assess cell-type specific transcriptomic changes in hiPSC-derived glutamatergic (iGLUT) neurons and measure neuronal network activity in iGLUTs using arrayed calcium imaging across 15 mutants. Finally, we conduct deep phenotyping of estradiol effects in a single zebrafish mutant through bulk RNA sequencing, seizure assays, and whole-brain activity mapping.

Results: We uncover estradiol-dependent effects across genes and modalities, revealing convergent and divergent gene-by-estradiol interactions at the transcriptomic, circuit, and behavioral levels. Estradiol broadly ameliorates ASD/NDD gene expression patterns across all knockouts examined, selectively rescues behavioral phenotypes (*ASH1L*, *SCN2A) in zebrafish,* and dampens network hyperexcitability phenotypes in human neurons in a subset of ASD/NDD genes (*ASH1L*, *SCN2A, ANK2, CACNA1G, SHANK3)*. Two genes, *ASH1L* and *SCN2A*, show comprehensive estradiol rescue of differential gene expression and network bursting activity in human neurons as well as sleep-wake and visual-startle phenotypes in larval zebrafish mutants. Moreover, estradiol rescues seizures and regional increases in brain activity in *SCN2A* larval zebrafish mutants.

Conclusion: Altogether, we describe novel roles of estradiol in modulating network hyperexcitability in the context of loss of function mutations of select ASD/NDD genes.

80

Vps13b-deficiency disturbs retinal tissue homeostasis and differentiation in zebrafish

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The vacuolar protein sorting protein 13 b (VPS13B) belongs to the VPS13 proteins, a family of bulk-lipid-transfer proteins. Mutation of VPS13B leads to the autosomal recessive Cohen Syndrome. This rare disease manifests in a very complex clinical picture. Patients suffer from congenital neutropenia, microcephaly, and develop severe ophthalmic symptoms over time. The latter comprise myopia, coloboma, and retinal degeneration, which worsen with age and can severely impair patients' quality of life. VPS13B has recently been reported to function in Golgi-associated lipid transport and mitochondrial fission. Its precise function in the eye and how dysfunctional VPS13B causes the eye defects seen in Cohen Syndrome, remain unclear.

To investigate the molecular mechanisms possibly underlying the eye defects in Cohen Syndrome, we cloned fragments of zebrafish Vps13b and identified also the open reading frame. Using *in situ* hybridization, we found that *vps13b* is expressed in the developing eye from early on. We developed transient loss-of-function models of Vps13b deficiency and observed that Vps13b loss-of-function embryos developed in general smaller eyes and coloboma. Toluidine staining indicated a retinal lamination defect at 72 hours post fertilization (hpf) persisting until 120 hpf. We detected decreased proliferation and increased apoptosis in Vps13b-deficient embryos, which was accompanied by loss of several retinal markers such as *prox1a*, *isl2b*, and *vsx1*. In addition, loss of Vps13b prevents photoreceptor outer segment development, as seen using electron microscopy analyses and immunofluorescence.

In summary, we successfully developed loss-of-function zebrafish models for further analyses of the molecular mechanisms leading to compromised vision and retinal function in Cohen Syndrome. Our data suggests that Vps13b is not only required in the mature eye but affects eye development already early on.

82

Screening and Validation of Small-Molecule Chaperone Drugs for Mucopolysaccharidosis Using a Zebrafish Animal Model

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Topic

1 Disease Models

Abstract Content

Background:

Mucopolysaccharidosis Type I (MPS I) is a lysosomal storage disorder caused by mutations in the alpha-L-iduronidase (IDUA) gene, leading to systemic glycosaminoglycan (GAG) accumulation and severe CNS complications. To overcome the short half-life and poor blood-brain barrier (BBB) permeability of current enzyme replacement therapy (ERT), this study designed small-molecule pharmaceutical chaperones (PCs) through 3D structural analysis of IDUA active sites to enhance catalytic stability and therapeutic efficacy.

Methods:

We established an *in vivo* screening platform using zebrafish embryos. Initially, endogenous z-Idua activity was suppressed via z-*idua*-morpholino (MO) injection. At 48 hours post-fertilization (hpf), the clinical drug ALDURAZYME (human IDUA protein) was injected to establish an enzyme-rescue model. A series of novel PC candidates (ACK 600–622) were administered to evaluate synergy. Dynamic enzymatic activity was monitored from 72 to 120 hpf to assess the biological stability and longevity of each candidate.

Results:

Five candidates—ACK 601, 603, 604, 620, and 622—significantly boosted IDUA activity in synergy with ALDURAZYME at 72 hpf. Notably, ACK 620 exhibited the most superior stabilizing efficacy. Longitudinal tracking revealed that repeated dosing of ACK 620 sustained high-level enzymatic activity through 120 hpf.

Conclusion:

This confirms that structurally optimized PCs can significantly extend the therapeutic window of clinical enzymes, potentially reducing injection frequency and total dosage for patients. This study validates the zebrafish embryo as a robust platform for rapid PC screening. The discovery of ACK 620 provides critical pilot data for developing CNS-targeting therapies, opening new opportunities for precision medicine strategies in treating lysosomal storage diseases.

83

MeCP2 mutant zebrafish shows increases anxiety like behavior, a model for Rett syndrome.

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Topic

7 Neural Circuit Behavior

Abstract Content

Rett syndrome (RTT) is a rare neurodevelopmental disorder occurring in about 1 in 10000 female births, being one of the most common causes of intellectual disability in females (Gold W.A. 2024). The principal cause of RTT is the loss of function of *MECP2,* a gene located in the X chromosome encoding for methyl-CpG-binding protein 2 (MeCP2). Zebrafish are social animals, with many advantages as a model animal, including their high spawn rate, optical accessibility of the embryos and readily available genetic tools, and as a result have been used extensively to study neuropsychiatric disorders (Washbourne P. 2023) including RTT.

Using a wide range of behavioral tests on adult fishes, we found an increase in anxiety-like behaviors as well as reduced thigmotaxis while their social preference remained unaffected. Notably, we also found an increase in fear behaviors in response to the alarm substance, a reduction in the brain levels of certain metabolites, specifically epinephrine and norepinephrine, similarly to what was found in human studies. Using imaging analysis, we identified an increased in cell density, in particular of glutamatergic neurons in a region in the telencephalon, the Dm (dorsal medial part of the dorsal telencephalon) functionally equivalent to the mammalian basolateral amygdala. And we confirmed that this hypertrophic region is involved in the elevated fear stimulus response in the mutants, showing a clear link between the mutants affected behavior and a region of the brain whose anatomy was altered by the loss of function of MeCP2.

These results consolidate zebrafish as an ideal animal model to study RTT as well as other neurophysiological disorders and an invaluable tool to decipher the mechanisms that control emotions and behaviors.

84

The Antioxidant Paradox: Compartment Specific ROS modulation by Well Characterized Compounds in the Early Zebrafish Embryo

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Topic

9 Germline and Early Development

Abstract Content

Reactive oxygen species (ROS) have transitioned from being viewed as damaging agents to recognized as signalling molecules that regulate cell behaviour. In zebrafish, we showed that ROS are critical for cytoskeleton polymerization, protein localization, and cell migration during early development. While NADPH oxidase (Nox) inhibitors and antioxidants are widely used to deplete ROS, our observations revealed that the inhibitor **VAS2870 paradoxically increases ROS** in the Enveloping Layer (EVL) cells, while it decreases extracellular ROS. These findings led us to systematically characterize widely used antioxidants within the compartmentalized environment of the early embryo.

We performed a comparative analysis of Nox inhibitors (VAS2870, Apocynin, DPI) and antioxidants (EUK-134, TEMPOL, N-acetyl cysteine [NAC]). Treatments began prior to epiboly onset. We established survival curves to identify sublethal concentrations and assessed phenotypes at 24 hpf. ROS dynamics were monitored using **CM-H2DCFDA (DCF)** for extracellular/general ROS and the ratiometric sensor **HyPer7** for H_2O_2 fluctuations in the EVL and Deep Cell Layer (DCL).

Most compounds decreased pigmentation by 24 hpf. VAS2870, Apocynin, EUK-134, and TEMPOL significantly delayed epiboly, whereas DPI and NAC had no effect. Strikingly, ROS sensors revealed highly compartmentalized responses: while VAS2870 and EUK-134 decreased extracellular DCF oxidation, they increased the signal within the EVL cells. Similarly, NAC unexpectedly increased DCF oxidation in both compartments, while Apocynin increased signal only extracellularly. HyPer7 imaging corroborated these discrepancies; while Apocynin and DPI decreased H_2O_2 levels, VAS2870 treatment caused a surprising increase in HyPer7 oxidation in both cell layers.

Well-characterized redox modulators can exert divergent, paradoxical effects depending on the embryonic compartment and the ROS species measured. These data serve as a cautionary note: pharmacological effects must be empirically validated within the specific spatiotemporal context of the developing embryo to avoid misinterpreting redox signalling dynamics.

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86

From muscle architecture to locomotor behavior: a zebrafish model reflecting patient-like symptoms of McArdle disease

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Topic

1 Disease Models

Abstract Content

Glycogen phoshorylase (PYGM) is a key enzyme in the first step of glycogenolysis. It is responsible for converting glycogen into glucose, the primary energy source for skeletal muscle activity. Mutations in this gene lead to McArdle's disease. Patients affected by this condition are unable to engage in excessive physical exertion due to the lack of energy.

To investigate structural and functional consequences of glycogen phosphorylase deficiency, we generated a zebrafish (*Danio rerio*) line with CRISPR/Cas9-mediated knock-out of the *pygm* gene (*pygm-/+*). We hypothesize that zebrafish can closely mimics human McArdle disease.

Detailed morphological analyses revealed abnormalities in skeletal muscle organization in *pygm-/+* individuals, including disturbed myofibrillar alignment, altered muscle fiber integrity, and changes in overall body posture. These structural alterations were accompanied by significant biochemical changes in glycogen storage and behavioural impairments. The observed phenotype closely reflects key clinical features of McArdle disease, particularly exercise intolerance resulting from compromised muscle performance. Our findings demonstrate that *pygm*-deficient zebrafish provide a robust platform for studying the structural basis of muscle dysfunction and its functional consequences in vivo.

This model offers a valuable opportunity to further dissect disease mechanisms and evaluate potential therapeutic interventions targeting muscle performance and metabolic adaptation.

87

Breathing Beyond Water: Development of the Labyrinth Organ in Paradise Fish

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Air breathing evolved multiple times independently in teleost fish as an adaptation to aquatic hypoxia. The paradise fish (*Macropodus opercularis*) possesses a specialized air-breathing structure, the labyrinth organ, which arises as an epibranchial modification of the first gill-bearing arch and enables aerial respiration after metamorphosis.

Although the morphology of the labyrinth organ has been described, its developmental origin and regulatory basis remain unknown. This gap limits our understanding of how evolutionary innovations emerge from conserved branchial arch structures.

To address this, we performed detailed anatomical analyses from larval to juvenile stages to characterize the remodeling of the first epibranchial during labyrinth organ formation. In parallel, we generated single-cell RNA sequencing and 10X multiome datasets from adult gill and labyrinth organs to define their cellular composition and regulatory landscape. These data reveal distinct cell populations and candidate gene regulatory programs associated with labyrinth organ identity and maintenance. Comparative observations further suggest that the epibranchial region may represent an evolutionary hotspot for functional diversification across teleosts.

Building on these findings, we are initiating functional validation of candidate regulators using transgenic approaches in zebrafish. I will present our anatomical framework, adult single-cell atlas, and emerging regulatory hypotheses.

Together, this work establishes paradise fish as a complementary system for studying the evolution of aerial breathing and organ-level innovation, while leveraging zebrafish genetics to interrogate underlying mechanisms.

89

Fishy traits: decoding paradise fish behaviour using deep learning algoritms

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Ethology, the study of animal behaviour, seeks to understand the minds of animals. With recent advancements in technology, particularly in the field of machine learning (ML), researchers now have powerful new tools at their disposal. AI-based video analysis allows for the automated and non-invasive collection of behavioural data without the need for physical markers. This approach offers an alternative to traditional methodologies, enabling the analysis of larger datasets with minimised human bias.

In this project, open-source AI algorithms were used to study paradise fish (*Macropodus opercularis*), a species known for its genetically encoded complex behaviours. Algorithms can be trained to do two main tasks: (1) describe what the fish was doing (classification) (2) track its body movements over time (quantification).

As a proof-of-concept study, we chose monitoring air-breathing, a characteristic behavior of this species. We performed the supervised training of multiple algorithms (DeepLabCut, SLEAP and Labgym) on videos acquired from naturally behaving fish. The analysis of the resulting datasets suggests that the algorithms can readily detect air breathing, and this behavior is accompanied by a consistent arching of the body, likely due to anatomical constrains.

Our findings highlight the potential of AI-driven approaches to uncover subtle patterns in animal behaviour that would often remain undetected following the traditional methodologies. Due to the method described being adaptable -- from early behavioural assessment of larvae to in-depth behaviour comparison of mutant lines with wild-type individuals -- it could potentially allow us to investigate the underlying genetic components of behaviour.

92

In toto monitoring of slow myofiber dynamics during post-embryonic growth in zebrafish

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Topic

9 Germline and Early Development

Abstract Content

Skeletal muscle growth after embryogenesis remains poorly understood, particularly for slow-twitch myofibers. Here, we generated transgenic zebrafish lines *Tg(smyhc1: H2A-EGFP, smyhc1: mApple-CAAX)* enabling in toto visualization of every single slow myofibers. Our findings reveal that during the post-embryonic stages, the number of slow myofibers did not increase significantly; however, their volume expanded markedly, suggesting that slow myofibers primarily undergo hypertrophic growth. We observed that double and triple nucleated myofibers appeared simultaneously. Over time, the mononucleated myofibers gradually transitioned into binucleated myofibers, with the latter becoming predominant as development progressed. Moreover, while fast myofibers in zebrafish are completely replaced during development, we investigated whether a similar phenomenon occurs in slow myofibers. To track individual slow myofibers over time, we developed a multicolor labeling system *Tg(smyhc1:brainbow; ubi:palmbow; smyhc1:Tet3G-Cre-ERT2)* that allows long-term tracking of myofiber lineage. Using tissue clearing techniques, we were able to observe slow myofibers continuously from early development through to adulthood. Together, these tools provide a powerful platform for monitoring slow muscle tissue development from the post-embryonic to adult stages, offering new insights into the growth and differentiation of slow myofibers.

94

Polr1c-Mediated Modulation of Ribosome Biogenesis Is Essential for Retinal Regeneration in Zebrafish

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Topic

3 Neurobiology

Abstract Content

Background: Ribosome biogenesis is a fundamental process required for cell growth and proliferation. Transcription of ribosomal RNAs) by RNA polymerases I and III represents a key step in ribosome production, and mutations in their subunits, such as *POLR1C*, cause Treacher Collins syndrome. Despite its importance in development, the role of ribosome biogenesis in regenerative processes, including retinal regeneration in zebrafish, remains unexplored.

Methods: The localization and distribution of *polr1c* in the zebrafish retina were examined using immunohistochemistry. To evaluate the regenerative response in polr1c-deficient fish, adult zebrafish were intravitreally injected with *polr1c* vivo morpholino (VMO), followed by retinal injury induced by mechanical needle puncture. The regenerative response was assessed at 4 days post-injury (dpi).

Results: polr1c was localized in the inner and outer segments of photoreceptors, as well as in the corneal and ciliary epithelium. Double immunostaining further confirmed its co-localization with ZPR1, ZPR3, and ZS-4, indicating that polr1c is expressed in both cone and rod photoreceptors. In polr1c-deficient retinas, the expression of polr1c in photoreceptors was markedly reduced, and ribosome biogenesis, as indicated by Y10b staining, was suppressed. Following retinal injury, the expression of glutamine synthetase (GS) and brain aromatase (cyp19a1b), markers of Müller glia (MG), as well as polr1c and Y10b, was increased at 4 days post-injury (dpi). In contrast, these responses were markedly reduced in polr1c-deficient retinas. Notably, co-treatment of polr1c-deficient fish with estradiol restored injury-induced apoptosis ($TUNEL^+$ cells) and Müller glia–associated proliferative responses compared with polr1c-deficient fish alone. However, this restoration was abolished when estradiol was co-incubated with fulvestrant, an estrogen receptor (ER) antagonist.

Conclusion: polr1c-mediated ribosome biogenesis regulates local estrogen production in the retina through modulation of brain aromatase during retinal regeneration.

97

In Toto Imaging to Study Basal Epithelial Cell Behavior in Zebrafish Skin

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Topic

14 Stem Cells and Regeneration

Abstract Content

During post-embryonic growth, zebrafish skin undergoes significant expansion, requiring massive proliferation and differentiation of the stem cell population. In the zebrafish epidermis, this growth is driven by basal epithelial cells (BECs), which continuously proliferate or differentiate to replenish non-basal skin cells in the epidermis. Recent lineage-tracing studies have demonstrated that zebrafish BECs exhibit clonal dominance during skin growth, suggesting that BECs are a heterogeneous population. However, due to technical challenges in tracking each and every BEC in a growing individual, their degree of heterogeneity, lifespan, and spatiotemporal expansion dynamics remain poorly understood. Here, by establishing stable transgenic lines using the *itgb4* promoter, I determined that the promoter is an ideal promoter for ubiquitously visualizing all ~500,000 basal cells in a living adult zebrafish. Building on this, I am establishing a panel of *itgb4*-driven, *Brainbow*-based reporter lines and live imaging platforms that will enable in toto, long-term tracking of every single BEC across the entire zebrafish epidermis at single-cell resolution. I expect these tools and approaches to determine collective BEC lineage dynamics tailored to distinct skin growth conditions, including post-embryonic growth, adult tissue homeostasis, and regeneration, and to facilitate the identification of key regulatory mechanisms governing BEC lineage behavior. Altogether, these findings may reveal basic principles explaining how individual stem cells collectively respond to varying growth stresses, thereby providing a conceptual framework for understanding and instructing stem cell populations in diseased conditions.

98

Ca2+ influx through muscle-type nicotinic acetylcholine receptors contributes to contractions and development of slow muscle cells in early zebrafish development

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Topic

3 Neurobiology

Abstract Content

Although the mechanisms of muscle contraction have long been studied, the differences in the contraction process between fast and slow muscles are not yet fully understood. Recent studies in zebrafish have revealed that the subunit composition of nicotinic acetylcholine receptors (AChRs) at the neuromuscular junction differs between slow and fast muscles. However, the physiological functions of slow muscle-type AChRs remain unclear. In the present study, we analyzed the channel properties of slow muscle-type AChRs using the two-electrode voltage clamp method using *Xenopus* oocytes. We found that the slow muscle-type AChRs showed much higher Ca^{2+} permeability than fast muscle-type ones. To clarify the physiological roles of Ca^{2+} influx through the slow muscle-type AChRs, we generated a transgenic (Tg) zebrafish line expressing Ca^{2+}-impermeable AChRs specifically in slow muscles. Loss of Ca^{2+} permeability markedly decreased locomotor activity at 1-3 days post fertilization (dpf). Interestingly, this impairment improved during development, and 5dpf Tg showed locomotor activities comparable to those expressing wild-type (WT) AChRs. Ca^{2+} imaging of the Tg line suggested that Ca^{2+} influx via AChRs is crucial for sustained Ca^{2+} responses during muscle contraction in 2 dpf larvae, as the slow muscle cells in the Tg line showed shortened Ca^{2+} responses. Furthermore, the slow muscle fibers of the Tg line were thinner than those expressing Ca^{2+}-permeable AChRs. These results suggest that Ca^{2+} influx through AChRs contributes to sustained Ca^{2+} release from the sarcoplasmic reticulum and plays an important role in the contraction and development of slow muscles during early developmental stages.

101

Bridging Drug Exposure and Efficacy: LC-MS/MS Based Measurement of Doxorubicin Uptake in Zebrafish Breast Cancer Xenografts

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Topic

1 Disease Models

Abstract Content

Doxorubicin is a widely used chemotherapy drug for the treatment of many cancers, including breast cancer. Studies using zebrafish xenograft models have shown that doxorubicin reduces tumour growth. However, since doxorubicin is usually added directly to the fish water, the exact amount of drug absorbed by the zebrafish larvae remains unclear.

In this study, we developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure how much doxorubicin is taken up by zebrafish larvae. This allowed us to estimate the effective drug exposure needed to reduce tumour growth in triple-negative breast cancer (MDA-MB-231) zebrafish xenografts. First, sublethal doxorubicin concentrations were determined by evaluating embryo survival and developmental abnormalities. Zebrafish larvae implanted with MDA-MB-231 cells were then treated with the optimized sublethal concentration, and therapeutic efficacy was evaluated by monitoring reduction in tumour fluorescence over three days. Drug accumulation in the larvae was quantified using our optimized LC-MS/MS protocol.

LC-MS/MS method was optimised and validated as per the ICH Q2 (R2) guidelines. The method showed a limit of detection and limit of quantification for doxorubicin of 2 µg/L and 5 µg/L respectively, with accuracy ranging from 82% to 114%, meeting international validation standards. Interestingly, although the amount of drug up taken by the larvae was relatively low (2.06 ± 1.01 ng/larva), a clear reduction in tumour fluorescence was observed after three days of treatment, indicating that doxorubicin remained effective even at low levels of uptake.

This study provides a sensitive method to quantify doxorubicin in zebrafish larvae and helps to link actual drug exposure with therapeutic response in zebrafish xenograft cancer models . This approach may help improve dose–response studies and interpretation of drug efficacy in zebrafish-based cancer research.

102

Molecular delineation of a retina-dependent photoneuroendocrine pathway controlling background adaptation in zebrafish

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Topic

7 Neural Circuit Behavior

Abstract Content

Many vertebrates, including fish, amphibians, and reptiles, dynamically adjust their body color to the perceived brightness of the ambient background. This response takes tens of minutes and involves the aggregation or dispersion of pigment granules within the melanophores of the skin, resulting in the pale or dark appearance of the animal, respectively. In teleosts, ambient light detection depends on the retina, which transmits the signal to the hypothalamus, leading to the secretion of peptide hormones via the pituitary gland into the bloodstream. Melanin-concentrating hormone (MCH) is released upon light stimulation, resulting in the blanching of the skin. Alpha-melanocyte-stimulating hormone (α-MSH, encoded by the pomca gene and produced in the hypothalamus and pituitary) has the opposite effect: light inhibits its secretion, leading to melanin dispersion in the skin. Because "dark appearance" is an easy-to-score phenotype in larval zebrafish, defective visual background adaptation (VBA) has been used in genetic screens as a proxy for retinal defects, but the responsible neuroendocrine circuit remained elusive. Here, we identified the molecular and cellular components underlying this response. We found that intrinsically photosensitive retinal ganglion cells (ipRGCs), expressing the homeobox transcription factor Onecut1, project to the neuropil region of the preoptic area, where their axons overlap with the dendrites of pmchl-expressing hypothalamic neurons. Signals from these ipRGCs increase the transcription of pmchl, one of two genes encoding MCH isoforms, and repress pomca. Ablation of either onecut1-positive ipRGCs or pmchl-expressing hypothalamic neurons prevents the fish larva from adapting to a bright background.

104

Differential Neurotoxic Mechanisms of Anxiety-Like Behaviour Following Acute Exposure to Organophosphate and Organochlorine Contaminants in Zebrafish

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Topic

10 Toxicology

Abstract Content

Contaminants of Emerging Concern (CECs) pose increasing risks to environmental and human health due to their neurotoxic potential. Among them, the organophosphate Roundup and the organochlorine triclosan are widely used chemicals frequently detected in aquatic environments. However, the mechanisms through which chemically distinct contaminants induce similar anxiety-like behaviour remain poorly understood.

Adult zebrafish of mixed gender were exposed to triclosan (0.6 mg/L) and Roundup (12.8 mg/L) for 6 h. Anxiety-like behaviour was assessed using our in-house behavioural tracking system ZebraTrack. The underlying molecular mechanisms were investigated through gene expression analysis and enzymatic assays including ELISA.

Acute exposure (6 h) to both triclosan and Roundup induced significant anxiety-like behaviour in adult zebrafish, as indicated by reduced distance travelled (Untreated: 29.2 ± 7.9 m; Treated: triclosan: 20.1 ± 5.2 m, Roundup: 14.5 ± 7.9 m; n = 21-32; p = < 0.0001). This behavioural phenotype was accompanied by increased freezing duration and freezing bouts. Despite producing similar behavioural outcomes, the two contaminants acted through distinct neurochemical pathways.

Roundup exposure resulted in significant inhibition of brain acetylcholinesterase (AChE) activity (Treated: 32 % reduction; n = 15-16; p = < 0.0001) without detectable changes in reactive oxygen species (ROS) levels, indicating disruption of cholinergic neurotransmission as a primary mechanism underlying anxiety-like behaviour.

In contrast, triclosan exposure did not alter AChE activity, nor did it affect expression of brain-derived neurotrophic factor (BDNF), corticotropin-releasing hormone (CRH), its receptor CRHR1, or ROS levels after 6 h exposure. Instead, triclosan significantly reduced serotonin levels, indicating a serotonergic mechanism underlying the behavioural phenotype.

These findings show that similar behavioural phenotypes arise through distinct neurochemical mechanisms, with Roundup disrupting cholinergic signalling and triclosan acting through serotonergic pathways independent of oxidative stress. This distinction may aid identification of pathway-specific biomarkers for assessing contaminant-induced neurobehavioral toxicity.

105

Creating zebrafish models to study defects in nucleophagy repair *in vivo*

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Topic

18 Genomic Resources

Abstract Content

DNA-protein crosslinks (DPCs) interfere with essential DNA transactions, including replication, transcription, and chromatin remodelling, thereby promoting genomic instability. Persistent DPCs are resolved through multiple repair mechanisms, including replication-coupled and transcription-coupled pathways. Recently, new repair pathway termed nucleophagy has been described, in which crosslinked proteins are excised from DNA and targeted for lysosomal degradation. This pathway is centred on the TEX264 protein. TEX264 functions as a molecular adaptor that links the AAA+ ATPase VCP/p97 to DPCs and promotes their resolution via the SPRTN protease or lysosomal degradation. Despite these advances, the organismal role of TEX264-dependent nucleophagy remains poorly understood.

To address this question, we use zebrafish as an *in vivo* model. Morpholino-mediated silencing of the zebrafish *tex264a* orthologue in embryos results in increased accumulation of TOP1 cleavage complexes (TOP1ccs), suggesting that nucleophagy-mediated repair is conserved in vertebrates. To further investigate the function of TEX264 at the organismal level, we generated *tex264a* crispants carrying a premature stop codon early in the coding sequence. TEX264-ko zebrafish strains are under development and will be characterised for developmental phenotypes and DNA damage signatures, including DPC accumulation, DNA breaks and hepatocellular carcinoma. To investigate whether TEX264 has a role in HCC, the primary cancer associated with defective DPC repair, we will examine the role of *tex264a* in liver tumorigenesis using a zebrafish model in which HCC is induced by $kras^{G12V}$ overexpression. This approach will allow us to determine how *tex264a* loss affects tumour initiation, progression, and metastasis during embryonic development and adulthood. In addition, combinatorial use of *tex264a* morphants in the DUAL FUCCI background will allow us to assess how nucleophagy deficiency impacts cell-cycle progression and tissue development *in vivo*.

Collectively, our work establishes the first *in vivo* model of nucleophagy-mediated DPC repair deficiency and will provide insights into its role in organismal development and carcinogenesis.

107

Presence of sialic acid terminal sugar throughout early development of zebrafish (Danio rerio) embryos

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Topic

5 Tissue Formation

Abstract Content

Background: The zebrafish has recently been shown to be an extremely favorable model on which even rare diseases such as Lysosomal Storage Disorders, LSDs can be successfully investigated. However, infantile sialic storage disease, ISSD is not among them with lysosomal transporter, Sialin missing. Genes of enzymes necessary in synthesis and terminal glycosylation with sialic acid sugar residues are proven to be present in zebrafish. Plan to delete Sialin, transporter of sialic acid, belonging the Slc family (SoLute Carrier family 17 member A5, Slc17a5) will be performed using the Crispr/Cas9 method that would lead to model of ISSD. Currently, aim is to established presence of target sugar in wild type zebrafish embryos for future monitoring of developmental changes throughout the early life of $Slc17a5^{-/-}$ knockout.

Methods: Terminal glycosylation was followed by using probes both for sialic acid specific *Sambucus Nigra* lectin, SNA labeled with a fluorescent dye CY3, SNA-CY3 parallel with phalloidin-FITC dye for actin staining throughout developmental stages 1-5dpf of zebrafish embryos.

Results: Presence of sialic acid was found in each dpf of zebrafish embryo in extracellular matrix of developing tissues. Interesting finding was intense sialic acid staining in apical parts of mucous glands in skin that dominated through images under fluorescent microscope.

Conclusion: Zebrafish has present sialic acid possible as terminal sugar in various structures of developing embryos that could be followed in planed $Slc17a5^{-/-}$ knockout zebrafish.

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108

From darkness to vision: models of laser-induced retinal injury to study regeneration

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Topic

1 Disease Models

Abstract Content

Background: Retinal degenerative diseases are multifactorial diseases with different etiological aspects but common degeneration pattern. Progressive cell death results in irreversible vision loss due to the formation of a glial scar in mammals. Zebrafish, on the other hand, possess the ability to regenerate functional retinal tissue. Therefore, we intend to identify key genes and regulatory pathways that could be modulated to induce regeneration in mammals.

Methods: Two models of laser-induced retinal degeneration (zebrafish and mouse) using a 532 nm laser system (Micron 5, Phoenix) were setup. Thereby, progression and kinetics of scar formation and its resolution were followed using optical coherence tomography (OCT) imaging. Additionally, histological and immunohistological analyses were performed at baseline as well as days 0, 3, 7, 14, 21, 35 in mouse and days 0, 1, 7, 10, 14, 17, 23 and 28 in zebrafish.

Results: The system was effective to generate mouse and zebrafish models of focal retinal degeneration. Laser power and pulse duration were optimized to 70 mW for mice and 50 mW for zebrafish, both with a 100 ms pulse. This induced replicable damages without altering the Bruch's membrane integrity recapitulating dry age-related macular degeneration. OCT and fundus imaging showed the development of damage over time. Regeneration of retinal structures was thereby observed in the zebrafish at 28 dpi. Changes in the different retinal layers in H&E stainings show tissue degeneration in the outer nuclear layer (ONL) within hours of observation. Expression of GFAP and IBA1 proteins indicate Müller cell activation as well as microglia migration to the injury site early upon injury.

Conclusion: These results present two comparable models of retinal degeneration that allow to study the differences in the molecular pathways activated upon damage induction in species with or without regenerative capacity.

110

A Zebrafish Model To Study Prolonged Exposure To Aminoglycoside Antibiotics

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Topic

10 Toxicology

Abstract Content

Hair cells are sensitive to chemicals such as cisplatin and aminoglycoside antibiotics, used to treat cancers and life-threatening infections, causing hearing loss and/or vestibular dysfunction as secondary effect. Hair cell loss is irreversible in most mammals. In rodents, we have shown that chronic systemic exposure to a nitrile or streptomycin initially cause damage to vestibular hair cells that is reversible and this is associated with changes at the transcriptomic level.

Zebrafish larvae are a good model to study hair cells due to their external localization in the neuromasts of the lateral line. Cisplatin and aminoglycoside antibiotics damage zebrafish larvae hair cells after acute exposures. Fish neuromasts contain support cells able to regenerate the lost hair cells already 24 hours post-exposure. Most studies have focused on acute exposures, finding that hair cells loss results from hair cell death by mitochondrial dysfunction and oxidative stress.

We aimed to develop a model to study long exposures to ototoxins using zebrafish larvae before the regenerative response is effective. To this end, we exposed 5dpf zebrafish larvae to low concentrations of four different aminoglycosides and, after 6h and 24h, we assessed qualitatively eight neuromasts of the anterior lateral line. All of the aminoglycoside antibiotics used caused a clear dose-response loss of hair cells. Then, we have confirmed these results by counting the number of hair cells after treatment using the transgenic line *myo6b:nlsEos* that specifically labels hair cell nuclei. The Eos protein is photoconvertible, changing from green to red fluorescence after UV irradiation. We have used this property to identify which hair cells have survived the whole exposure period in order to isolate them by FACS. We will use this approach to study gene expression changes in these surviving hair cells.

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114

Zebrafish Embedding Mold (ZEM) for standardized optical imaging of zebrafish embryos and larvae (Danio rerio)

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Topic

2 Emerging Technologies

Abstract Content

Biological imaging plays a critical role in understanding living systems by revealing key biological processes. Among model organisms, zebrafish are particularly valuable due to their transparency, which allows direct visualization of internal structures and physiological responses. Despite these advantages, achieving consistent specimen orientation across multiple individuals during imaging remains a persistent challenge, demanding substantial time and manual effort. To address this, we developed the Zebrafish Embedding Mold (ZEM), a standardized agarose-based fixation system designed for high-quality, reproducible zebrafish imaging. The embryo mold contains 36 half-sphere chambers, each holding a single embryo, supporting observation through 48 hpf. The larval mold accommodates specimens from 3–7 dpf in both lateral and dorsal views, enabling consistent tracking of developmental and phenotypic changes. We validated the tool by exposing zebrafish to 50 nm polystyrene nanoplastics across multiple concentrations. Clear fluorescence signals were detected in both embryos and larvae, confirming the system's suitability for high-throughput substance screening. Beyond nanoplastics, ZEM is readily applicable to studies involving chemical compounds, heavy metals, and microplastics, supporting both pharmacological and toxicological investigations under well-controlled conditions. The standardized mold design also facilitates continuous, high-throughput experimental workflows, reducing variability between experiments. Collectively, ZEM improves imaging accuracy, efficiency, and reproducibility, establishing it as a practical and versatile tool for zebrafish-based biomedical and environmental toxicology research.

115

Sod3a as an Extracellular Antioxidant Defense Against Polystyrene Nanoplastic Toxicity in Zebrafish

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Topic

10 Toxicology

Abstract Content

Nanoplastics have been recognized as emerging environmental contaminants with potential adverse effects on human health. These particles have been detected in a wide range of human tissues, where their nanoscale dimensions facilitate both cellular uptake and tissue accumulation. Exposure to nanoplastics has been shown to induce oxidative stress; however, the specific contribution of extracellular accumulation to overall toxicity remains poorly understood. In this study, we investigated the role of extracellular antioxidant defense mechanisms in polystyrene nanoplastic (PSNP; 50 nm)-induced oxidative stress using zebrafish (*Danio rerio*), a well-established transparent vertebrate model widely employed in toxicological research. We focused specifically on superoxide dismutase 3 (SOD3), an extracellular enzyme that catalyzes the dismutation of superoxide radicals and thereby regulates reactive oxygen species (ROS) in the extracellular environment. Sod3a in zebrafish is an ortholog of human SOD3 and retains conserved metal-binding residues essential for catalytic activity. To examine the functional significance of Sod3a in nanoplastic-induced toxicity, we generated *sod3a*-deficient zebrafish and assessed their biological responses following PSNP exposure. Compared with wild-type (WT) controls, *sod3a*-deficient larvae exhibited significantly greater tissue accumulation of PSNPs, which was associated with elevated oxidative stress, increased apoptotic cell death, and impaired intestinal function and immune responses. Collectively, these findings demonstrate a critical role for Sod3a in modulating PSNP-induced oxidative stress and provide novel insights into extracellular antioxidant mechanisms underlying nanoplastic-induced toxicity.

116

In toto single-cell-resolution monitoring of whole-liver growth dynamics in developing and adult zebrafish

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Background

Vertebrate organs undergo drastic increases in overall size and cell number during the post-embryonic growth period. However, due to technical challenges in monitoring the entire growing organs at sufficient cell resolution, it remains little understood how individual cells behave to make adult organs with their distinct size and shape.

Methods

Here, we developed WAO-ExM (Whole Adult-Organ Expansion Microscopy), which combined tissue clearing, expansion, lightsheet illumination, and macro photography to visualize each and every single hepatocyte in developing and adult zebrafish.

Results

Using transgenic lines that fluorescently label hepatocyte nuclei, we show that WAO-ExM markedly improve the z-axis resolution of nuclear signals, allowing unambiguous visualization of every hepatocyte throughout the entire 0.9 cm thick expanded adult zebrafish liver. We further determined that hepatocyte number increases by 538-fold during post-embryonic growth, reaching a total of 1,265,206 cells in the adult. By integrating this method with a brainbow-based cell barcoding system, we captured the emergence of dominant hepatocyte clones and determined their cell numbers. Through temporal inactivation of extracellular matrix laminins, we further demonstrated that liver shaping process and hepatocyte number growth are not intrinsically coupled. This approach is generally applicable to adult zebrafish organs, including the heart and pancreas.

Conclusion

Altogether, we establish a streamlined pipeline for sample processing, centimeter-scale volumetric imaging, and quantitative analysis that readily enable adult vertebrate organ morphogenesis to be resolved at bona fide single-cell resolution.

119

Morphodynamics of germinal niches in the medaka ovary: from larva to adult

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Topic

9 Germline and Early Development

Abstract Content

In teleost fish, lifelong fertility depends on continuous oocyte production sustained by germinal niches located at the ovarian surface. These niches maintain and renew the oogonial pool throughout adulthood. While their cellular composition and organization have been described in mature ovaries, it remains unclear how they emerge and become organized during post-embryonic development.

We investigated germinal niche morphogenesis in the medaka (*Oryzias latipes*) from hatching to adulthood, using whole-organ three-dimensional imaging combined with immunolabelling for Sox9 (somatic supporting cells), Vasa (germ cells), and nuclear staining with methyl green. Ovaries were imaged by confocal or light-sheet microscopy to reconstruct ovarian architecture at successive developmental stages.

At hatching (10 days post-fertilization, dpf), the ovary displayed a transient bilobed organization that rapidly resolved into a single lobe. The early ovary consisted of a thin, dense and continuous surface epithelium enriched in germ cells surrounded by Sox9-positive somatic cells. During juvenile development, this epithelium progressively reorganized: elongated Sox9-positive germinal cords emerged and subsequently segmented to generate individualized germinal niches at the adult ovarian surface. Niche individualization was first detected between 50 and 60 dpf, concomitant with ovarian expansion. This transition correlated with increased follicle number and size, particularly with the emergence of stage III follicles. These observations indicate that niche formation is tightly associated with stromal remodeling driven by follicular growth. Furthermore, a gradual diversification of niche composition in oogonia and newly formed follicles, represented by diplotene-stage oocytes (Gdip), was quantified throughout development, indicating increasing structural and functional heterogeneity of niches over time.

Altogether, our findings reveal that germinal niches are not pre-existing structures but arise through a coordinated morphogenetic program coupled to follicular expansion. This study provides a developmental framework for understanding how ovarian organization supports continuous oocyte renewal in teleost fish.

120

Chaperoning Wnt on Cytonemes: A Relay Mechanism for Long-Range Patterning in the Zebrafish Embryo

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Topic

5 Tissue Formation

Abstract Content

Wnt signalling is a central mechanism driving vertebrate embryonic patterning, yet how hydrophobic Wnt ligands are delivered with precise spatial control remains a major unresolved question. Increasing evidence points to thin, cellular threads called cytonemes, signalling filopodia that directly transfer Wnt components from a producing cell to a recipient. Here, we investigate how hydrophobic Wnt ligands are loaded, protected, transported, and handed over along cytonemes during zebrafish development.

Using high-resolution live imaging techniques, PAC2 zebrafish fibroblasts and in vivo imaging in zebrafish embryos, we uncover a sequential chaperone relay for Wnt transport. Our data indicate that the intracellular chaperone Wntless (WLS/Evi/Sprinter) promotes the formation and loading of Wnt-positive cytonemes in the producer cells. At the cytoneme tip, WLS presents and hands over Wnt to the extracellular chaperone, notably sFRP2, which shields Wnt from extracellular degradation and facilitates receptor engagement at the plasma membrane of the receiving cell.

Unexpectedly, the interplay between WLS and sFRP2 does more than protect Wnt during its journey across tissues: WLS actively enhances cytoneme formation in the producer cell, whereas sFRP2 enhances cytoneme length in receiving cells, effectively doubling cytoneme reach and enabling highly specific signalling through cytoneme-cytoneme contacts. This bidirectional extension creates a precise and robust mechanism for long-range Wnt delivery across larger tissues.

Together, our findings reveal a coordinated intracellular–extracellular chaperone system that controls Wnt dissemination via cytonemes and directly impacts anteroposterior patterning in the vertebrate embryo via precise and long-range morphogen dissemination.

121

Jagged2-mediated Notch signaling requires Mib and Pex1

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Topic

3 Neurobiology

Abstract Content

Maintenance of neural progenitors requires Notch signaling in vertebrate development. A previous study has shown that Jagged2-mediated Notch signaling maintains proliferating neural progenitors in the ventral spinal cord. However, components for Jagged-mediated signaling remain poorly defined during late neurogenesis. Here we investigate how Mib and Pex1 are involved in the regulation of Jagged2-mediated long-range Notch signaling. Co-IP assay revealed that Mib physically interacts with Jagged2, but not with the intracellular domain itself. Cell transplantation experiments showed the formation of extracellular vesicles containing Jagged2 was promoted by the co-expression of Mib:EGFP. We also performed yeast-two hybrid screening by using the intracellular domain of zebrafish Jagged2, and investigated a possible role of PEX1 as a component of Notch signaling for the differentiation of neural precursors in p3 domain. Western blotting shows that zebrafish PEX1 might interacts with the intracellular domain of zebrafish Jagged2 physically. PEX1 MO-injected embryos showed an increased number of GABAergic KA” neurons as well as the ectopic expression of secondary motor neurons in the p3 domain. The increased number of KA” neurons was also observed in the zebrafish embryos with PEX1 mutation induced by CRISPR/Cas9. These phenotypes resemble those of Jagged2 morphant. Our observations imply that a critical role of Mib and PEX1 in the cell-fate decision of proliferating neural precursors in the p3 domain during the continuing growth and development of the vertebrate nervous system.

122

A common organizational feature at electrical and chemical synapses

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Topic

3 Neurobiology

Abstract Content

Background: The molecular organization of gap junction (GJ)-mediated electrical synapses is perceived to be simpler than that of chemical synapses. Because of their experimental accessibility, auditory synapses on the teleost Mauthner cells are considered a valuable model to investigate the properties and molecular organization of electrical synapses in vertebrates. Transmission at these synapses is mediated by multiple GJs at which channels formed by connexin proteins (Cxs) are under the control of a complex molecular scaffold. The zebrafish genome contains four homologs of the neuronal Cx36: Cx35.5 (*gjd2a*) and Cx35.1 (*gjd2b*), and Cx34.1 (*gjd1a*) and Cx34.7 (*gjd1b*). Cx mutations suggested that GJs are formed by heterotypic GJ channels, made of a presynaptic Cx35.5 hemichannel and postsynaptic Cx34.1 hemichannel.

Methods: We generated transgenic fish at which Cxs were tagged with fluorescent proteins and studied them combining electrophysiology, immunolabeling and expansion microscopy.

Results: and found that, in addition to Cx35.5 and Cx34.1, Cx34.7 is present in combination with Cx34.1 at some but not all postsynaptic GJs within these terminals. Given that Cx34.7 does not compensate for the loss of transmission in Cx34.1-/- fish, the finding reveals an novel functional organization for GJ channels, at which while Cx34.1 is obligatory and Cx34.7 seems to play an accessory function, likely assembling with Cx34.1 in the postsynaptic hemichannel under specific functional contexts. The functional role of Cx34.7 does not result from its inability to form channels with Cx35.5. That is, our results also show that both Cx34.1 and Cx34.7 when exogenously expressed can form channels with Cx35.5, suggesting that this novel Cx functional assignment is likely to defined by the postsynaptic molecular machinery.

Conclusions: Thus, electrical synapses can share a organizational feature with chemical synapses, similar to how they combine multiple postsynaptic receptor types to modify synaptic function.

123

In Vivo Small Molecule Screening Identifies Calcium Channel Modulation as a Driver of Cardiomyocyte Proliferation

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Topic

4 Cardiovascular

Abstract Content

Acute myocardial injuries, such as myocardial infarction, result in the irreversible loss of millions of cardiomyocytes (CMs). In the adult human heart, CMs are terminally differentiated and lack the regenerative capacity to replace lost tissue in a sufficient manner. Consequently, the heart responds to injury by forming a permanent fibrotic scar, which leads to reduced cardiac function and eventually progresses to heart failure. A promising therapeutic approach to boost regeneration is the induction of proliferation in surviving CMs at the wound border.

Unlike humans, adult zebrafish (*Danio rerio*) possess the remarkable ability to fully regenerate their hearts and resolve fibrotic scars after injury. This regenerative process is driven by the dedifferentiation and subsequent cell cycle re-entry of surviving CMs, which revert to a more embryonic-like state. Therefore, identifying the molecular drivers of CM proliferation during embryonic development is a crucial strategy for discovering novel targets to stimulate heart regeneration.

To identify such pro-proliferative cues, we conducted an *in vivo* high-throughput screen (HTS) of 485 small bioactive molecules using zebrafish embryos. One of the most striking hits identified was Cilnidipine, a dual L- and N-type calcium channel blocker primarily used as an antihypertensive medication in several Asian countries. Our analysis revealed that Cilnidipine treatment in zebrafish embryos leads to an enlarged ventricle characterized by a significant increase in ventricular CM number and proliferation rate, rather than an increase in cell size. Calcium imaging demonstrated severely reduced Ca^{2+} flux in treated embryos, suggesting that calcium channel inhibition and the resulting decrease in intracellular Ca^{2+} levels act as a potent trigger for CM proliferation. Our ongoing research focuses on characterizing the Ca^{2+}-dependent downstream signaling pathways involved. Furthermore, we aim to translate these findings to human iPSC-derived cardiomyocytes to investigate the potential of calcium signaling as a therapeutic target to bolster regeneration.

125

tafazzin and *abhd18* genetic interactions in a zebrafish model of Barth Syndrome

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Topic

1 Disease Models

Abstract Content

Background: Mitochondrial dysfunction plays a central role in many diseases, including cardiomyopathies. Barth syndrome (BTHS) is a rare X-linked disorder caused by mutations in the *TAFAZZIN* gene, which encodes a mitochondrial transacylase essential for the remodeling of monolysocardiolipin into mature cardiolipin, a signature phospholipid critical for mitochondrial function. BTHS leads to severe mitochondrial defects, cardiomyopathy, and early mortality. A recent cell-based genetic screen from the Moffat lab identified a strong positive genetic interaction between *TAFAZZIN* and *ABHD18*, an uncharacterized enzyme, where *ABHD18* loss rescued the fitness of *TAFAZZIN*-deficient cells.

Methods: To explore this genetic interaction *in vivo*, I will establish a zebrafish BTHS model system. To date, I developed an antisense morpholino knockdown strategy targeting Tafazzin to recapitulate BTHS-related cardiac phenotypes. As well, I tested a small covalent inhibitor of ABHD18, ABD646, as a potential therapeutic agent. I am now in the process of generating zebrafish *tafazzin* and *abhd18* mutant lines to explore this genetic interaction throughout development.

Results: The Tafazzin morphants exhibited severe cardiac phenotypes consistent with mitochondrial dysfunction. Remarkably, treatment with ABD646 rescued these cardiac abnormalities, supporting a functional *in vivo* interaction between *tafazzin* and *abhd18*. In contrast, a *tafazzin* indel mutant line generated via CRISPR editing displayed no deleterious phenotype, suggesting the presence of compensatory genetic mechanisms. Ongoing efforts aim to characterize these mechanisms and evaluate the phenotype of *abhd18* mutants alone and in combination with *tafazzin* deficiency.

Conclusion: This research reveals a novel *in vivo* genetic interaction between *tafazzin* and *abhd18*, and identifies ABHD18 as a promising therapeutic target to mitigate mitochondrial dysfunction in BTHS. These findings demonstrate the power of zebrafish to uncover genetic modifiers and drug targets relevant to mitochondrial disease. Ultimately, this work may inform new strategies to treat cardiomyopathies and heart failure linked to impaired mitochondrial lipid remodeling.

126

Time-dependent repression of mTORC1 drives dendritic pruning to enable central nervous system axon regeneration

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Topic

3 Neurobiology

Abstract Content

Background
Dendritic degeneration is an early hallmark of axonal injury in the mammalian central nervous system and is typically associated with neuronal dysfunction and cell loss. However, dendritic remodeling is also observed in regeneration-competent systems, suggesting that it may contribute to successful axonal repair. How dendritic dynamics are coordinated with axonal regeneration remains poorly understood.

Methods
Using sparse single-cell labeling of retinal ganglion cells (RGCs) in adult *Tg(gfi1ab:gal4;UAS:lynGFP)* zebrafish, we quantitatively analyzed dendritic morphology following optic nerve crush. Axonal regeneration, dendritic architecture, and mTOR signaling dynamics were examined using imaging, molecular analyses, and pharmacological manipulation of the insulin–mTOR pathway.

Results
Following injury, regenerating RGCs undergo a transient phase of dendritic pruning beginning around 2 days post-injury and peaking during active axonal elongation. This pruning is primarily characterized by reduced dendritic branching and total dendritic length, while the overall field area of the arbor remains largely preserved. Dendritic complexity is restored only after optic tectum reinnervation. Molecular analyses revealed a biphasic mTOR response: mTOR activity increases immediately after injury but is rapidly suppressed during the dendritic pruning phase. Pharmacological prolongation of mTOR activation through insulin or direct pathway stimulation partially preserved dendritic complexity but significantly delayed axonal regrowth and tectal reinnervation. Co-administration of the mTORC1 inhibitor rapamycin abolished the dendrite-preserving effect of insulin, demonstrating that injury-induced dendritic pruning is mTORC1-dependent.

Conclusion
Our findings identify transient dendritic remodeling as a temporally regulated component of successful axonal regeneration. A controlled, time-dependent repression of mTORC1 signaling appears to drive the dendritic pruning phenotype, revealing a

trade-off between dendritic maintenance and axonal regrowth that may inform strategies to enhance CNS repair.

127

Fishing for new immunotherapy compounds to boost innate-tumor rejection

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Topic

17 Chemical Biology and Drug Discovery

Abstract Content

Despite significant advances in cancer immunotherapy, tackling immunosuppression within the tumor microenvironment (TME) remains critical for more effective responses. Using the zebrafish xenograft model, we established that tumor engraftment is governed by innate immunity: some cell lines are recognized and cleared by the host ("regressors"), while others evade it ("progressors"). This platform allowed for an *in vivo* phenotypic drug screen to identify compounds that induce clearance by boosting innate-tumor rejection.

Zebrafish xenografts of human colorectal (CRC) and breast cancer (BC) progressor cell lines were used to screen an FDA-approved small compound library, enabling drug repurposing. Tumor cells were injected into 2 days post-fertilization embryos and subjected to compound testing. Tumor clearance was quantified 3–5 days later and hits were defined as compounds that reproducibly increased it.

Screening of >500 drugs identified 23 confirmed hits, several effective in both CRC and BC models. Mechanism-of-action (MOA) enrichment analysis revealed an overrepresentation of autonomic nervous system-targeting drugs, such as muscarinic receptor antagonists, dopaminergic agonists, and PDE inhibitors. Using transgenic zebrafish lines, we observed the selected hits modulate the innate TME by increasing myeloid cell recruitment and repolarizing macrophages towards a pro-inflammatory phenotype. The top hit, a muscarinic receptor antagonist, was further validated in a CRC xenograft mouse model, where it significantly reduced tumor growth as monotherapy and synergized with αPD-1 checkpoint blockade to improve survival. Macrophage depletion in zebrafish and use of NSG mice abolished drug-induced clearance, confirming immune dependence. Ongoing studies include MOA characterization and immune profiling of treated mouse tumors.

We identified a repurposable FDA-approved compound that, when combined with checkpoint blockers to engage both innate and adaptive immunity, may overcome TME-mediated immunosuppression and enhance therapy efficacy.

131

Molecular control of retinal neurogenesis in zebrafish: identifying and characterizing immediate retinoic acid target genes with molecular and computational approaches

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Topic

12 Education and Science Communication

Abstract Content

Despite numerous links between retinoic acid (RA) and neurogenesis, our understanding of how RA stimulates the differentiation of neurons is incomplete. To address this question, we leveraged molecular and computational approaches to identify novel and understudied RA-target genes. We first performed a bulk RNA-Seq screen to identify immediate and indirect targets of RA during the earliest stages of retinal neurogenesis. We treated zebrafish embryos with the RA-receptor agonist AM580 in the presence or absence of the translation blocker, cycloheximide, and then isolated retinal cells, purified RNA, constructed cDNA libraries, and performed Illumina sequencing. By comparing the sequencing profiles of these treatments, we were able to identify known immediate RA-targets, including *cyp26a1* and *cyp26a2*. In addition we identified two understudied transcription factors, *tshz1* and *nr6a1b,* as immediate transcriptional targets of RA. To identify all genes putatively regulated by RA in the developing retina, we created a direct repeat motif finder in python called RAREdar that identifies genes with transcriptional start sites near retinoic acid response elements (RARE). By subjecting a curated list of zebrafish eye genes from publicly-available single-cell RNA-Sequencing datasets to RAREdar, we identified 8 additional transcription factors and 14 additional genes. With the goal of gaining new insight into how the RA pathway contributes to the shape and cellular composition of vertebrate eyes, we have begun examining the expression of these genes by in situ hybridization and RT-qPCR. For genes that appear to be bona fide immediate RA targets, we have begun to disrupt them with CRISPR/Cas9 and examine phenotypes including cell cycle kinetics and photoreceptor differentiation in the developing eye. All of this work has carried out by undergraduates working in the lab during the summer, their senior thesis, and/or as part of an advanced Developmental Biology lecture-lab course.

135

Deciphering the zebrafish hepatic duct heterogeneity and cell plasticity using lineage tracing and single-cell transcriptomics

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background
Liver possesses remarkable regenerative potential, yet the contribution, heterogeneity and functional roles of hepatic ductal cells, a.k.a. biliary epithelial cells, remain incompletely understood. Zebrafish provide powerful tools for deciphering these mechanisms due to their genetic tractability and regenerative capacity. This study provides a comprehensive examination of molecular and cellular mechanisms underpinning liver ductal development and liver regeneration in zebrafish, with a spotlight on the functional roles of *her* family genes.

MethodsUsing state-of-the-art knock-in zebrafish models, multiple transgenic reporter lines, single-cell transcriptomics and lineage-tracing strategies, we identified and characterised heterogeneity, plasticity and molecular signatures of hepatic ductal cells. We also revealed morphological and spatial features of ductal subtypes by confocal microscope imaging. By further single-cell RNA-seq analyses of lineage-traced *her9*-expressing hepatic ducts in homeostatic and regenerative states, we uncovered ductal remodelling processes and regenerative dynamics of liver.

ResultsDistinct expression patterns of *her2*, *her6* and *her9* were identified as specific molecular signatures that distinguish ductal cell subtypes with unique morphology and spatial distribution. Particularly, *her9* emerged as a pan-ductal marker and showed responsiveness to the synergistic effect of Notch and BMP signalling, while *her2* served as a specific marker of the highly branched Notch-responsive intra-hepatic ductal network. In addition, *krt4* was found enriched in large luminal ducts. In a homeostatic state, *her2*$^{+}$ intra-hepatic ducts were genetically fate-mapped to contribute to the formation of luminal ducts. Under extreme loss of intra-hepatic ducts, *krt4*$^{+}$ luminal ducts could be remodelled into intra-hepatic-duct-like cells, underscoring the plasticity of ductal cells. Single-cell transcriptomics during regeneration demonstrated the dynamics of liver regeneration, highlighting temporal activation of signalling pathways and cellular states.

ConclusionOur findings provide a comprehensive framework for understanding ductal cell heterogeneity and plasticity in zebrafish liver during development and regeneration. The identification of molecular signatures suggests how ductal remodelling and liver repair occur.

138

Investigating the role of Rnd1b in mesendoderm internalization during gastrulation

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Vertebrate gastrulation represents a fundamental morphogenetic process, where cell migration and fate determination are coordinated to establish the primary body plan. One critical movement in this context is the internalization of mesendoderm cells at the embryonic margin. Although it is well established that inductive signals—notably Nodal and TGF-β pathways—specify germ layers and initiate internalization events, the identity of the downstream cytoskeletal effectors specifically driving motility remains elusive. Unraveling the core cytoskeletal regulators required for germ layer internalization will broaden understanding of conserved tissue remodeling mechanisms and help clarify the events driving the acquisition of invasive cellular behavior.

To address these questions, this research investigates potential cytoskeleton regulators, acting downstream of Nodal signaling and their roles in directing mesendoderm internalization. Our approach integrates transcriptomic screening to identify candidate regulators characterized by mesendoderm-enriched, Nodal-dependent expression, followed by functional validation through morpholino knockdown, CRISPR-Cas9 mutagenesis, and rescue experiments. In vivo two-photon microscopy in zebrafish embryos provides high-resolution insight into both cellular and whole-embryo gastrulation dynamics, while cell transplantation assays allow quantitative evaluation of internalization efficiency and cell-autonomous effects.

Preliminary experiments, including collaborative screening during Xenopus gastrulation, have identified Rnd1b as a Nodal-dependent potential cytoskeleton regulator. Rnd1b is a small GTPase associated with regulating cortical tension and C-cadherin levels. We have observed its specific expression at the zebrafish embryonic margin at the onset of gastrulation, consistent with a role in internalization. Notably, Rnd1b-deficient embryos display morphological changes, such as a thickened dorsal axis, irregular morphology of internalizing cell mass, and cryptic cellular protrusiveness, with further analyses being pursued to fully delineate its role in germ layer formation and cell-autonomous behavior.

140

A Novel Zebrafish Model Reveals Biological Insights into Alkaptonuria

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Topic

1 Disease Models

Abstract Content

Genetic disruptions in Phenylalanine/Tyrosine (Phe/Tyr) degradation pathway cause several human metabolic diseases, including alkaptonuria (AKU). It results from accumulation of homogentisate (HGA), a toxic metabolite that deposits as black pigment in connective tissues. HGA degrades collagen, causing osteoarthritis and other skeletal pathologies. Our lab generated a zebrafish AKU mutant line that faithfully replicates key human symptoms, including visible tissue blackening in adulthood. Elevations of HGA levels in AKU zebrafish blood were comparable to those in human patient body fluids, with Raman miscopy confirming same pigment composition. Using zebrafish transparency, increasing HGA deposition in connective tissues was detected from the juvenile stage, before visible blackening appeared. To evaluate this model's utility for drug development, we treated zebrafish with nitisinone, an approved drug that lowers HGA levels in patients. Nitisinone treatment significantly decreased circulating HGA levels in adults and reduced connective tissue HGA accumulation when administered at juvenile stages. Combined with the ease of genetic manipulation and transgenic rescue approaches in zebrafish, this model provides a powerful platform for investigating new therapeutic targets within the Phe/Tyr pathway and advancing drug development for AKU.

142

Link between DNA-protein crosslinks and neurodegeneration: Tdp1-deficient zebrafish model

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Topic

3 Neurobiology

Abstract Content

Interactions between DNA and proteins are dynamic and essential for replication, transcription, and DNA repair. Because of various endogenous and exogenous causes, proteins covalently and irreversibly bind to chromosomal DNA, forming DNA-protein crosslinks (DPCs). If DPCs are not properly repaired, they accumulate in cells, causing genome instability and cytotoxicity, which is associated with cancer, neurodegeneration, and aging. There are multiple mechanisms of DPC repair depending on DPC type. Here, we focus on tyrosyl-DNA phosphodiesterase 1 (TDP1), an enzyme involved in the processing of topoisomerase 1 (TOP1)-DPCs. Under physiological conditions, TOP1 relieves torsional stress by introducing single-stranded breaks (SSBs) during DNA replication and transcription. If TOP1 encounters DNA helical alterations, is in the vicinity of elevated aldehyde concentrations due to metabolic processes or after exposure to chemotherapeutics such as camptothecin, it becomes irreversibly bound to DNA and forms TOP1-DPCs. TDP1 hydrolyzes the bond between the tyrosine residue of TOP1 and the DNA 3'-phosphate, generating the 3'-OH end of DNA. However, it cannot remove the whole protein crosslink before upstream processing by proteases. A point mutation in TDP1 causes human syndrome, SCAN1 (spinocerebellar ataxia with axonal neuropathy 1). This autosomal recessive neurodegenerative disease is characterized by a slowly progressive ataxia and cerebellar atrophy with distal sensorimotor axonal neuropathy. Our aim is to determine molecular cause behind the neurodegeneration caused by TDP1 deficiency. To this end, we created TDP1-deficient zebrafish strain in which we analyse TOP1-DPC formation in neurons using the modified RADAR assay and immunostaining, DNA breaks and apoptosis. Neurodegenerative phenotypes in adult and old zebrafish are assessed in respect to swimming patterns, memory and learning ability using the ZANTIKS system.

TDP1-deficient zebrafish model enables us to investigate whether neuronal TDP1 deficiency leads to DPC accumulation in the brain and whether this causes neurodegeneration.

143

Regulation of gene expression by enhancer RNAs during zebrafish development

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Enhancers are key regulatory elements that control gene transcription in a spatially and temporally precise manner, which is why they play fundamental roles in cell differentiation and cell fate decisions. Over the past decade, it became evident that many enhancers are actively transcribed by RNA Polymerase II, producing short, unstable transcripts known as enhancer RNAs (eRNAs). Despite growing evidence that suggests that eRNAs contribute to transcription activation, their precise functional role remains poorly understood. Importantly, commonly used approaches to study enhancer function - such as CRISPR interference (CRISPRi) or the CRISPR-Cas9-mediated deletion of enhancer regions - disrupt the DNA regulatory element itself. They are therefore not well suited to specifically dissect the contribution of the eRNA transcript to target gene activation. Our study thus aims to address this gap by investigating the functional role of eRNAs in vivo. To achieve this, we first identified transcription start sites genome-wide across several developmental stages using capped small RNA sequencing (csRNA-seq), a technique that enables the detection of low-abundance, unstable, and short-lived transcripts such as eRNAs. To experimentally test how eRNAs influence the expression of their target genes, we employed the Rfx-Cas13d system to selectively deplete specific eRNAs. The resulting effects on target gene expression were analyzed using quantitative PCR (qPCR). Our findings will be further validated by phenotypic characterization using whole-mount in situ hybridization (WISH). Finally, we aim to establish a direct functional connection between the presence of eRNAs and mRNA transcription using single-molecule fluorescence in situ hybridization (smFISH). Together, our approach will allow us to directly assess the regulatory contribution of eRNAs to transcription activation in an in vivo vertebrate model.

144

Optogenetic Tracking of Energy Status During Zebrafish Heart Regeneration

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Topic

4 Cardiovascular

Abstract Content

Background: Optogenetic sensors to measure intracellular metabolites (e.g. GCaMP for Ca2+) allow for non-invasive analysis in cells and tissues. Here we developed a transgenic zebrafish line for real-time monitoring of cellular energy status in cardiomyocytes using PercevalHR, a genetically encoded sensor that detects the relative abundance of ATP and ADP.

Methods: We generated a transgenic zebrafish line expressing PercevalHR specifically in cardiomyocytes. Using ex vivo live imaging of injured cardiac slices, we compared metabolic activity between the border zone (BZ) and remote zone (RZ) during heart regeneration. Slices were treated with 2-deoxy-D-glucose (2DG) to block glycolysis, or with etomoxir to inhibit fatty acid import, at multiple timepoints post-injury.

Results: At 3 days post-injury (dpi)—when cardiomyocytes in the BZ begin proliferating—2DG treatment triggered a localized metabolic response in the BZ but not the RZ. This effect was absent at 7 days (proliferation peak) and 21 days (late regeneration). Moreover, while the RZ at 3 dpi showed no response to 2DG treatment, it showed a drastic reduction in ATP production upon blocking fatty acid import, showcasing that these CMs retain their mature metabolic profile.

Conclusions: PercevalHR enables spatially resolved, time-sensitive measurement of metabolism in regenerating zebrafish hearts. This approach reveals dynamic, region-specific metabolic shifts and can identify pathway-specific contributions to cardiac regeneration.

145

Assessment of the Regenerative Potential of Nigella sativa seeds Using the Zebrafish Caudal Fin Regeneration Model

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background:
Natural products constitute an important source of bioactive compounds with potential regenerative and pharmacological properties. The zebrafish (Danio rerio) caudal fin regeneration model is widely used as an in vivo platform for screening compounds influencing tissue repair processes. The aim of this study was to evaluate the regenerative activity of selected plant extracts, fractions and reference natural compounds using this model.

Methods:
Zebrafish larvae at approximately 72 hours post fertilization underwent caudal fin amputation and were subsequently incubated with tested samples dissolved in embryo medium. After 48 hours of exposure, the regenerative response was assessed by measuring the regrowth of the amputated tail fin. The study included extracts and fractions obtained from Nigella sativa seeds. Additionally, pure natural compound thymoquinone was evaluated.

Results:
Among the tested samples, the CO_2 extract obtained from Nigella sativa seeds and thymoquinone significantly enhanced caudal fin regeneration compared with untreated controls.

Conclusion:
The obtained results indicate that thymoquinone and Nigella sativa seed, may stimulate tissue regeneration in vivo. The zebrafish caudal fin regeneration assay proved to be a useful and sensitive model for screening the regenerative potential of plant-derived compounds and extracts.

Founding: The project 'Innovative Products Based on Natural Substances for Use in the Prevention of Selected Civilizational Diseases' no. KPOD.07.07-IW.07-0100/24 financed by the European Union from the National Reconstruction and Resilience Plan, Component D: Efficiency, Accessibility, and Quality of the Healthcare System, which is part of Investment D3.1.1 Comprehensive Development of Research in Medical Sciences and Health Sciences.

148

Non-coding RNAs in zebrafish sex determination

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Topic

9 Germline and Early Development

Abstract Content

MicroRNAs (miRNAs) are a class of non-coding small RNAs that regulate mRNA expression by binding to their 3' untranslated region (3'UTR). The expression of the targeted mRNA is reduced, rendering miRNA regulation a fine-tuning mechanism. One of these miRNAs, *miR214*, caught our interest in a genetic screen in which only around 10% of homozygous carriers were female, a phenotype that we replicated with additional alleles.

miRNAs exert their function by targeting the mRNAs' 3'UTR. Therefore, depleting parts of the 3' UTR in potential target genes should mimic the *miR214* mutation phenotype. We tested three candidate genes, but only mutations in the *gsdf* 3'UTR could replicate the *miR214* phenotype, rendering *gsdf* a likely target of *miR214*.

Thus, *miR214* binds the *gsdf* 3'UTR and restricts its expression. However, the story is more complex: *miR214* originates from an evolutionary conserved long noncoding-RNA (lncRNA) precursor called *dnm3os*. Deleting either a larger fragment surroung *miR214* or the entire lncRNA had no phenotype.
When deleting a short fragment upstream of *miR214,* it resulted in a female bias. This allele is dominant over the originally identified male-biased *miR214* allele, yet does not have elevated *miR214* expression or affected *gsdf* levels, suggesing that different elements witihin the dnm3os lncRNA affect sex determination in opposing directions, making it a factor that can truly balance sex-ratios.

Additionally, we encountered a second surprise: the *miR214* loss of function phenotype was nearly lost in offspring of the rare females that developed from heterozygous incrosses. This was evident at the phenotypic and molecular levels, as judged by mRNA sequencing. A similar effect was also observed in *gsdf* 3'UTR mutants.

Our studies imply *dnm3os* and *miR214* as interesting players in zebrafish sex determination, and hint to a maternal balancing effect on this pathway.

152

Altered Circadian Regulation of the Mevalonate Pathway in Glioblastoma

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Topic

1 Disease Models

Abstract Content

Background
Circadian clocks coordinate brain physiology by imposing a temporal structure on metabolic processes. However, their role in malignant transformation remains poorly understood. In pa rticular, it is unclear whether disruption of circadian regulation contributes to the transition f rom glial cells to glioblastoma
(GBM). As cholesterol biosynthesis is essential for both glial homeostasis and GBM growth, we investigate whether circadian regulation of the mevalonate pathway represents a mecha nistic link between clock dysfunction and malignant transformation.

Methods
To address this, we combine an in vivo zebrafish brain model that preserves physiological cir cadian regulation with human GBM cells cultured in 2D monolayers and 3D tumour spheroid s. Following circadian synchronisation, temporal expression of key mevalonate pathway gen es, including *hmgcs*, *hmgcra*, and *fdps*, is analysed using RT-
qPCR. The functional consequences of altered temporal regulation are assessed by monitorin g proliferation and invasion using live-cell–based assays and by analysing cell-
cycle distribution and stem-
cell marker expression via flow cytometry. In parallel, CRY- and REV-ERB–
targeting compounds are used to pharmacologically modulate circadian clock function to exa mine the influence of clock gene activity on the mevalonate pathway and GBM-
associated phenotypes.

Results
Non-malignant zebrafish brain tissue exhibits time-dependent regulation of cholesterol-
associated genes consistent with circadian control, whereas GBM models display reduced te mporal organisation. These observations suggest that malignant transformation is accompan ied by metabolic reprogramming and a loss of circadian control over metabolic timing.

Conclusion
Overall, our findings indicate that disruption of circadian regulation of the mevalonate pathw ay may represent a previously overlooked aspect of glioblastoma biology and could contribut e to metabolic changes associated with tumour progression.

156

Insulin-Dependent Regulation of Hepatic Glucokinase Revealed by In Vivo Bioluminescence Imaging in Zebrafish

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Topic

8 Metabolism

Abstract Content

Glucokinase (Gck) is a pivotal regulator of systemic glucose homeostasis and a therapeutic target for type 2 diabetes. In pancreatic β-cells, GCK functions as a glucose sensor for insulin secretion, while in hepatocytes it controls glucose uptake and glycolytic flux in an insulin-responsive manner. In our recent work, we demonstrated conserved *gck* regulation in zebrafish and showed that pharmacologic activation with the clinical GCK activator Dorzagliatin enhances Gck activity and improves glycemic control, supporting the translational utility of this model.

Here, we extend these findings by dissecting the regulatory mechanisms governing hepatic *gck* expression *in vivo*. We generated a *gck:luciferase* transgenic reporter line enabling non-invasive, longitudinal bioluminescence imaging (BLI) from larval to adult stages. This platform overcomes the limitations of fluorescence imaging in older animals and permits quantitative, organ-resolved analysis of metabolic responses.

Using *gck* reporter lines, we visualize robust induction of hepatic *gck* expression in response to high nutrient feeding and glucose exposure. Importantly, acute pharmacologic blockade of insulin receptor signalling using the antagonist S961 suppresses hepatic *gck* expression, reduces glycolytic gene expression, and induces hyperglycemia, demonstrating that insulin signalling is required for proper *gck* regulation in zebrafish. Insulin receptor antagonism produces more pronounced metabolic dysregulation in juvenile fish, coinciding with the maturation of adipose tissue and systemic glucose handling.

Together, these findings establish zebrafish as a tractable *in vivo* system to study both activation and inhibition of Gck and reveal conserved insulin-dependent control of hepatic *gck* expression. Our bioluminescence imaging-based platform provides a powerful tool for evaluating organ-specific responses to *gck* modulators and for modelling insulin resistance across developmental stages.

158

Molecular identification and functional characterization of a novel regulator of blood-brain barrier formation in zebrafish

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Topic

4 Cardiovascular

Abstract Content

The blood-brain barrier (BBB) refers to a set of unique adaptations acquired by endothelial cells (ECs) lining cerebral blood vessels, which play a crucial neuroprotective role. It protects the central nervous system (CNS) from blood-borne neurotoxins and pathogens, and safeguards cerebral homeostasis for proper neuronal function. These characteristics are not intrinsic to brain ECs but are rather induced and maintained by the surrounding neural tissue. The Wnt/β-catenin signaling pathway is well-known to regulate brain angiogenesis and barriergenesis. Neural-derived Wnt7a/b ligands bind specifically to Reck/Gpr124/Fzd/Lrp5/6 receptor complexes present at the surface of cerebral ECs. However, regulators other than Wnt signaling are also likely to contribute to BBB formation. To investigate this possibility, we combined whole-mount *in situ* hybridization with confocal imaging of fluorescent reporter lines and discovered that a set of Wnt-regulated BBB marker genes seems to be also controlled by an additional, yet-to-be-identified factor. To uncover the molecular identity of this regulator, we generated a single-cell RNA sequencing (scRNA-seq) dataset of the zebrafish vasculature at 72 hours post-fertilization (hpf) by isolating ECs using FACS. Bioinformatic analysis of this dataset revealed several candidate regulators, which we will functionally test using CRISPR/Cas9-mediated mutagenesis. In parallel, we generated knock-in BBB marker reporter lines with which we will conduct a small-molecule discovery screen. Identifying novel regulators of BBB formation will provide key insights into the mechanisms controlling barrier integrity and may ultimately reveal new strategies to modulate BBB function in neurological disorders.

159

Scaling zebrafish husbandry under budget constraints: operational strategies for sustainable growth in Poland's largest facility

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Topic

21 Husbandry

Abstract Content

The development of zebrafish research infrastructure in Central and Eastern Europe has often relied on targeted external funding. Zebrafish Core Facility (ZCF) at IIMCB was established in 2012 through the EU FP7 FishMed grant, which enabled the establishment of the initial infrastructure. Over fourteen years, ZCF expanded from a single rack to 23 racks in 2026, becoming the largest zebrafish husbandry center in Poland. Growth supported by grant funding was complemented by standardization of workflows, personnel training, and colony management practices. As the facility matured, structured biological monitoring was introduced to ensure expansion aligned with best husbandry practices, maintaining healthy colonies and reproductive stability.

To support sustainable growth under limited resources, the facility adopted operational strategies to enhance efficiency and reduce errors. A locally developed database enables precise tracking and management of zebrafish lines, improving oversight and planning. Husbandry procedures—including fin clipping, SF and IVF, live feed culture, larval rearing, and specialized care for phenotypes such as casper—are standardized. Most routine tasks are performed by trained personnel rather than individual researchers, minimizing handling variability, reducing line misidentification, and ensuring consistent feeding. Space-based user fees encourage researchers to plan colony size responsibly, reducing unnecessary backup lines and overall animal use. The facility also supports external groups by providing embryos and offering paid line maintenance for collaborators without dedicated infrastructure. This flexible model optimizes space while partially supporting facility development.

Our experience demonstrates that the sustainable operation of a large zebrafish facility requires coordinated development of infrastructure, standardized husbandry practices, and efficient management strategies. By combining external funding with operational self-sustainability, facilities can scale responsibly while maintaining colony health, reproducibility, and long-term support for the research community.

161

THE ROLE OF PERIPLAKIN IN EPITHELIAL ZEBRAFISH SKIN HOMEOSTASIS

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Periplakin (Ppl) is a cytolinker protein that connects filamentous actin and keratin in epithelial cells, responsible for the apical cell protrusion morphogenesis, namely microridges. However, its function in maintaining skin epithelial homeostasis remains unexplored.

Here, we investigated *ppl* mutant zebrafish generated by the CRISPR/Cas9 system to determine the role of Ppl in skin epithelial homeostasis. High-resolution confocal imaging was performed to analyse quantitative cell surface area across developmental stages, as well as the number of goblet cells in adults. Concurrently, Ppl localization and cell junction proteins were examined by using immunostaining. Protein and gene expression levels were analyzed using RT-qPCR and LC-MS/MS.

Ppl mutant demonstrated a difference in the size of cell surface area and cell number throughout all developmental stages; the periderm cell surface area was smaller from larval to adult, and a reduction in goblet cell number compared to WT. In adult WT fish, we found that Ppl is mainly localized in the apical region of the periderm cell. In the *Ppl* mutant, the localization of junctional proteins was disrupted. E-cadherin protein was mislocalized; it is not tightly localized to the cell-cell junction, but its distribution was wider than WT. It also partially affects ZO-1 protein localization; the junctions appear with a zig-zag structure instead of the linear lines of contact between neighboring cells. In contrast, the localization of desmoplakin and N-cadherin remains unaffected. From RT-qPCR, we found that expression of *ppl* and *mucin5.1* was significantly downregulated. Proteomics profiling identified that the loss of Ppl proteins led to a reduction in the levels of proteins that regulate actin filament-based processes. All findings suggest that Ppl contribute to the assembly of apical actin networks, thereby maintaining epithelial cell structures.

Together, our findings suggest Ppl as an important cytolinker protein to maintain the epithelial scale homeostasis in adult zebrafish.

163

Investigating the effects of development and conspecific interactions on the sleeping behaviour of *Danionella cerebrum*

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Most organisms spend a large fraction of their lives sleeping; however, the amount and intensity varies among different organisms. Environmental stimuli, conspecific interactions, aging, and seasonal changes are a few factors, among others, that might cause variations in an organism's sleeping behaviour. However, it is technically challenging to assess the effects of these factors on the behaviour and on the underlying neuronal activity of a sleeping animal. We aim to leverage the life-long transparency of the tiny teleost fish, *Danionella cerebrum*, to investigate the effects of development and aging on the sleeping behaviour and neuronal activity in selected sleep and wake circuits. The innate sociability of these fish, also make it interesting to observe the effects of conspecific interactions on the sleeping behaviour of fish in groups. We aim to observe how day-time activity might affect the corresponding night-time sleep in groups of fish, as opposed to fish in isolation. In this regard, we aim to develop a three-dimensional behavioural imaging and machine learning pipeline to observe social interaction dynamics in fish, across large timescales, followed by employing whole brain 2-photon imaging to observe the long-term effects of development and aging on the neuronal firing activity. Finally, we aim to combine these methods to observe how the behaviour and neuronal activity occurring during sleep varies with age in the social context.

165

Drug repurposing in Duchenne Muscular Dystrophy: estrogen receptor modulators ameliorate the dystrophic phenotype of *sapje* zebrafish

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Topic

17 Chemical Biology and Drug Discovery

Abstract Content

Duchenne Muscular Dystrophy (DMD) is a severe X-linked disorder characterized by progressive muscle degeneration and premature mortality, caused by mutations in the *dmd* gene encoding dystrophin protein. Current therapies largely rely on corticosteroids, which provide limited benefit and are often associated with significant side effects. Therefore, the identification of novel pharmacological strategies remains an urgent unmet need. The *sapje* zebrafish mutant is a well-established *in vivo* model that recapitulates key pathological features of human DMD and enables rapid drug screening.

To enhance these readouts*, sapje* larvae were outcrossed with transgenic reporter lines, allowing real-time visualization of muscle structure, vasculature, and cell death. This integrated platform was used to screen a small library of 11 estrogen receptor modulators. Compounds were evaluated for their ability to improve muscle integrity, locomotor performance, survival, and cardiac function.

Several estrogen receptor modulators significantly ameliorated the dystrophic phenotype in *sapje* larvae. In particular, (Z)-Tamoxifen, Endoxifen, 17β-estradiol, Fulvestrant, and Toremifene markedly improved muscle structure and functional performance, with detectable effects at low micromolar concentrations. Treated larvae showed improved muscle fiber organization, reduced cell death, and enhanced locomotor activity. These findings reveal a previously underexplored role of estrogen signaling in modulating muscular dystrophy.

Overall, our results identify estrogen receptor modulators as promising candidates for therapeutic repurposing in DMD. Furthermore, the use of *sapje* fluorescent biosensors enhances phenotypic resolution and strengthens the zebrafish platform for translational drug discovery and preclinical screening in muscular dystrophies.

166

Exploring the Role of Innate Immunity to Support Cardiac Repair and Regeneration

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Topic

4 Cardiovascular

Abstract Content

Background

Multiple cellular and molecular interplays are being involved in the complex but inefficient process of cardiac repair, while heart failure (HF) is the final effect of multiple converging pathways of inadequate repair following cardiac injury or stress.

Methods

Using a mouse genetic HF model we have previously shown that macrophages and their secretome reflect cardiac injury and HF progress and affect cardiac fibroblasts during post-injury cardiac remodeling, thus modulating associated outcomes. We further used a cross-species approach to demonstrate that the polarization status of mouse macrophages differentially regulates, in a paracrine manner, cell proliferation in regenerating zebrafish cardiac explants.

Results

Proteomic profiling of the secretomes of pro-regeneration M2a polarized macrophages revealed their enrichment with molecules, including ECM components, known to induce adult mouse cardiomyocyte proliferation and maturation (FGL2, Agrin, Fibronectin 1), or to facilitate the regenerative potential of zebrafish fibroblasts (ALDH1a2). Notably, markers of M2 polarization were also overproduced, along with molecules of unknown function whose contributions in cardiac regeneration are being currently investigated.

Conclusion

We will present the phenotypic analyses of knocking down and knocking out the two zebrafish genes *out at first homolog* (*oafa* and *oafb*). We have selected the *Oaf* as a novel protein, whose secretion was specifically upregulated in the M2 supernatant and its role in cardiac development and regeneration still unknown. The protein contains a BRICHOS domain that is proposed to act as chaperones to prevent amyloid-like aggregation of the mature polypeptides and other members of the

family have been linked to a long list of pathologies including cancer, obesity and amyloid-like disorders.

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167

Comparative assessment of locomotor behavior in zebrafish (*Danio rerio*) exposed to common laboratory solvents

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Topic

10 Toxicology

Abstract Content

BACKGROUND: Organic solvents are widely used in zebrafish assays to dissolve poorly water-soluble compounds, but they may affect physiology and behavior, potentially complicating the interpretation of results. Despite their frequent usage, comparative studies examining solvent-induced behavioral effects under standardized conditions remain limited. This study assessed the behavioral effects of seven commonly used solvents – dimethylsulfoxide (DMSO), ethanol (EtOH), methanol (MeOH), acetone (Ace), acetonitrile (ACN), isopropanol (IPA), and ethyl acetate (EtoAC), on locomotor behavior of zebrafish at 120 h post-fertilization.

METHODS: Experiments were conducted in accordance with the OECD 236 (2013). Embryos at the 4-64 cell stage were individually placed in 96-well plates containing 300 µL of test solution per well. Embryos were exposed to seven organic solvents, each tested at four concentrations (1, 0.5, 0.1, and 0.05%, *v*/*v*). Larval activity was quantified using a DanioVision observation chamber under alternating light-dark conditions, assessing distance moved, velocity, acceleration, mobility states, and turning behavior. Multivariate analysis was performed using principal component analysis (PCA) and hierarchical clustering on principal components (HCPC) to investigate solvent-dependent behavioral patterns.

RESULTS: EtOH, MeOH, Ace, and IPA induced hyperactivity at ≥ 0.5%, while DMSO showed biphasic effects, stimulating activity at 0.5% but suppressing it at 1%. ACN demonstrated a significant inhibitory response even at 0.5%, while EtOAc was tolerated only at ≤0.1%. PCA revealed clear clustering of behavioral responses primarily driven by light–dark transitions and further modulated by solvent type and concentration.

CONCLUSION: Concentration-dependent behavioral effects highlight the importance of careful solvent selection in zebrafish assays. Under the tested conditions, ≤0.1% Ace and EtOAc, and ≤0.05% DMSO, EtOH, MeOH, ACN, and IPA can be considered suitable solvent concentrations for behavioral studies. These results promote experimental reproducibility and reliability by establishing reference values for solvent selection and concentration limits in zebrafish behavioral assays under the investigated experimental conditions.

168

Modelling Mucopolysaccharidosis type 1 in zebrafish for development of local retinal therapies

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Topic

1 Disease Models

Abstract Content

Background: Mucopolysaccharidosis type 1 (MPS1) is a severe lysosomal storage disorder caused by mutations in the *IDUA gene*. Hematopoietic stem cell transplant, although life-saving, does not impede progressive retinopathy. Lipid nanoparticles (LNPs) are a promising delivery vehicle for gene editing technologies, thus potentially enabling permanent correction of the mutation in the retina. We aim to generate an MPS1 zebrafish model carrying a severe patient mutation and use it as a platform to evaluate retinal delivery strategies with LNPs, assessing gene-editing efficiency through targeted gene correction.

Methods: MPS1 zebrafish knock-in lines were generated by co-injecting Cas9 protein, a gRNA, a DNA template containing the Q70X in wild-type zebrafish eggs. F0 founders were outcrossed, and progeny was screened by Sanger sequencing and raised. Cy5-labelled LNPs were used to encapsulate GFP mRNA. 1nl of LNP solution was injected into one eye of anesthetized 3dpf albino larvae. After 24h and 48h larvae were fixed for either whole-mount microscopy or immunofluorescent staining.

Results: We successfully generated an *idua* Q70X fish and a knockout line carrying a 10nt deletion in exon 6, leading to a premature stop codon. Both lines are currently being characterized for disease-relevant phenotypes. In parallel, we tested LNPs as a potential delivery system in zebrafish larvae by injecting GFP-LNPs into the yolk of freshly fertilized embryos or in the eye of 3dpf larvae. Both approaches yielded positive GFP signal, confirming that LNPs can successfully deliver GFP mRNA into different tissues in zebrafish.

Conclusion: We have confirmed that zebrafish can be used as a platform to assess retinal LNP delivery efficiency. Together with our newly generated MPS1 lines, we aim to develop and optimize retinal gene therapy strategies, as well as to improve our understanding of *in vivo* gene therapy for treatment of rare metabolic disorders.

169

Interspecies variation in phlorotannin content and antioxidant activity of Adriatic Ericaria macroalgae evaluated using zebrafish *Danio rerio*

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Topic

10 Toxicology

Abstract Content

BACKGROUND: Phlorotannins are polyphenolic compounds unique to brown macroalgae and have attracted considerable attention due to their diverse bioactivities. However, their content and bioactivity may vary considerably among closely related species. The aim of this study was to investigate interspecies variation in phlorotannin-rich fractions obtained from three Adriatic species, *Ericaria crinita, Ericaria mediterranea*, and *Ericaria amentacea*, and evaluate their antioxidant potential using both *in vitro* assays and a zebrafish model.

METHODS: Phlorotannin-rich fractions were isolated from *Ericaria* species collected in the Adriatic Sea by extraction with 80% ethanol, followed by solid-phase extraction. Total phenolic content was determined using the Folin–Ciocalteu method, while chemical characterization of the obtained fractions was performed using UHPLC-ESI-HRMS. Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays. *In vivo* assays were performed using zebrafish embryos, where oxidative stress was induced with hydrogen peroxide to evaluate the protective antioxidant effects of tested fractions.

RESULTS: Considerable interspecies variation was observed among the investigated *Ericaria* fractions. Across the analyzed fractions, the total phenolic content ranged from 0.037-0.2017 g PGE/g fraction. The strongest *in vitro* antioxidant activity was also observed for *E. amentacea* (DPPH IC_{50}=0.09 mg/mL; ABTS IC_{50}=0.06 mg/mL). UHPLC-ESI-HRMS profiling further confirmed interspecies differences in phlorotannin composition. Zebrafish embryotoxicity test revealed species-dependent differences in toxicity profiles, with *E. crinita* and *E. mediterranea* showing more favorable safety profiles, whereas *E. amentacea* exhibited higher toxicity and a narrower non-toxic range. A similar species-dependent trend was observed in the *in vivo* antioxidant assay, reflecting differences in both antioxidant potential and safety profiles.

CONCLUSION: The results demonstrate pronounced interspecies variability in phlorotannin content, antioxidant capacity, and biological effects among *Ericaria* species. The zebrafish embryo model proved to be a useful *in vivo* platform for evaluating the activity and safety of phlorotannin-rich fractions.

170

Toward therapeutic approaches for Usher syndrome type 2: development of a zebrafish ush2a model

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Topic

1 Disease Models

Abstract Content

Usher syndrome is the most common cause of inherited deaf-blindness, characterized by congenital or early-onset sensorineural hearing loss and progressive retinal degeneration. Mutations in the **USH2A** gene account for the majority of cases of Usher syndrome type 2. Animal models that accurately reproduce disease-associated mutations are essential for studying the underlying pathophysiological mechanisms and for evaluating emerging therapeutic strategies. The zebrafish (*Danio rerio*) represents a powerful vertebrate model for studying sensory disorders due to the high conservation of genes involved in auditory and visual function.

In this study, we aimed to generate a zebrafish model of USH2A-associated disease by inducing a targeted deletion within the zebrafish ortholog ush2a. Using CRISPR/Cas9 genome editing, gRNAs were designed to flank exons 58–63, enabling removal of this genomic region following microinjection of Cas9 ribonucleoprotein complexes into one-cell stage embryos. This region was selected to generate an out-of-frame deletion, resulting in a predicted premature termination codon and functional loss of the Ush2a protein.

Genomic analysis of injected embryos revealed the presence of the expected deletion event. Sanger sequencing confirmed precise excision within the **ush2a** locus in F0 mosaic embryos. The resulting frameshift is predicted to mimic **loss-of-function mechanisms associated with pathogenic USH2A variants,** including nonsense mutations such as **c.11864G>A (p.Trp3955Ter)** reported in patients from the Slovenian and broader Balkan population.

The generation of this zebrafish model represents an important step toward investigating the functional consequences of ush2a disruption *in vivo*. Ongoing work will focus on establishing stable mutant lines and characterizing sensory phenotypes, as well as evaluating experimental therapeutic approaches, including gene electrotransfer (GET) to enable efficient plasmid uptake in retinal cells.

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171

Elucidating SNRNP70-TARDBP interactions and their contribution to neuronal development and disease.

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Topic

3 Neurobiology

Abstract Content

SNRNP70 (small nuclear ribonucleoprotein 70) is a major spliceosome protein in the core of the U1 snRNP complex, where it plays a key role in initiating pre-mRNA splicing. Intriguingly, SNRNP70 is also detected within extra-nuclear regions where it co-localises with RNA in axons and dendrites while being necessary for neuromuscular junction assembly. However, the overall cytoplasmic function of SNRNP70 remains unclear.

To determine the molecular function of SNRNP70, proteomic approaches were previously conducted in the lab, which indicated that cytoplasmic SNRNP70 interacts with various proteins, including the RNA and DNA binding protein TARDBP. TARDBP, a predominantly nuclear protein, has key roles in mRNA processing, trafficking and the suppression of cryptic exon splicing. Similar to SNRNP70, TARDBP is known to shuttle between nuclear and cytoplasmic areas where it has been found to traffic ribosomal protein mRNAs to the ribosome. We demonstrated that SNRNP70 directly binds to TARDBP and that these protein-protein interactions can be mapped to nuclear, soma cytoplasm and axonal regions of neurons.

We hypothesise that SNRNP70-TARDBP interactions play a critical role within neurons. Interestingly, both SNRNP70 and TARDBP are well known to mis-localise/aggregate in neurodegenerative diseases, specifically ALS and Alzheimer's disease (AD), suggesting that a potential breakdown of such interactions could contribute to disease. Our current research focuses on investigating these interactions further to elucidate how they regulate axonal growth and maintenance of neuronal connections.

174

Impact of neuroactive chemicals on vertical positioning and swim bladder size in zebrafish larvae

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Topic

7 Neural Circuit Behavior

Abstract Content

Neuroactive chemicals are frequently detected in European waterbodies and may pose a risk to non-target species like fish by altering neuronal function and behavior. Substances with anxiolytic-like properties have been shown to increase surface-oriented behavior in zebrafish larvae, a response that similarly occurs under hypoxic conditions or when the function of anterior lateral line is impaired. Moreover, vertical positioning and buoyancy control, which rely on proper swim bladder regulation, are essential for finding resources, mating, and predator avoidance.

In this study, we tested eight chemicals with diverse modes of action (Aldicarb, Buspirone, Caffeine, Carbamazepine, Fentanyl, Fluoxetine, Nicotine, Pentylenetetrazol) and found that six of them induced swim bladder hyperinflation (SBHI) after 16 h of exposure, and a significant change in vertical positioning over 4 h of behavioral tracking. For four chemicals, SBHI was also present at the end of the 4-hour behavioral assay, indicating that increased surfacing preceded SBHI.

The mechanistic relationship between surfacing behavior and SBHI remains unresolved, but this project currently investigates two potential mechanisms: (i) chemical interference with oxygen sensing modalities driving hypoxia-like responses, or (ii) disruption of the lateral-line mediated surface detection leading to excessive air intake.

By linking chemically altered vertical positioning with SBHI, this work not only identifies sensitive early-life behavioral and morphological endpoints relevant for environmental risk assessment, but also provides valuable insight into fundamental mechanisms governing sensory integration, buoyancy regulation, and organ function in zebrafish larvae.

178

The KCNC3^{R420H} SCA13 causative mutant causes the inhibition of autophagosome-lysosome fusion

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Topic

3 Neurobiology

Abstract Content

Spinocerebellar Ataxia Type 13 (SCA13) is an autosomal dominant neurodegenerative disease caused by mutated alleles encoding the voltage-gated potassium channel KCNC3. *KCNC3* is highly expressed in cerebellar Purkinje cells (PCs), the sole output neurons in the cerebellum. The R420H mutation results in a dominant-negative non-functional channel subunit, which leads to mid-age-onset ataxia manifested by progressive cerebellar atrophy. Endogenous KCNC3 is located on the plasma membrane, whereas KCNC3^{R420H} is retained in the Golgi perinuclear region, likely associated with an altered glycosylation pattern of this mutant.

To establish an *in vivo* animal model system for SCA13, we generated a stable transgenic zebrafish strain with PC-specific co-expression of *KCNC3*^{R420H}, together with fluorescent reporters, revealing progressive degeneration of PCs during larval stages. 3D analysis of cleared brains from adult zebrafish expressing *KCNC3*^{R420H} indicated a decreased cerebellar volume. Furthermore, using a fluorescent LC3 reporter, which marks autophagosomes, we observed an accumulation of autophagosomes in KCNC3^{R420H}-affected PCs.

Further analysis using murine NIH3T3 fibroblasts showed that expression of *KCNC3*^{R420H} phenocopied autophagic alterations observed in zebrafish in these cells, consistent with autophagy impairments caused by a *de novo* function of KCNC3^{R420H}. Studies investigating a) autophagy induction, b) autophagic flux using the autophagy inhibitor Bafilomycin A1, and c) lysosomal acidification and cargo destruction, revealed that in *KCNC3*^{R420H} expressing fibroblasts, the fusion of the autophagosome with lysosomal vesicle is inhibited. Furthermore, metabolic analysis in these *KCNC3*^{R420H}-expressing SCA13-cells revealed an increase in oxidative stress, a depletion of ATP, and an altered redox potential. These findings provide a potential mechanistic explanation for the neurodegenerative effects of KCNC3^{R420H} *in vivo*.

Our comprehensive analysis of zebrafish PCs and cultured mouse fibroblasts indicates a link between SCA13 and autophagy dysregulation, which is currently being examined in neuronal cells and zebrafish to reveal mechanistic insights into the neuronal degeneration process occurring in SCA13 *in vivo*.

179

Identification of pathogenic variants in the BORCS5 and BORCS8 genes associated with rare neurodevelopmental disorders

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Topic

3 Neurobiology

Abstract Content

The BLOC-One-Related (BORC) complex is a ubiquitously expressed protein complex composed of eight subunits called BORCS1-8. Consistent with its role in axonal lysosome transport, mutations in BORC subunits cause neurological defects in mice. However, the physiological significance of BORC in humans remains to be determined.

We have identified twelve patients with BORCS5 gene mutations and five patients with BORCS8 gene mutations, all of whom have severe neurodevelopmental disorders. Individuals affected by BORCS8 mutations exhibited global developmental delay, progressive muscle wasting, and optic atrophy. Patients with BORCS5 mutations had brain malformations, microcephaly, optic atrophy, and progressive motor disorders.

We generated a borcs8 F0 KO model in zebrafish using CRISPR/Cas9 editing. Mutant larvae showed a marked reduction in body, head and eye size, as well as a locomotor defect associated with a decrease in dorsal and ventral myotomes and shorter axons.

We also developed borcs5 F0 KO models and overexpression models via RNA injection. These reproduced early morphological defects (reduced body, head and eye size), as well as locomotor abnormalities. The mutants presented microcephaly and axonal and muscular alterations.

Overall, we have identified mutations in BORCS5 and BORCS8 for the first time, and our results demonstrate that they are essential for the development and proper functioning of the central nervous system.

180

Modelling spinal muscular atrophy in zebrafish model

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Topic

3 Neurobiology

Abstract Content

Spinal Muscular Atrophy (SMA) is a rare autosomal recessive neurodegenerative disease primarily caused by mutations in SMN1. It is the leading genetic cause of infant mortality. Despite recent advances in the SMA therapeutics, there remains a need to identify potential therapeutic compounds that can exert their effects via non-SMN dependent mechanisms. Morpholino-based knockdown is commonly used in zebrafish to study SMA pathogenesis. However, this smn knockdown model is transient and not appropriate for efficient drug discovery purposes. Thus, development of a stable model for screening candidate drugs would be valuable for identifying of lead compounds as potential therapeutics for SMA. Here we generated and characterized a smn KO zebrafish mutant using the CRISPR/Cas9 genome targeting. The smn mutants displayed impaired locomotor activity, axon branching defects and abnormal NMJ structures. We demonstrated that loss-of-function of smn specifically in motoneuron is principally the cause of motor deficits, motoneuron loss and muscle atrophy in zebrafish. As a proof-of-principal, we showed that treatment of the smn mutant larvae with salubrinal significantly improved locomotor activity. These findings show the potential value of using this zebrafish SMA model for efficient in vivo screening of potential therapeutic compounds.

181

Modeling Glycogen Storage Disease XV in Zebrafish: Insights into Local Glycogen Storage and Tissue Homeostasis

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Topic

1 Disease Models

Abstract Content

Glycogen plays a central role in glucose homeostasis, yet its regulation outside classical metabolic tissues such as liver and muscle remains poorly understood. We identified a previously uncharacterized population of mesenchymal dermal fibroblasts (DFs) enriched in enzymes involved in glycogenesis and gluconeogenesis, suggesting a potential role in local glycogen metabolism.

To investigate this metabolic relationship, we are using zebrafish as a model system. Due to evolutionary divergence, zebrafish possess two paralogs of glycogenin-1: ***gyg1a*** (enriched in the liver) and ***gyg1b***, (expressed in mesenchymal dermal fibroblasts). We are applying CRISPR/Cas9-mediated mutagenesis to disrupt ***gyg1b*** in order to determine whether DFs can convert Phe/Tyr amino acids into glycogen and to assess their contribution to local glucose and glycogen metabolism.

Transcriptomic analyses confirm that dermal fibroblasts are enriched for genes involved in glycogen metabolism as well as enzymes of the Phe/Tyr degradation pathway. The selective expression of ***gyg1b*** in these cells suggests a specialized metabolic role distinct from classical glycogen-storing tissues, indicating a possible link between amino acid catabolism and glycogen synthesis in these cells.

This study aims to clarify whether dermal fibroblasts contribute to local glucose and glycogen production through amino acid catabolism. Understanding these cell-specific metabolic pathways may provide new insight into systemic glucose homeostasis and improve our understanding of the mechanisms underlying Glycogen Storage Disease XV (GSDXV).

182

Investigating the origins of heart defects in Adams-Oliver Syndrome – identifying requirements for a dock6 intron in cardiac development.

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Topic

1 Disease Models

Abstract Content

Background: Adams-Oliver Syndrome (AOS) is a rare multisystemic congenital disorder. To-date mutations in 6 genes have been associated with AOS (*DOCK6*, *EOGT*, *NOTCH1*, *RBPJ*, *ARHGAP31* and *DLL4*), however only ~50% of AOS patients receive a genetic diagnosis, typically through targeted exome sequencing. Congenital heart defects (CHDs) are observed in ~20% of AOS cases, however for individuals with mutations in *DOCK6* this rises to ~50%. In many AOS cases the molecular aetiology is unclear, and the mechanisms underpinning CHDs in DOCK6-associated AOS are ill-defined.

Methods and Results: To investigate the role of *dock6* in cardiac development we analysed its expression in zebrafish embryos. Using *in situ* hybridisation we identified that *dock6* is expressed in a subset of embryonic tissues, including vascular structures. We further observed expression of several unannotated intron-retaining *dock6* transcripts, including an intron1 transcript that is expressed in the developing heart. Analysis of published CHIP-Seq data revealed that part of this cardiac intronic transcript overlies putative enhancer marks. To test whether this region of *dock6-intron1* has enhancer activity, we generated stable transgenic zebrafish lines expressing *dock6-intron1* enhancer constructs containing a minimal promoter and fluorophore. We consistently observed tissue-specific fluorescence driven by *dock6-intron1*, particularly in vascular and cardiac structures consistent with those observed by *in situ* hybridisation, suggesting that *dock6-intron1* has enhancer activity. To test the function of *dock6-intron1* in development we generated a zebrafish line harbouring an INDEL mutation in this intronic region. Analysis of $dock6^{intron1}$ mutants identifies cardiac-specific defects, including abnormal chamber alignment during early heart morphogenesis.

Conclusions: Together our data identify a new cardiac-specific transcript of *dock6,* alongside an intronic enhancer, that are important for zebrafish heart development. We suggest that a broader approach to sequencing in AOS patients may identify non-coding variants that could underpin AOS phenotypes in individuals without a current genetic diagnosis.

187

Genetic rescue of FAH deficiency in zebrafish reveals systemic origins of Tyrosinemia type I toxicity

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Topic

1 Disease Models

Abstract Content

Tyrosinemia type I is a rare inherited metabolic disorder caused by deficiency of fumarylacetoacetate hydrolase (FAH), the enzyme catalyzing the final step of the phenylalanine/tyrosine (Phe/Tyr) degradation pathway. Loss of FAH leads to accumulation of toxic metabolites, including succinylacetone, and without treatment the disease is usually fatal within the first year of life.

Using in situ hybridization, we identified expression of the zebrafish *fah* ortholog in the expected hepatic domain and unexpectedly in craniofacial dermal fibroblasts. To investigate its function, we generated a *fah* mutant line. At 5 days post fertilization (dpf), *fah*$^{-/-}$ larvae showed normal body morphology and swim bladder inflation. However, similar to the human condition, mutants died rapidly and failed to survive beyond 8 dpf. Skeletal staining with Alcian Blue and Alizarin Red revealed pronounced deformation of the opercular bone in *fah*$^{-/-}$ larvae.

To test whether blocking earlier steps in the Phe/Tyr degradation pathway could rescue the phenotype, we genetically targeted upstream enzymes. Disruption of *hgd* (homogentisate 1,2-dioxygenase) throughout the organism fully rescued the lethal phenotype, producing viable and fertile *fah*$^{-/-}$ *hgd*$^{-/-}$ double mutants. In contrast, tissue-specific disruption of *hpd* paralogs in either the liver (*hpda*) or dermal fibroblasts (*hpdb*) alone was insufficient to rescue survival.

These findings suggest that toxic metabolites produced in multiple tissues contribute to systemic intoxication in FAH deficiency. Our results establish zebrafish as a powerful model for Tyrosinemia type I and provide a platform for future pharmacological studies, while highlighting the contribution of extrahepatic metabolism to whole-organism physiology.

188

Modelling mitochondrial tRNA deficits in zebrafish as a translational model: towards concept and clinical insights

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Topic

1 Disease Models

Abstract Content

Background
Mitochondrial amino-acyl tRNA-synthetase (mt-ARS) deficiencies are rare nuclear-encoded mitochondrial diseases with wide phenotypic variability and are often characterized by severe central nervous system involvement including epilepsy, spastic paraplegia and developmental delay. Although the canonical function of mt-ARS enzymes is well established, their impact on oxidative phosphorylation (OXPHOS) dysfunction does not seem to explain the full phenotype. Given that mitochondrial function and neuroanatomy are well conserved in zebrafish (Danio rerio), this represents an excellent model to study the implications of mt-ARS deficiencies.

Methods
Crispant and knock-out (KO) zebrafish models for human mt-ARS disorders, specifically phenylalanyl- and asparaginyl-tRNA synthetase (fars2 and nars2, respectively), are created using CRISPR/Cas9 genome editing. Biochemical assays of OXPHOS complex abundance and activities, Seahorse analysis and real-time quantitative PCR (RT-qPCR) are used to evaluate mitochondrial respiration and functions, whereas a transgenic neuronal-GFP labeled line, fluorescent mitochondrial labeling and swimming behavior analysis are used to gain insight into neurological and locomotor phenotypes.

Results

Preliminary biochemical data showed a wide biological variety for OXPHOS complex activities in wild-type zebrafish, whereas preliminary Seahorse data of crispant zebrafish show reduced mitochondrial function, indicating the research approach is promising for modeling mt-ARS disease. In addition, behavioral data of crispant larval zebrafish revealed a shorter distance moved compared to scrambled treated zebrafish. Adult crispant zebrafish show a wide phenotypic variety including length differences, irregular pigmentation and heart edema formation. Mitochondrial fluorescent labeling decreased dose-dependently with mitochondrial inhibitors, thus representing a useful platform to track dysfunctional mitochondria within the zebrafish. Additionally, preliminary RT-qPCR data reveal differential regulation of mitochondrial genes.

Conclusion
These preliminary results show this approach, with extensive biochemical, transcriptome and behavioral phenotyping of mt-ARS deficient zebrafish models could provide valuable insight into disease mechanisms, which can be correlated to phenotypic features in humans and used to explore future therapeutic options.

190

Exploring the interplay between tempo and reproducibility in embryonic development

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Embryonic development is precisely timed and coordinated to ensure a robust outcome in a changing environment. However, a growing amount of evidence suggests that accelerating development by using cues like high temperature increases error rate and leads to a loss of reproducibility. In the present work, I am exploring whether the fidelity of developmental processes depends on the rate at which development proceeds by testing the opposite approach: whether deliberately slowing down developmental tempo increases the reproducibility of embryogenesis, with a particular focus on developmental gene regulatory networks (GRNs) that define the different cell type lineages in a developing embryo.

Here, we used low-dose global pharmacological inhibition of protein synthesis to slow down development in zebrafish embryos and staged them using an automated deep learning-based model. Preliminary results suggest quantifiable differences in the extent to which inhibitors with different mechanisms of action (e.g. ones targeting translation initiation vs elongation) globally delay developmental progression in contrast to a control treatment, and indicate that some parts of the translation process are more tunable for controlling developmental rate than others. I predict that translation inhibition affects the tempo of development via transcription factor, and thus GRN, activity, a hypothesis I will test using individually-resolved sci-ATAC-seq. With this approach, I hope to determine whether errors in developmental progression can be rescued or even further reduced by slower GRN progression.

191

Roles of myelin in live neurofilament dynamics

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Topic

3 Neurobiology

Abstract Content

In the vertebrate system, myelinating glia form myelin to surround large axons and allow for efficient saltatory conduction and neuronal communication. In humans, myelination defects can cause debilitating neurodegenerative diseases including multiple sclerosis and some forms of Charcot-Marie-Tooth (CMT) disease. A common hallmark of neurodegenerative diseases is accumulation of neurofilaments (NFs), the major cytoskeletal component in neurons, and mutations in neurofilaments have also been linked to CMT. *In vitro* and *ex vivo* studies have revealed multiple relationships between myelination and neurofilaments. Both are intrinsic to axon radial expansion and the establishment of nodes of Ranvier, and neurofilament subunits become phosphorylated at the onset of myelination. Neurofilament transport is slower in myelinated areas when compared to nodes of Ranvier *ex vivo* and unmyelinated sections *in vitro*. However, this relationship has not been studied in a live *in vivo* model and little is known about the mechanisms involved. To study neurofilament dynamics *in vivo,* our lab has developed a transgenic zebrafish line which expresses neurofilament tagged with a photoconvertible fluorophore. With this transgene, we can perform pulse-escape experiments to measure neurofilament movements in live zebrafish larvae. To explore the relationship between neurofilaments and myelination, I will combine this photoconversion technique with established methods to manipulate myelination, including demyelination injury and inhibition of myelinating cell development. Once established, this model will allow us to explore many open questions, such as potential differences between the central and peripheral nervous systems and the mechanisms involved in neurofilament and myelin interactions. These studies may also help us better understand neurodegenerative diseases and provide us with potential targets for treatment.

192

Propagation of phenotypic variability in early zebrafish embryogenesis

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Topic

9 Germline and Early Development

Abstract Content

Embryogenesis has evolved to produce highly stereotyped forms despite biomolecular stochasticity and environmental variations. However, environmental factors such as thermal stress may nonetheless impose challenges on maintaining consistent developmental outcomes. While the influence of temperature on developmental rate and shifts in average phenotype is known, its impact on phenotypic *variability* (i.e., the propensity of a trait to vary around an average phenotype) from the molecular to the organismal scale remains poorly understood. While variability under stochastic or environmental perturbations can influence cell fate decisions or be buffered against to stabilize developmental outcomes, most measurements of variability are often studied in isolation and are not linked across molecular and cellular scales to understand their consequences on organismal phenotypes.

To investigate the molecular and cellular basis of embryo-to-embryo variability in zebrafish development, we generated an individually-resolved time-series atlas of early zebrafish embryogenesis at different temperatures (16 embryos x 8 timepoints, 2–16 hpf, 28°C and 34°C) using single-nucleus combinatorial barcoding and indexing (sci-RNA-seq3). This approach simultaneously captures information on mRNA abundance, cell type abundance, and whole-embryo cell type composition, enabling the quantification of phenotypic variability at each scale. We leverage the unique structure of this dataset to develop a hierarchical framework that tracks how variability propagates through these layers.

In a preliminary analysis, we apply this statistical modeling of variance to track how both gene-specific expression variability within a cell type and individual-to-individual variability in cell type proportions shifts over development. We compare estimates of variance at both gene- and cell-levels to ask whether variability is dampened, maintained, or amplified across these scales. Overall, we provide an individually-resolved zebrafish atlas of early embryogenesis, develop a statistical framework to integrate multiple layers of phenotypic variability, and plan to use these resources to relate baseline variability to responsiveness under temperature stress.

194

Development of Social Preference in a Shoaling and a Territorial Fish Species

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background: This study compares the social preference of zebrafish (*Danio rerio*), a shoaling species, and of the Siamese fighting fish (*Betta splendens*), a territorial and aggressive species, across ontogenetic development. Behavioural methods were developed for measuring social preference of larvae and adult to derive comparable quantitative indexes of social affiliation. Comparing species that diverge in social affiliation presents an opportunity to investigate the proximate mechanisms underlying social behaviour.

Methods: Social preference was measured from 5 days-post-hatching onwards. Larvae were placed in U-shaped binary choice arenas and, after 15 minutes for acclimation, their choice to stay alone or close to a conspecific was recorded for 15 minutes. Tests ran daily until 15 days-post-hatch then weekly until 10 weeks-post-hatch. For 9–12 months adults, a linear choice arena was used to measure the affiliation choice (empty versus one male and one female). To account for long-term affiliative preferences, the choice test ran for 7.5 hours.

Preliminary results: Both species develop social preference around 4–5 weeks-post-hatch. Surprisingly, the social preference index of fighting fish was stronger than for zebrafish around 6–9 weeks-post-hatch. Fighting fish are also more reluctant to leave the affiliation area once they enter. This result can reflect the development of agonistic behaviour during this period. Preliminary data for adults seems to confirm the switch from affiliative to solitary behaviour in fighting fish while the zebrafish maintains their social preference.

Conclusion: The two species associate with conspecifics during the first weeks of development, but differences in social behaviour seem to already emerge as larvae. Adults' behaviour seems to indicate the shoaling behaviour of zebrafish and the more solitary behaviour of fighting fish. If confirmed, these results allow establishing the two species as appropriate models for investigating the mechanisms of social preference in fish.

195

Understanding the mechanical basis of morphogenetic pace during vertebrate gastrulation

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Embryogenesis relies on the timely succession of differentiation and morphogenetic steps. Remarkably, the pace of these conserved steps varies significantly across species. A striking example is gastrulation, the crucial process that specifies and shapes the germ layers, which takes 2x longer in medaka (Oryzias latipes) than zebrafish (Danio rerio) in the same laboratory conditions. While such pace differences are well-known, how time is kept during development remains poorly understood. Recent work using *in vitro* models revealed a key role for interspecies differences in biochemical reactions in defining the pace of differentiation. It is unknown, however, whether and how these molecular parameters set the pace of morphogenesis. Morphogenesis is a tissue-scale process, set by the interplay between cell- intrinsic forces and resisting tissue material properties, which are not easily derived from subcellular molecular turnover.

In this project, we aim to define which biophysical parameters scale the pace of gastrulation movements in medaka and zebrafish. For this, we are using in vivo single plane illumination microscopy and quantitative image analysis to characterize the pace of gastrulation across species. Our preliminary results suggest that the cell movements that power internalization are indeed slower in Medaka. In the future, we will combine interspecies cell transplantations, in vitro force measuring and theoretical modeling to define whether the pace of morphogenesis to define the relative contributions of cell-intrinsic forces and tissue material properties. This project aims to uncover the biophysical mechanisms pacing the tissue movements shaping the vertebrate embryo, the first step in understanding how developmental pace is controlled from the molecular to the tissue scale.

196

Exploring the role of Mohawk homeobox A in the zebrafish musculoskeletal system

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Topic

5 Tissue Formation

Abstract Content

Background

Locomotion in vertebrates relies on the coordinated function of muscles, bones, and connective tissues, including tendons and ligaments that transmit force across the musculoskeletal system. While the transcription factor Scleraxis is well established as a regulator of tendon development, the functional role of Mohawk homeobox (Mkx) in the musculoskeletal system is much less well characterized. Although Mkx has been associated with connective tissues in mammals, its broader developmental and physiological contributions have not been extensively characterized in fish.

Methods

A reporter knock-in zebrafish line was generated to visualize mkxa expression while simultaneously disrupting gene activity. Endogenous activity of mkxa was characterized in the craniofacial and axial musculoskeletal systems, and loss of mkxa was assessed through analyses of growth, viability, and locomotor performance under controlled environmental conditions. In parallel, we are performing musculoskeletal tissue characterization, differential expression analysis and non-destructive chemical analyses to determine how mkxa inactivation affects surrounding tissues and co-localized tissue-specific markers.

Results

Loss of mkxa was associated with reduced viability and altered developmental progression compared to wild-type siblings. Behavioural analyses suggested differences in locomotor activity during larval stages, and mutant larvae appeared smaller than wild-type siblings during later developmental stages, suggesting impaired growth.

Conclusion

Together, these findings suggest that mkxa may influence survival, growth, and locomotor behaviour in zebrafish. Future analyses, including metabolomics, single-cell transcriptomics and Raman-based chemical imaging, will help determine the underlying developmental or physiological mechanisms responsible for these phenotypes

The authors thank Beata Filipek-Górniok at DanioReadout for generating knock-in line.

199

Matrix Metalloproteinases and Heat shock protein 70 in Diabetic Retinopathy

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Topic

1 Disease Models

Abstract Content

Diabetic retinopathy (DR) is a major complication of diabetes characterized by vascular and neuronal damage in the retina. Retinal neurodegeneration has been hypothesized to contribute to DR pathogenesis either independently or as a consequence of vascular injury. Although hyperglycaemia affects both vascular and neuronal cells, the molecular mediators involved remain poorly defined. Inflammatory processes regulated by matrix metalloproteinases (Mmps) are central to both tissue injury and regeneration, and emerging evidence implicates Mmps in DR-related tissue damage as well as in photoreceptor (PR) regeneration. Moreover, heat shock protein 70 (Hsp70), a key regulator of cellular stress responses and proteostasis, was suggested to interact with activated Mmp9, thereby contributing to mitochondrial injury.

We currently examine the interplay of Mmp9 and Hsp70 in an established zebrafish model of DR, where PR restoration occurs alongside vascular damage and neurodegeneration. Early results show that Hsp70 and Mmp9 proteins localize to the retinal photoreceptor layer, in proximity to mitochondria. We are furthermore quantitating protein levels and using in situ methods to detect and localize Mmp protein activity. Studies have shown that *hsp70* and *mmp9* are both expressed by Muller glial cells, which play a central role in retinal homeostasis, inflammation and regeneration. To better understand transcriptional changes in specific cell types, single-cell RNA sequencing of diabetic and control retinae was performed. We are characterizing transcriptional states of Müller glia and photoreceptor progenitor populations, to define disease-related modulation of inflammation- and regeneration-associated pathways.

Understanding how Mmps and Hsp70 contribute to both degeneration and cell restoration will clarify their role in maintaining retinal homeostasis during diabetes and other retinal pathologies. These studies additionally help identify novel mediators of injury and repair operating under altered metabolic conditions.

200

Heart injury-induced Fibronectin 1 confers liver dysfunction in zebrafish through hepatic Pparβ signaling

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Topic

8 Metabolism

Abstract Content

Background: Organ-organ crosstalk mediated by secretory factors plays crucial roles in maintaining physiologic homeostasis. Heart-related diseases, such as heart failure and cardiac ischemia/reperfusion, significantly impact liver pathological progression, yet the exact molecular mechanisms remain unclear.

Methods: Utilizing a zebrafish ventricle ablation model, we investigated the effects of heart injury on liver development and function.

Results: Our results revealed heart injury induced liver stunted growth and aberrant lipid accumulation in zebrafish. Mechanistically, heart injury increased cardiac and serum Fn1 protein levels and activated hepatic integrin-Src axis, while reduced hepatic Ampkα phosphorylation and Pparβ abundance. Heart-specific Fn1 knockdown reduced the activation of hepatic integrin-Src axis, elevated hepatic Ampkα phosphorylation and Pparβ protein levels, and ameliorated these liver defects. Moreover, Ampkα activation increased Pparβ protein and β-oxidation gene expression, whereas hepatic Pparβ knockdown abolished the liver protection conferred by cardiac Fn1 knockdown.

Conclusion: Our study demonstrated that heart injury-induced liver stunted growth and aberrant lipid accumulation is conferred by cardiac-derived Fn1, which acts through the suppression of hepatic Ampkα–Pparβ signaling. Further understanding of the molecular mechanisms underlying the inter-organ communication could provide new insight to treat liver disorders induced by heart diseases.

201

Germplasm decoded – how does the germplasm instruct germline formation and function?

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Topic

9 Germline and Early Development

Abstract Content

Background:

In sexually reproducing organisms, establishing the germline is the first critical step towards reproduction and species survival. Instructions for germline specification can come either from the soma (epigenesis) or from within the future germ cells (preformation). In animals employing preformation, such as zebrafish and other teleost fish, the substance containing instructions is called germplasm (GP), a sub-cellular condensate of maternally-provided mRNAs and proteins. While we know that GP is essential for germline establishment, we do not know how those instructions contained within the GP are extracted to dictate germline development.

Methods:

To investigate the mechanism of GP-resident maternal mRNA translation and its impact on germline specification, I am using zebrafish embryos. First, using complementary immunoprecipitation techniques followed by RNA sequencing and proteomics, I am generating a catalogue of GP mRNA and protein composition before and after zygotic genome activation, when the GP transitions from a discrete condensate to dispersed punctae. This information is crucial to understanding how the molecular identity of GP changes over time, and how these changes may underpin the accessibility of maternal mRNAs.

To explore how maternal mRNAs are accessed by the translational machinery, I am using high-resolution microscopy to test the hypothesis that GP-resident mRNAs, such as *nanos3* and *dnd*, are selectively shifted to the GP periphery to interact with ribosomes and initiate localized translation. This will be done by imaging the 5'-3' mRNA orientation in fixed samples to determine how the conformation of mRNAs changes between non-translated and translated species, and by ALFA-tag-based live-imaging of translation to determine where these maternal mRNAs are translated in relation to the GP.

Results/Conclusion:

Taken together, this work will reveal the mechanisms by which the maternal mRNAs in the GP are decoded to instruct early germ cell development.

202

Whole-brain functional imaging of zebrafish larvae using a microfluidic chip reveals distinct neural dynamics during chemically induced brain states

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Topic

2 Emerging Technologies

Abstract Content

Microfluidic devices and light-sheet microscopy provide complementary capabilities for controlled stimulation and large-scale monitoring of neuronal activity in small model organisms. We recently introduced NeuroExaminer 2.0, a monolithic glass microfluidic chip compatible with a commercial light-sheet microscope that enables reversible loading of zebrafish larvae, precise delivery of soluble compounds, and whole-brain calcium imaging at cellular resolution.

Using this platform, we performed functional imaging of zebrafish larvae during controlled exposure to the anesthetic tricaine (MS-222) or the convulsant pentylenetetrazol (PTZ). After motion correction, neuron segmentation, and atlas registration, brain-wide activity dynamics were quantified across tens of thousands of neurons. PTZ induced rapid and widespread neuronal activation accompanied by seizure-like synchronization across brain regions. In contrast, tricaine caused strong suppression of forebrain activity and mild activation in some midbrain and hindbrain areas. During recovery, neural activity gradually returned to baseline after PTZ treatment, whereas tricaine washout produced a transient global increase in activity consistent with awakening from anesthesia. Despite inter-individual variability in response amplitude, both compounds elicited reproducible temporal activity patterns, demonstrating the robustness of the platform for brain-wide pharmacological studies.

These results establish NeuroExaminer 2.0 as a powerful platform for combining microfluidic stimulation with whole-brain calcium imaging to investigate brain-wide neural responses to pharmacological perturbations and brain-state dynamics in zebrafish larvae.

204

The Rhythm of Development – Quantifying temporal coordination across scales at single-cell resolution

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Topic

19 Cell fate

Abstract Content

Background

Vertebrate embryogenesis requires precise temporal coordination to ensure robust tissue patterning and morphogenesis. During somitogenesis, cells in the presomitic mesoderm (PSM) exhibit oscillatory expression of segmentation clock genes that regulates the timing of somite formation. While these oscillations provide a well-characterized temporal framework at the tissue level, it remains unclear how segmentation clock dynamics relate to developmental progression at single-cell resolution and how synchronized development is maintained across individual embryos. I aim to investigate how oscillatory gene expression can be leveraged to quantify developmental timing and synchrony using embryo-resolved sci-RNA-seq dataset spanning early developmental stages in *Danio Rerio*.

Methods

The analysis focuses on *her*-expressing somitic progenitor cells within the PSM, where tightly regulated oscillatory gene expression provides a natural temporal reference. By inferring the oscillation phase of *her* gene expression, individual embryos can be positioned along the the segmentation clock cycle rather than chronological time. Consequently, cells originating from the same embryo can be analyzed within a shared temporal coordinate system defined by PSM oscillation dynamics. This approach allows developmental trajectories and transcriptional programs to be compared across embryos and tissues with improved temporal resolution.

Results

Preliminary analyses suggest that notochord cells display coordinated transcriptional dynamics, with multiple genes showing expression patterns correlated with oscillatory programs in the PSM within the same embryo. This observation indicates that segmentation clock–defined developmental time may capture coordinated progression beyond somitic lineages.

Conclusion

Using oscillation-based embryo staging, we aim to quantify developmental synchrony during somitogenesis and assess variability in developmental progression across embryos and environmental conditions. This framework will enable identification of coordinated versus decoupled developmental programs and evaluation of how perturbations such as temperature affect temporal precision. Together, this work establishes a single-cell approach linking segmentation clock oscillations to developmental timing and robustness during vertebrate embryogenesis.

206

Dietary Self-DNA Delivered via Spirulina Impairs Reproduction, Metabolism and Neurogenic Markers in Zebrafish.

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Topic

8 Metabolism

Abstract Content

DNA can act as an immunologically active molecule, functioning as both pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively). Recent studies suggest that environmental or organism-released fragmented DNA may exert inhibitory effects on individuals of the same species. This phenomenon has been described in plants and invertebrates, including *Caenorhabditis elegans* and *Drosophila melanogaster*, where exposure to self-DNA induces defects in reproduction, growth and metabolic regulation.

In this context, we investigated the effects in zebrafish (*Danio rerio*) of dietary self-DNA delivered through spirulina (*Arthrospira platensis*), a widely used feed-supplement biostimulant in fish nutrition. Three experimental groups were established: C, control group fed a standard diet; A-25, fed a diet containing 25% wild-type spirulina and 75% standard feed; and S-25, fed a diet containing 25% spirulina enriched with self-DNA and 75% standard feed.

Metabolomic analysis showed only for the S-25 group an unbalanced purine metabolism, including increased levels of deoxyadenosine, guanine, xanthine, and uric acid, thus suggesting enhanced purine catabolism and potential oxidative stress. These changes were accompanied by higher levels of L-cystathionine, reduced phospholipids such as glycerophosphocholine, and increased asymmetric dimethylarginine (ADMA), indicating possible impairment of nitric oxide signalling in the S-25 group.

Preliminary transcriptomic analysis revealed differential expression of genes associated with metabolic regulation and oxidative stress responses, including SOCS3, FABP7, CEBPD, and CCS, supporting the occurrence of metabolic imbalance and activation of antioxidant pathways.

Reproductive assays showed that exposure to self-DNA reduced egg production by 38% and larval hatching by 55%. Molecular analyses further revealed reduced expression of gonadotropins and their receptors in the S-25 group, suggesting disruption of the reproductive endocrine axis. In addition, brain analyses showed reduced expression of the neurogenic markers Sox2 and PCNA.

Overall, these preliminary findings suggest that self-DNA exposure may impact metabolic homeostasis, reproductive physiology and neurogenic processes in zebrafish.

207

Conservation of miR-202-regulated reproductive phenotypes and targets in teleost fish

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

MicroRNAs are small regulatory RNAs that post-transcriptionally regulate gene expression through sequence-specific interactions with target mRNAs. miR-202 is a highly conserved gonadal microRNA amongst vertebrates that has been shown to play a key role in reproductive success in teleost fishes, particularly in the Japanese medaka (*Oryzias latipes*). In medaka, loss of miR-202 leads to a strong reduction in female egg number and ability of the eggs to be fertilised, and to a decrease in sperm quantity and quality in males. Two direct miR-202 targets, tead3b and vgll4b, were identified in gonads and associated with the number of spawned eggs per female and male sperm quality, respectively.

To validate the interspecies conservation of miR-202 function, we analyzed its loss-of-function phenotype in zebrafish (*Danio rerio*) and performed *in silico* predictions to identify conserved targets between medaka and zebrafish.

In zebrafish, deletion of miR-202 results in a similar reproductive phenotype to that observed in medaka, with reduced egg number and fertilization success in females and decreased fertilization success in males. *In silico* analyses indicate that the medaka miR-202 targets tead3b and vgll4b are also predicted miR-202 targets in zebrafish, supporting the hypothesis that the same genes are involved in controlling female fecundity and male fertility in both species.

Our work suggests that miR-202 regulates conserved genetic networks underlying reproductive success in teleost fish. The convergence of phenotypic data and target predictions in medaka and zebrafish supports the idea that specific miRNA–target gene interactions can be conserved through evolution to control similar reproductive phenotypes in different vertebrate species.

209

Fishing for complaints: Investigating the cause of early lethality in *pitx1* mutant larvae

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The homeobox gene *paired-like homeodomain 1* (*pitx1*) is highly conserved among vertebrates and encodes a transcription factor with well-established roles in limb development. In mice, loss of Pitx1 leads to severe skeletal defects, including shortened hindlimbs and the absence of the patella. In humans, mutations or altered regulation of PITX1 have been linked to limb abnormalities such as polydactyly and familial clubfoot. In the teleost fish *Danio rerio*, Pitx1 is thought to regulate the formation of pelvic fins, the evolutionary counterpart of tetrapod hindlimbs. *pitx1* may also participate in additional developmental processes beyond hindlimb formation like regulating the growth of mandible in mice. To investigate the function of *pitx1* during zebrafish development, we generated a knockout line using CRISPR–Cas9 gene editing. The mutation consists of an 8-base-pair deletion that introduces a frameshift and a premature stop codon. While heterozygous animals develop normally without morphological abnormalities, homozygous mutants consistently fail to reach adulthood and die approximately three to four weeks post-fertilization. The cause of lethality is currently unknown. To identify homozygous mutants prior to the onset of dying, we established a live genotyping approach followed by PCR analysis using polyacrylamide gel electrophoresis. To explore mechanisms underlying the observed lethality, we analyzed single-cell RNA sequencing datasets to identify *pitx1*-expressing cell populations and potential downstream targets.Cryosection analyses revealed *pitx1* expression in the pharyngeal region, prompting examination of several candidate genes, including *muc5.1*, *muc5.3* and *agr2*. While *agr2* and *muc5.3* showed no consistent changes in expression, *muc5.1* needs to be studied in greater detail in order to draw any meaningful conclusions. In addition, we have started to investigate whether other pharyngeal-derived tissues, such as the thymus, develop normally. Although the cause of early lethality is presently unresolved, the *pitx1* loss-of-function mutant provides a foundation for future studies of *pitx1* in development.

210

ACRC/GCNA is an essential protease that repairs DNA-protein crosslinks during vertebrate development.

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Topic

9 Germline and Early Development

Abstract Content

Background. DNA-protein crosslinks (DPCs) are toxic DNA lesions that block all DNA transactions including replication and transcription, and the consequences of impaired DNA-Protein Crosslink Repair (DPCR) are severe. At the cellular level, impaired DPCR leads to the formation of double strand breaks, genomic instability and cell death, while at the organismal level, it is associated with cancer, aging and neurodegeneration. Despite its importance, the mechanisms of DPCR at the organismal level are largely unknown. Proteases play a central role in DPCR, as they remove proteinaceous part of the DPCs, while the peptide remnant crosslinked to DNA is subsequently removed by other repair factors. Here we characterized the role of the putative protease ACRC/GCNA (ACidic Repeat Containing/Germ Cell Nuclear Antigen) in DPCR at the organismal level using the zebrafish as model organism. **Methods.** We created zebrafish mutants with inactive Acrc using CRISPR/Cas. Maternal zygotic *acrc* mutants are early embryonic lethal and we performed rescue experiments by injecting mRNAs encoding mutated and truncated forms of Acrc, Human and mouse Acrc, as well as zebrafish Sprtn, another protease implicated in DPCR. We also analyzed DPC levels and composition in rescued embryos using the RADAR assay. **Results.** We showed the vital importance of the catalytic function of Acrc and identified Histone H3, Topoisomerases 1 and 2, Dnmt1, Parp1, Polr3a and Mcm2 as putative DPC substrates of Acrc *in vivo*. **Conclusion.** Taken together, our results demonstrate the vital role of the Acrc protease in DPC repair in zebrafish during early embryonic development.

211

Origins of primitive erythropoiesis in zebrafish

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Topic

19 Cell fate

Abstract Content

Background:

The first blood cells generated during embryogenesis are primitive erythrocytes, thought to originate from the lateral plate mesoderm (LPM) and closely linked to endothelial cell specification. However, the precise origin of primitive erythrocytes and their relationship to the endothelial lineage remain poorly understood. Prevailing hypotheses propose their specification from bipotent hemangioblast progenitors or from multipotent mesoderm at earlier developmental stages. An alternative theory suggests that primitive erythrocytes arise through transdifferentiation from a subset of committed endothelial cells, analogous to the emergence of adult hematopoietic stem cells.

Methods & Results:

To address these questions, we employed fate-mapping techniques, single-cell RNA sequencing, and chromatin accessibility profiling to trace the origins of primitive erythrocytes during early development. We identified the earliest erythroid progenitors arising directly from multipotent mesoderm at the onset of gastrulation (50–75% epiboly, approximately corresponding to mouse E6.5), earlier than previously recognized.

Conclusion:

Our data challenge the classical hemangioblast model. Despite notable similarities between endothelial and erythroid transcriptional profiles, both fate-mapping and single-cell transcriptomics reveal no evidence of a shared bipotential progenitor cluster. We therefore propose that the apparent relatedness of primitive erythroid and endothelial lineages reflects a transient shared mesodermal state rather than a discrete bipotential progenitor. Together, these findings redefine the developmental origin of primitive erythrocytes and demonstrate that erythroid fate commitment is initiated at the very onset of gastrulation.

212

Automated imaging of zebrafish embryos using robotic embryo handling and light-sheet microscopy

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Topic

2 Emerging Technologies

Abstract Content

Zebrafish models of disease offer unique advantages due to their optical transparency, genetic tractability, and suitability for high-throughput imaging. However, analysis would benefit from improved methods for rapid handling, imaging and drug discovery. Mutations in the zebrafish adhesion G-protein-coupled receptor (aGPCR) gene *adgrg6* lead to morphological defects in the ear and hypomyelination of the peripheral nervous system. In humans, disease-causing *ADGRG6* mutations are known to result in peripheral hypomyelination. We are using *adgrg6* mutant variants and pharmacological agents to modulate Adgrg6 pathway activity in the zebrafish model, and imaging development of peripheral myelination and the ear as readouts of Adgrg6 function.

Selective plane illumination microscopy (light-sheet fluorescence microscopy) enables fast and gentle image acquisition; however, sample handling remains a manual and time-consuming bottleneck. We have developed a light-sheet microscope based on the OpenSPIM concept, incorporating scanning mirrors to generate the light sheet, and an electrically tunable lens in the detection arm for rapid focusing. We have successfully imaged zebrafish embryos using a 16x 0.8NA water dipping objective lens, resolving developing organ structures including the otic vesicle. To enable high-throughput assays, we will integrate the light-sheet microscope with a robotic sample handling system. This system uses image recognition to locate and aspirate zebrafish embryos, together with translation and rotation within the imaging cuvette, facilitating multi-angle image acquisition. Custom software will automate the imaging workflow and control hardware operations. The completed platform will autonomously capture high-quality images from zebrafish cohorts and is designed with future commercial release in mind.

215

Genetic variation shapes developmental diversity across medaka strains at single-cell resolution

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Phenotypic diversity arises through the interplay of genetic and environmental factors and spans multiple levels of biological organization, from molecular and cellular processes to whole-organism traits. Embryonic development represents a critical window during which variation—such as differences in cell-type specification—must be balanced with robustness: developmental programs must reliably generate functional organisms while retaining plasticity to accommodate environmental change. While fundamental developmental programs are remarkably conserved, genotype-dependent variation in developmental dynamics and responses to environmental change remain understudied. Dissecting these differences is particularly relevant in externally developing teleost fish, where factors such as temperature strongly influence developmental rate and cellular processes.

Medaka (*Oryzias latipes*) provides a powerful complementary model to zebrafish, offering hundreds of wild-derived inbred strains from distinct latitudes, capturing naturally occurring genetic variation. These resources enable controlled, genotype-resolved investigations of developmental diversity across environmental contexts. Here, we examine how developmental progression differs among five genetically distinct medaka strains. Using single-cell RNA sequencing with combinatorial indexing and embryo-specific hashing, we profile cell-type composition and transcriptional dynamics across embryogenesis in over 100 embryos sampled at three timepoints.

Our preliminary analyses reveal strain-specific differences in developmental rate and transcriptomic ages across cell-types, along with variation in cell-type composition and gene expression dynamics. Together, these findings suggest that natural genetic variation can influence the tempo and coordination of embryonic developmental programs.

Building on these observations, we will test whether strain-specific differences in developmental coordination and tempo are linked to developmental robustness across temperatures due to climatic adaptation. Strains from different geographic regions will be exposed to a temperature gradient (16–34 °C) to assess whether adaptation influences developmental resilience across temperatures. By linking genotype to developmental dynamics at single-cell resolution, this work provides

insights into principles of teleost embryogenesis and the impact of genetic variation on developmental programs under changing climates.

216

Dissecting Tumor–Haematopoietic Crosstalk in a Zebrafish Glioma Model

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Topic

1 Disease Models

Abstract Content

Gliomas are the most common cancers of the central nervous system (CNS) in children. Among them, paediatric high-grade gliomas are particularly aggressive and are associated with a poor prognosis. These tumours frequently display an immunosuppressive tumour microenvironment (TME) that limits therapeutic efficacy. Consequently, there is a critical unmet need for simple and rapid paediatric models that enable the study and manipulation of interactions between neoplastic cells, the TME, and the haematopoietic compartment.

Here, we characterise a zebrafish glioma model initiated by tissue-specific expression of the oncogene $HRAS^{G12V}$ in the spine and brain. Transformed cells can be observed as early as the larval stage, and approximately 12% of adult fish develop tumours following mosaic injection. Using flow cytometry, single-cell RNA transcriptomics, and live imaging, we analysed immune cell development during tumorigenesis and dissected the interactions between immune cells, fibroblasts, and cancer cells within the tumour microenvironment.

Oncogene expression in the CNS of developing larvae led to immediate changes in haematopoiesis. As early as 4 days post-fertilisation (dpf), we observed an increase in myb^{+} haematopoietic stem cells and myeloid populations. In adult tumour-bearing fish, we explored the dynamic adaptation of the haematopoietic compartment in response to tumour growth. While there was an overall reduction in neutrophils in the whole-kidney marrow, a tumour-specific immature neutrophil population emerged. Within the TME, we observed a strong type 2 immune signature and the accumulation of macrophages and microglia.

This zebrafish model provides a valuable platform to investigate the complex and dynamic interactions between tumour cells and cells of the microenvironment, as well as their effects on haematopoiesis. Deciphering the roles of these interactions will be key to understanding glioma progression.

217

Robotic injection in zebrafish embryos

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Topic

2 Emerging Technologies

Abstract Content

Microinjection is a widely used technique for genetic manipulation and for generating disease models. Manual injection is labor-intensive, low-throughput, and constrained by the narrow developmental window of zebrafish development.

To overcome these limitations, we developed a user-friendly automated zebrafish injection robot. In this poster we demonstrate two designs that are used to inject in eggs and dechorionated embryos. We use deep learning-based image recognition and visual feedback for robot control.

Comparison with manual injection demonstrates similar efficiency and mortality, but an increase in throughput. I addition the operation of the robot is easier to learn than manual microinjection, leading to quicker results after training.

The robot allow to increase throughput and scale up injections for screens.

218

Oxygen is for the weak: Comparative analysis of developmental responses to environmental perturbations across species

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EMBL, Heidelberg, Germany

Topic

6 Evolution and Alternative Fish Models

Abstract Content

Oxygen deprivation is lethal to the majority of vertebrate organisms while zebrafish (*Danio rerio*) embryos can survive prolonged anoxia. How embryos maintain this state and resume development upon reoxygenation remains poorly understood. To investigate the cellular and molecular basis of this phenomenon, we study zebrafish and their distant relatives medaka (*Oryzias latipes*) as these species occupy distinct habitats differing in their oxygen availability and temperature. These ecological differences suggest that the two species may have evolved divergent molecular strategies to cope with low-oxygen conditions.

Our results suggest that medaka larvae also possess the ability to enter an anoxia induced paused state that has been described before in zebrafish by Padilla et al. We compared the viability of embryos from different stages in both species exposed to prolonged anoxia and we found not only that medaka can enter a similar paused state, but also that it exhibits a longer developmental window during which this pause can occur.

Previous studies suggest that cells enter pause at specific cell-cycle phases in zebrafish embryos exposed to prolonged anoxia. To characterize this paused state in both species, we will employ Fluorescence-activated cell sorting (FACS).

We will also apply single-cell combinatorial indexing RNA sequencing to study the pause state in both species. This approach will enable us to define cell-type–specific transcriptional programs associated with entry and maintenance of the anoxia-induced paused state.

We aim to identify medaka- and zebrafish-specific molecular pathways involved in development under suboptimal environmental conditions and gain a deeper understanding of the evolution of anoxia tolerance associated molecular pathways by comparing two distantly related teleost species.

To summarize, this work establishes a framework for vertebrate suspended animation–like states, with the aim to investigating transcriptional changes and energy-sensing pathways in a species-specific manner at a cell type resolution.

219

Targeted enrichment and cultivation of primary zebrafish cells for high-resolution *in vitro* analysis

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Topic

2 Emerging Technologies

Abstract Content

The zebrafish (*Danio rerio*) has become a powerful *in vivo* model for studying cell biological processes and for various human diseases and pharmacological testing. Due to its transparency, the zebrafish is ideal for *in vivo* imaging. One particular limitation is the inability to observe small individual cellular structures and dynamic processes, particularly in neurons, such as intracellular vesicle transport and autophagy. In rodent models, an interplay between *in vivo* studies and primary cell culture is used, but such cultures are not routinely available in zebrafish.

Microdissection of larval tissue yields limited cell numbers due to the small size of the organism. To overcome these limitations, we used Magnetic-Activated Cell-Sorting to enrich specific cell populations from zebrafish larvae between 2- and 5-days post-fertilization. We demonstrate that neural progenitors can be isolated in sufficient numbers with high purity from pooled clutches. Furthermore, electrophysiological recordings demonstrated that neurons were actively firing and could be stimulated to elicit action potentials.

In parallel, we fabricated microfluidic platforms that enable directed axon outgrowth from primary neurons to physically segregate somata and axons. This configuration facilitates high-resolution live imaging of individual neurons and dynamic subcellular processes, such as axonal vesicle trafficking. Moreover, these cultures have been integrated into microelectrode arrays to enable electrophysiological recording and stimulation. These cultivation chambers will allow targeted manipulation of specific neuronal compartments via pharmacological treatments, electrophysiological recordings, or optogenetic stimulation.

To extend this approach beyond neural cells, we developed a modular transient transgenic system expressing transmembrane affinity tags under tissue-specific regulatory elements. This enabled the purification and culture of various cell types. The combination of the expression system and culture of specific cell types in microfluidic chambers will permit co-culture of different cell types to study mechanisms of cell-type interactions, neural circuit formation, or signal transduction events under defined conditions.

220

Development and characterization of a new zebrafish model to better understand the physiopathology of Charcot-Marie-Tooth type 2A disease.

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Topic

3 Neurobiology

Abstract Content

Charcot-Marie-Tooth disease (CMT) is a rare genetic disease affecting the peripheral nervous system. CMT type 2A (CMT2A) is one of the most frequent and severe form of CMT that often leads to wheelchair dependency. In addition to motor deficits, some CMT2A patients show optical atrophy or hearing loss. CMT2A originates from mutations in *MFN2* which encodes Mitofusin-2 (MFN2), a highly conserved, mitochondrial GTPase, localized in the outer mitochondrial membrane where it regulates mitochondrial fusion. To date, no treatment is available. Therefore, we hypothesized that Zebrafish may be a relevant model to identify new therapeutic molecules. We developed a zebrafish overexpressing mutated MFN2 zebrafish model. To this purpose, we focused on one of the most prevalent CMT2A causing mutation: $MFN2^{R94Q}$. In order to characterize our CMT2A zebrafish model and underlying pathophysiological mechanisms, we analyzed multiple parameters: morphology (body, eye, otolith), locomotion, visual acuity, motor neurons size and mitochondrial integrity using multiple technical approaches. We found by using the touch escape response test that $MFN2^{R94Q}$ zebrafish had locomotor deficits. This was associated with a decrease of the axonal length of motoneurons, as assessed by immunocytochemistry. The optokinetic response test, that evaluates the visual acuity, also showed a visual impairment. Then, using the seahorse system to evaluate the mitochondrial function, we found a decrease in the maximal respiration of mitochondria in $MFN2^{R94Q}$ expressing larvae compared to control ones. To highlight pathways impacted by the mutation, we are performing an unbiased analysis of the proteome and the transcriptome (on going). In conclusion, the aim of this study was to develop a relevant model to better understand the physio-pathological mechanisms underlying CMT2A. This model will help us to identify therapeutic target and to easily test new molecules in this context.

221

Alternative dTTP homeostasis and cardiac dysfunction in DTYMK-deficiency

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Topic

1 Disease Models

Abstract Content

DTYMK deficiency was previously identified as the cause of a pediatric neurodegenerative disease. The *DTYMK* gene encodes the dTMPK (deoxythymidylate monophosphate kinase) enzyme which catalyzes the penultimate step in the biosynthesis of dTTP, completely blocking the only known pathway to generate this building block for DNA. In this study we investigated compensatory mechanisms in nucleotide homeostasis and characterize a previously unrecognized cardiac phenotype in a knockout zebrafish model.

Nucleotide content was analyzed in the pool of free nucleotides and genome-incorporated fraction. Surprisingly, levels of dTTP in *dtymk*-deficient embryos were unchanged, despite of a complete block in its biosynthesis, suggesting the presence of an alternative biosynthetic pathway for dTTP. We performed RNA sequencing on the heads of mutant and wildtype embryos to uncover the molecular identity of this compensatory pathway.

Secondly, we observed a cardiac phenotype in *dtymk* mutants which was not documented in patients. In addition to severe neurodegeneration, cardiac edema progressively develops in mutant embryos from 3dpf onwards, leading to lethality at 4-5dpf. The heart tube in *dtymk* mutants is elongated and severe cardiac edema is apparent. Functional cardiac parameters were derived from the analysis of short movies from the heartbeat in *dtymk -/-* embryos. Additionally, structural differences in the heart were documented using confocal microscopy.

Our results show that the role of dtymk is not limited to the brain but also has a severe impact on heart development and function. This study uncovers a compensatory mechanism in dTTP homeostasis and its implications for development and cardiac function.

222

Exploring the axonal mRNA processing role of the spliceosomal protein SNRNP70

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Topic

3 Neurobiology

Abstract Content

Introduction: mRNA is produced in the nucleus, but it must travel and undergo further processing in the cytoplasm before it can be eventually translated. The process of mRNA maturation is not static and can even occur in distant parts of neurons such as axons, dendrites and synapses. Small nuclear ribonuclear protein 70 (SNRNP70) is a core component of the U1 spliceosome complex and plays an important role in mRNA processing within the nucleus. Our group has previously demonstrated that SNRNP70 also localises to RNA granules located in motor neuron axons and this extra-nuclear pool is essential for motor neuron growth and neuromuscular connectivity. Interestingly, SNRNP70 has been found to modulate the axonal transcriptome, but the specific mechanisms involved are not yet understood. This project aims to uncover axonal mRNA targets of SNRNP70 to better understand the roles of SNRNP70 in extra-nuclear mRNA processing.

Methodology: Fluorescence-Activated Cell Sorting (FACS) of motor neurons was performed on two-day old zebrafish embryos from a transgenic line with overexpressed cytosolic SNRNP70. To investigate the impact of this overexpression on neuronal gene expression, we conducted transcriptomic analysis using bulk RNA-Seq on the sorted motor neurons. Furthermore, we employed Gene Set Enrichment Analysis to pinpoint significantly enriched Gene Ontology terms and identify key candidate genes within the core set of enriched terms.

Results: Overexpression of cytoplasmic SNRNP70 resulted in alterations in the neuronal transcriptome, including in pathways linked to neuronal development and axonogenesis. Candidate genes for further analysis have been selected. We now plan to further interrogate these mRNA targets and determine whether SNRNP70 directly binds to such mRNA targets, at single nucleotide resolution.

Conclusions: We expect that the results will enhance our understanding of the extra-nuclear roles of SNRNP70 in modulating the axonal transcriptome and the mechanisms by which it regulates neuronal connectivity.

223

Cell-type specific protein turnover dynamics during early development in zebrafish

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Topic

9 Germline and Early Development

Abstract Content

Developmental tempo, or the rate at which an organism develops, is a fundamental process in biology. While we know that protein dynamics, especially how fast proteins are made and broken down, play a major role in this timing, we still do not fully understand the relationship between protein turnover and the speed of development. Understanding this connection is essential to learning how organisms develop in a coordinated and temporally regulated manner. In this study, we use tandem fluorescent timers (tFTs) and advanced imaging to measure the age of proteins at a single-cell level. By imaging whole zebrafish embryos expressing a tFT construct, we can quantify the ratio of a slow-maturing red fluorescent protein to a faster-maturing green fluorescent protein in individual cells. This ratio represents the protein turnover rate for the cell. This approach allows us to track how protein stability changes in real-time. Our preliminary data suggest that tFTs are a robust method to quantify proteostasis across different cells. We will use this approach to investigate how protein turnover rates differ across cell types to determine if specific tissues drive the overall pace of development.

226

Archiving of zebrafish lines at the European Zebrafish Resource Center (EZRC)

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Topic

21 Husbandry

Abstract Content

Laws on animal experimentation aim to reduce the use of animals in European research, stimulating the use of alternative models such as zebrafish embryos. Centralized archiving of genetically modified zebrafish strains can make critical contributions to this aim, since it avoids redundant experiments and improves reproducibility of experimental results.

The European Zebrafish Resource Center (EZRC) at the KIT archives 27,000 knock-out mutations from the Sanger Institute (representing nearly half of all protein-coding genes), 2,000 mutations from forward-genetic screens at the MPI for Developmental Biology, and approximately 1,000 mutant and transgenic lines contributed by the community. It also serves a source for standard wildtype lines.

Genome editing by CRISPR can easily produce new mutant lines, however each CRISPR experiment requires a permit, molecular biological characterization of the mutations and evaluation of animals for harmful phenotypes. Therefore we offer free archiving of established CRISPR lines as frozen sperm together with the corresponding severity assessments.As a further contribution to standardization in the zebrafish field we provide hands-on training in cryopreservation methods and participate in the International Zebrafish and Medaka Courses (IZMC) held several times per year at the KIT.

Finally, European animal health legislation imposes certain requirements on shipping zebrafish embryos or adult fish. We will discuss how these are implemented at EZRC.

227

CRISPR/Cas9-mediated generation of a single-nucleotide lrrk2 mutation in zebrafish as a Parkinson's Disease model

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Topic

1 Disease Models

Abstract Content

Background

Mutations in LRRK2 are the most common genetic cause of familial Parkinson's disease (PD). The pathogenic G2019S mutation increases kinase activity and has been linked to mitochondrial dysfunction, calcium imbalance, and degeneration of dopaminergic neurons (DNs). However, mechanisms connecting LRRK2 hyperactivity to these pathologies *in vivo* remain incompletely understood. The aim of the project was to establish and characterize a zebrafish model for the G2019S variant.

Methods

We generated a zebrafish *lrrk2* G2010S mutant (functional equivalent of human G2019S) using CRISPR/Cas9-mediated genome editing. sgRNA targeting the conserved kinase domain were designed and validated. A plasmid donor construct was used to introduce the targeted mutation *via* homology-directed repair (HDR). Mutant founders were identified through genotyping and F1 offspring carrying the mutant allele were propagated.

Results

CRISPR/Cas9 editing successfully introduced the targeted mutation within the *lrrk2* gene encoding kinase domain. Preliminary phenotypic analyses focus on DNs integrity, mitochondrial function, and behavioral parameters relevant to PD. The *lrrk2* mutant line was crossed with *Tg(dat:EGFP)* transgenic zebrafish, in which DNs are fluorescently labeled. This enables direct visualization and quantification of dopaminergic neuron loss *in vivo*. Ongoing studies assess whether mitochondrial calcium uniporter (mcu) inhibition rescues DNs loss in this model.

Conclusion

This novel CRISPR-generated *lrrk2* G2010S zebrafish line provides a valuable tool to study the interplay between mitochondrial calcium influx, iron homeostasis, and ferroptosis in PD pathogenesis, and to evaluate neuroprotective strategies *in vivo.*

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suggestions and early guidance during the project. We also acknowledge all the members of Zebrafish Core Facility in IIMCB. All authors claim no conflict of interest.

230

Functional impact of β2 – adrenergic receptor polymorphisms in cardiovascular diseases using zebrafish model

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Topic

4 Cardiovascular

Abstract Content

Background: Cardiovascular diseases remain one of the leading causes of death worldwide. Despite a similar clinical presentation, patients often exhibit considerable variability in disease progression and response to pharmacological treatment. Genetic factors are believed to contribute substantially to this variability. The β2-adrenergic receptor (β2-AR) is a G-protein-coupled receptor involved in adrenergic signaling, plays an important role in regulation of cardiovascular function. Polymorphisms within the β2-AR gene may alter receptor activity and thereby contribute to interindividual differences in cardiovascular physiology and therapeutic response.

Methods: This study examined the functional consequences of two common β2-AR polymorphic variants, Arg16Gly (rs1042713) and Gln27Glu (rs1042714), which occur in approximately 40% and 30% of the population, respectively. Humanized zebrafish (lac. *Danio rerio*) were used to evaluate the physiological effects of these variants *in vivo*. Endogenous fish β2-AR receptors expression was suppressed using Morpholino oligonucleotides and subsequently rescued by introducing human β2-AR mRNA carrying the respective polymorphic variants. Heart failure (HF) was induced by exposing zebrafish larvae to doxorubicin, starting from 2 days post-fertilization. Key physiological parameters, including heartbeat, morphology and larval survival were monitored until day 5. Additionally, levels of inflammatory mediators and HF markers in zebrafish homogenates were quantified using ELISA and LC-MS. This experimental model enabled in vivo evaluation of the physiological impact of β2-AR polymorphisms, providing a platform for investigating their potential role in cardiovascular physiology.

Results: Preliminary stages of the project focus on the generation and molecular characterization of transgenic zebrafish lines expressing human β2-AR polymorphic variants. Ongoing works aims to assess potential differences in cardiovascular phenotypes.

Conclusion: Characterizing the functional impact of β2-AR polymorphisms may improve understanding of genetic factors underlying variability in cardiovascular

disease progression and treatment response. These findings may contribute to the development of more personalized strategies in cardiovascular medicine.

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231

Exploring a targeted pharmacological approach to boost Nrf2 pathway activity in a zebrafish focal demyelination model

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Topic

1 Disease Models

Abstract Content

Multiple Sclerosis (MS) is a complex neurodegenerative disease defined by progressive myelin loss, oligodendrocyte death, and axonal deterioration. While the precise triggers remain elusive, the pathogenic cascade is driven by a neuroinflammatory state characterized by the accumulation of reactive oxygen species (ROS). Recent evidence demonstrates that ROS buildup at lesion sites evokes an Nrf2-mediated antioxidant response in patients with MS forms (acute, progressive and relapsing-remitting). Given that this signaling pathway plays a pivotal role in promoting neuroprotection, we aim at modulating the antioxidant response within a zebrafish MS model to describe its potential beneficial effects in a pathological context. To induce acute MS-like lesions and gain insights into the dynamic molecular changes associated with demyelination and post-injury remyelination, we microinjected Lysophosphatidylcholine (LPC) in the spinal cord (SC) of multiple transgenic zebrafish lines and evaluated specific cell lineage perturbations. Specifically, 3 dpf LPC-injected larvae exhibited a massive recruitment of mpeg-positive macrophages, which showed a significant switch in gross morphology over time. Furthermore, we observed a marked reduction in medially placed Olig2-positive oligodendrocyte progenitor cells (OPCs), alongside the occurrence of ectopic mbp-positive structures, likely representing myelin-overloaded oligodendrocytes or aberrantly targeted neuronal cell bodies. Finally, by exploiting our recently established Tg(8xAORE:EGFP) reporter line, which traces Nrf2 signaling activation, we identified an enhanced antioxidant response specifically within the macrophages recruited at the lesion site. Building on these findings, we are currently testing pharmacological compounds to boost this endogenous defense, evaluating their ability to attenuate LPC-induced damage and accelerate the kinetics of lesion recovery.

233

Structural and Metabolic Dysfunction in Pygm-Deficient Zebrafish Muscles

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Topic

8 Metabolism

Abstract Content

McArdle disease (GSDV) is a genetic metabolic disorder caused by a deficiency in the muscle glycogen phosphorylase enzyme (PYGM). This defect results in the inability to break down glycogen into glucose molecules in skeletal muscles, leading to impaired energy production and reduced physical activity.

The aim of this study was to examine the structural and metabolic effects resulting from knocking out the zebrafish *pygm* gene, and to create an appropriate model for future research of human muscle glycogen phosphorylase deficiency.

Generation of the *pygm*-/- zebrafish *via* targeted mutation using the CRISPR/Cas9 gene-editing technique was followed by observations using light, fluorescent and transmission electron microscopy. The morphological results were supplemented by biochemical analysis.

The observations revealed severe changes in skeletal muscle, such as a disorganised, bent and torn structure. These changes are correlated with an increased amount of glycogen granules, an enlarged endoplasmic reticulum, and a disorganised mitochondrial network. Biochemical analysis indeed revealed increased levels of glycogen, ATP and Ca^{2+} in the skeletal muscles of *pygm*-/- zebrafish. Furthermore, our findings suggest some degree of skeletal muscle compensation mechanism and/or plasticity, as we observed increased expression of non-muscle glycogen phosphorylase isoforms in *pygm*-/- skeletal muscles.

The observed phenotype, which resembles the features of human McArdle patients, allows us to conclude that zebrafish could indeed be an efficient and valid animal model for this disease. The *pygm*-/- zebrafish line can be used for further research, including in-depth analysis of muscle metabolism.

The work was supported by the National Science Centre, Poland, (2021/43/D/NZ4/00081)

234

Using zebrafish models to understand hereditary spastic paraplegia caused by variants in PCYT2.

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Topic

1 Disease Models

Abstract Content

Hereditary spastic paraplegia type 82 (SPG82) is a complex progressive disease caused by bi-allelic hypomorphic *PCYT2* variants, which result in lower limb spasticity and neurological features including cerebral and cerebellar atrophy and ocular anomalies. PCYT2 is the rate-limiting enzyme in the Kennedy pathway, responsible for production of glycerophospholipids, including phosphatidylethanolamine (PE) which is enriched in the brain. SPG82 patients exhibit reduced PE and phosphatidylcholine (PC) levels, and elevated ether-PCs. The pathophysiological mechanisms underlying SPG82 remain poorly understood, primarily due to the absence of a viable animal model, as *Pcyt2*-deficient mice are embryonically lethal. However, zebrafish present a tractable model system for studying SPG82.

CRISPR-Cas9 targeting exons 3 and 13 of *pcyt2* were used to create two zebrafish models, one null (*pcyt2*^{ex3}) and one hypomorphic (*pcyt2*^{ex13}). Phenotyping of mutant animals revealed that they had significantly smaller body lengths and head sizes, and impaired mobility; and human *PCYT2* mRNA rescued these phenotypes. Lipidomic analysis of crispant larvae showed significant reduction in PC and PE species, with concomitant increase in ether-PC species. Furthermore, gene network analysis from RNAseq of mutant larvae revealed changes in expression of various neurological markers and an increased neuroinflammatory signature. Consistent with this, we observed increased recruitment of immune cells into the brain of *pcyt2* crispants using macrophage/microglia and neutrophil transgenic reporter lines. Lastly, confocal imaging in oligodendrocyte and myelin transgenic reporter lines revealed reduced myelination in the central nervous system of *pcyt2* crispants.

In summary, we have created two *pcyt2* zebrafish models that recapitulate the clinical and lipidomic phenotypes of SPG82. These models are being used to decipher the pathophysiology and devise new treatments for this disorder.

235

Chemical differences in treatment conditions influence outcome in zebrafish tumor xenograft model

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Topic

1 Disease Models

Abstract Content

Ewing Sarcoma (EwS) is a malignant pediatric bone and soft tissue cancer mainly driven by the EWS::FLI1 fusion oncogene. This fusion protein acts as a master regulator to maintain tumor-specific cell identities. Tumor cells in their high metabolic activity are often dependent on molecular chaperones, particularly heat shock protein 90 (Hsp90), to ensure correct folding of all the many and here especially the fusion protein.

In search of an actionable target to treat EwS, Hsp90 is an attractive candidate, as inhibition of one protein may reduce function of a whole lot of proteins as well as the oncogenic driver. However, traditional N-terminal Hsp90 inhibitors (Hsp90i) have faced clinical hurdles due to on-target toxicity and the induction of heat shock response (HSR).

Here, we aim to evaluate the tolerability and efficacy of novel allosteric C-terminal domain Hsp90i using an established zebrafish xenograft model for EwS.

Fluorescently labeled human EwS cell lines were microinjected into the perivitelline space of zebrafish larvae at 2 days post-fertilization (2dpf). Xenografted larvae were then treated with differently modified Hsp90i. Changes in tumor volume were quantified via automated fluorescence microscopy coupled to numerical image analysis on our zebrafish drug screening platform (ZANDR). No-observable-effect concentrations were determined beforehand to define treatment concentrations.

Of note, differences in the therapeutic efficacy of Hsp90 inhibitors were observed between their molecular (free base) or ionic (salt) forms. The low buffering capacity of the typical zebrafish media E3 itself might influence Hsp90i properties. Therefore, we probed if addition of HEPES would influence the outcome of the drug screens.

236

Establishing a zebrafish model for the pediatric bone tumor entity Ewing Sarcoma

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Topic

1 Disease Models

Abstract Content

Ewing Sarcoma is a highly aggressive pediatric bone and soft tissue tumor that requires suitable preclinical models for better understanding and therapeutic development. In this project a novel zebrafish model was established by generating a Cre-inducible Tg(loxP-EWS::FLI1) transgenic line combined with the optogenetic zHORSE system to enable spatially and temporally controlled expression of the oncogenic fusion gene EWS::FLI1. Tumor formation was observed in specific regions and confirmed through histological and immunohistochemical detection of the Ewing sarcoma marker FLI1. This model represents a promising in vivo model to study tumor development and the molecular mechanisms underlying Ewing sarcoma.

240

Investigating the impact of Environmental Chemicals of Human Health Concern on Neurodevelopment using the Zebrafish Model

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Topic

10 Toxicology

Abstract Content

Neurodevelopmental disorders (NDDs), such as Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder, manifest early in life, and can result in alterations in communication, social interactions, and learning. Although there is a strong genetic component, in recent years the incidence of NDDs has been increasing globally and genetics alone are insufficient to explain all cases. This rise in NDD rates has been linked with environmental exposure to certain chemicals. But despite reports of such links, there is little experimental evidence to support this. The zebrafish provides a useful model to help bridge this knowledge gap due to their rapid brain development and maturation, optical transparency, and amenity to small-scale chemical exposures. Here we used zebrafish to investigate the effects of developmental exposure to environmentally relevant concentrations of the metal, lead, and the pesticide, chlorpyrifos, (chemicals believed to impact brain development in mammals) on brain structure and behaviour. We exposed embryos to a range of concentrations of these chemicals, from <1 hour post fertilisation to 4 days post fertilisation (4dpf), alongside sodium valproate (a well-established developmental neurotoxin) as a positive control. Assessments on the developmental morphology of the brain, along with immunohistochemical analysis of neural structures, and video analysis of behavioural responses to light stimulation and thigmotaxis were conducted at 4dpf. Our data show that exposure to lead caused significant developmental impacts that are discernible in larval brain structure. Additionally, we observed alterations in swimming activity response to light from the exposures to both lead and chlorpyrifos. In our forthcoming work, we will assess the effects of chemical exposures on brain function using a transgenic zebrafish expressing a genetically encoded calcium (Ca^{2+}) indicator and whole brain Ca^{2+} imaging. Collectively, these data will help us understand how environmental chemical exposure may affect neurodevelopment and contribute towards the pathophysiology of NDDs in humans.

241

Cyclin Dx Is Required for Proper Lateral Line Development and Regenerative Capacity in Zebrafish

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background:

Mammals rely on inner-ear mechanosensory hair cells for hearing and balance, but cannot replace these cells once lost, leading to permanent functional loss. Zebrafish (*Danio rerio*) regenerate hair cells efficiently, making the posterior lateral line an informative system for studying the mechanisms that enable this regeneration. Following damage, zebrafish hair cells are regenerated by central support cells that differentiate into hair cell precursors and proliferate, while a distinct, peripheral support cell population repopulates the support cells. The genes that underlie the distinct roles of these cells during regeneration are an area of active research.

Methods:

We screened lines of fish with random GFP insertions in their genomes, and identified one that showed expression in a dorsal-ventral subset of support cells that contribute to hair cell regeneration. The insertion was determined to be in a cell cycle regulatory gene, *cyclin Dx* (*ccndx*). We used CRISPR mutagenesis and pharmacological and laser-based ablation techniques to characterize the role of *ccndx*.

Results:

Loss of *ccndx* results in lateral line developmental abnormalities, for instance, fewer neuromasts and reduced hair cell numbers, and impairs proliferative regeneration of hair cells, consistent with recent work that independently found a role for this gene in hair cell regeneration. We extend this by showing that *ccndx* is quickly expressed by other support cells during regeneration after ablation of cells expressing *ccndx*. Zebrafish with mutated *ccndx* exhibit a different pattern of reexpression compared to heterozygote siblings, suggesting that *ccndx* may play an important role in establishing the fate of support cell progeny in addition to controlling the proliferation of hair cell precursors.

Conclusion:

These findings offer insights into the mechanisms that regulate hair cell regeneration, identifying *ccndx* as a critical regulator of the regenerative capacity of

support cells and highlighting the interplay between proliferative control and cell fate decisions.

242

Studying *deltaA* as a signature of fate-indicating divisions in adult neural stem cells

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Topic

19 Cell fate

Abstract Content

In the adult vertebrate brain, neural stem cells (NSCs) ensure the generation of new neurons and maintain their own population throughout adulthood. As perturbations of this balance lead to either exhaustion of the NSC population or halt of neurogenesis, different mechanisms are in place to ensure homeostasis. Using intravital imaging and clonal analysis, the lab tracked NSC populations over their lifetime and identified a hierarchy of two behaviorally distinct NSC subpopulations in the adult zebrafish telencephalon. One subpopulation strictly self-renews and does not express the Notch ligand gene *deltaA*. It fuels, via asymmetric divisions, a second population that expresses *deltaA*. The latter population is shorter-lived and drives its progeny towards neurogenesis, indicating that *deltaA* expression marks a neurogenesis-headed fate. However, the molecular mechanisms regulating cell fate specification during this first asymmetric division remain elusive. Using BrdU pulse-chase labelling and single molecule RNA FISH, we specified the timing of *deltaA* mRNA expression: it rapidly increases within 24 hours post-division in the neurogenesis-headed daughter cell. This pattern matches the *deltaA*:GFP expression dynamics observed during live imaging, suggesting a primarily transcriptional control. These findings will be complemented by tracking a DeltaA-Scarlet fusion generated by Crispr-knock-in to follow protein turnover. Transcription factor binding sites on the *deltaA* minimal promoter (4.5kb) were systematically screened via literature search to identify candidate regulators. Foxn4, a forkhead transcription factor that directly activates Dll4-Notch signalling to introduce progenitor asymmetry in the spinal cord and retina, and Klf9, a Krüppel-like factor which acts as a brake on symmetric NSC self-renewal by modulating Notch1 in the adult hippocampus, were identified. Vivo morpholino experiments perturbing *foxn4* or *klf9* in adult NSCs are currently being analysed for their effects on *deltaA*:GFP and *deltaA* expression. Together, this work should reveal fundamental principles of NSC fate decisions that balance NSC maintenance at long-term.

243

Membrane transporters and microbiota-dependent toxicity in germ-free zebrafish larvae

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Topic

10 Toxicology

Abstract Content

The gut microbiota is an important modulator of host physiology, xenobiotic handling, and susceptibility to environmental contaminants, yet its role in transporter-mediated toxicological responses remains poorly understood in zebrafish. Germ-free (GF) zebrafish offer a unique opportunity to determine microbiota-driven ecotoxicological effects, particularly when combined with membrane transporter mutant lines. Here, we present an experimental platform to investigate how the absence of gut microbiota influences toxicity in wild-type (WT) and transporter-deficient zebrafish larvae under GF and conventional conditions, with particular emphasis on exposure to environmental contaminants. Building on our work with zebrafish membrane transporter mutants, including **Oatp1d1**$^{-/-}$ and **Oct1**$^{-/-}$, the planned approach will compare WT and mutant larvae reared in parallel under GF and normal conditions and exposed to perfluorooctane sulfonate (PFOS). This design will enable assessment of whether gut microbiota modifies toxicokinetic and toxicodynamic responses and what role membrane transporters play in these processes. Endpoints will include survival, developmental and morphological parameters, and behavioral phenotyping using automated video tracking, with a focus on locomotor activity and stimulus-evoked responses as sensitive early-life indicators of chemical toxicity. Molecular analyses will further examine pathways related to membrane transport, stress responses, and detoxification. At the current stage, the most advanced part of the study is the establishment and validation of the GF zebrafish model. Accordingly, 16S rRNA-based analyses are included in this initial phase to verify microbiological status and define baseline microbial profiles relevant to model establishment. These data will provide the necessary foundation for subsequent comparative exposure studies in GF and conventional WT and mutant larvae and for mechanistic evaluation of microbiota-dependent PFOS toxicity.

245

Characterizing a Noonan syndrome-associated mutation in SOS2 using zebrafish models

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Topic

1 Disease Models

Abstract Content

Noonan syndrome is a developmental disorder caused by dominant activating mutations in factors of the RAS/MAPK signaling pathway. Our lab has developed zebrafish models harboring Noonan syndrome-associated mutations, including the Sos2-specific mutation p. Met264Arg. Human patients with Noonan syndrome often have a short stature, which mirrors the reduced size seen in embryonic and adult $Sos2^{M264R/+}$ zebrafish. Additionally, human patients carrying a mutation in SOS2 exhibit a predisposition to cardiovascular and lymphatic defects and we are currently studying these potential phenotypes in zebrafish embryos with a $Sos2^{M264R}$ mutation. Furthermore, adult $Sos2^{M264R/+}$ zebrafish exhibited a distinctive jaw exostosis, first observed at approximately 3 months of age. Jaw cartilage development in wild-type and $Sos2^{M264R/+}$ embryos did not account for this phenotype, as the embryos did not show major craniofacial defects compared to our other Noonan syndrome fish models. We are currently following the development of the exostosis in juvenile and adult fish over time, which may give us more insights into the origin and growth of this tissue. Finally, interfering with the RAS/MAPK pathway by administering inhibitors to juvenile zebrafish may answer whether this phenotype is an effect of overactivation of this signaling pathway. Overall, our research offers valuable insights into the molecular mechanisms underlying Noonan syndrome and emphasizes the utility of zebrafish models in studying the diverse phenotypes of this disorder.

247

Early-life glucocorticoid overexposure primes adult stress hypersensitivity in distinct preoptic neuroendocrine subclusters

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Topic

3 Neurobiology

Abstract Content

Background

While early-life Glucocorticoid overexposure (eGC-OE) is implicated in lifelong stress hypersensitivity, the cellular substrates of this latent endocrine memory are poorly defined. These programmed signatures are typically not detectable at baseline, emerging only under challenge in adulthood. Here, we identify discrete cell populations and regulatory programs within the adult zebrafish preoptic area (POA, primary regulating hub for stress response) that may translate eGC-OE into adult stress hyper-reactivity.

Methods

We employed a "double-hit" paradigm, combining optogenetic developmental GC elevation with an adult predator-like stimulus (looming dots, LD). To identify cellular signatures of an acute stress response, we performed single-nucleus Multiome profiling on the adult POA. We integrated these data using co-expression network analysis and gene regulatory network (GRN) inference to identify eGC-OE-primed regulatory signatures. Finally, we tested a causal link between some of the identified molecular candidates and stress hypersensitivity through pharmacological perturbation of prioritized protein targets.

Results

Acute LD stress selectively recruited specific neuroendocrine subtypes characterized by robust immediate-early gene (IEG) induction and elevated GC-responsive gene expression. Most prominent among them are two-arginine vasopressin (*avp*+) subclusters and a glutamatergic somatostatin (*sst1.1*+) cell population. While eGC-OE had little impact on basal gene expression, it markedly potentiated stress-evoked transcriptional responses within these specific subtypes. GRN analysis revealed an increased convergence of GR-, CREB-, and MAPK-linked signaling onto IEG networks associated with neural activation and neuropeptide release. Pharmacological modulation of identified top LD-responsive factors attenuated the exaggerated cortisol response in eGC-OE fish, validating a functional link between primed transcriptional amplification and endocrine hypersensitivity.

Conclusion

Together, these findings identify discrete hypothalamic populations that encode latent stress sensitivity programmed by early-life GC exposure. By delineating the regulatory logic of these "primed" neurons, this study identifies specific mechanistic entry points underlying developmental programming of stress hypersensitivity.

248

Zebrafish red-head phenotype in Noonan Syndrome Shp2a-D61G line is associated with structural changes in gill morphology

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Topic

1 Disease Models

Abstract Content

Background: The RAS/MAPK pathway is a critical signaling cascade regulating cell proliferation, differentiation, and survival, and plays an essential role during development. Dysregulation of this pathway is associated with various diseases, including cancer and developmental disorders such as Noonan syndrome (NS). To study the consequences of aberrant RAS/MAPK signaling in NS in vivo, we generated a zebrafish model harboring a patient-derived gain-of-function mutation in the signaling protein Shp2 (*Shp2a*^{D61G}), which phenocopies key features observed in human patients. Unexpectedly, a subset of individuals develops a striking phenotype characterized by cranial reddening. Here, we aim to characterize this red-head phenotype and investigate the physiological processes contributing to its development.

Methods: Zebrafish siblings were monitored for phenotypic abnormalities and behavioral changes. To investigate the mechanisms underlying the phenotype, we are performing morphological analyses of gill and kidney structures, blood cell profiling, and gene expression analysis using qPCR at different time points.

Results: Approximately 10% of mutant zebrafish develop a phenotype characterized by red coloration in the head region, decreased sensitivity to the anesthetic tricaine methanesulfonate (MS222), and a behavioral preference for swimming near the water surface. Preliminary observations suggest structural changes in the secondary gill lamellae that may reduce the surface area available for oxygen exchange, potentially resulting in hypoxia. In addition, affected zebrafish appear to exhibit increased production of red blood cells accompanied by a reduction in myeloid cell populations.

Conclusion: These findings suggest that dysregulated RAS/MAPK signaling in *Shp2a*^{D61G} zebrafish results in a previously undescribed phenotype potentially involving gill remodeling leading to hypoxia and altered hematopoiesis. Ongoing analyses aim to further elucidate the mechanisms underlying this phenotype.

250

N-linked glycosylation is necessary for regeneration of hair cells and support cells in the neuromasts of the zebrafish lateral line

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background:

Loss of sensory hair cells in humans leads to permanent hearing loss since mammalian hair cells cannot regenerate. Zebrafish (*Danio rerio*) however can regenerate their sensory hair cells including those in the mechanosensory lateral line system. Regeneration of hair cells typically occurs through de-differentiation and proliferation of support cells that surround hair cells. In many cancer and stem cell populations, proliferative cells undergo a metabolic shift from oxidative phosphorylation to aerobic glycolysis, known as the Warburg Effect, though the role of this shift in hair cell regeneration is unknown.

Methods:

We have investigated this using pharmacological and laser-based ablation of lateral line cells and pharmacological manipulation of metabolic pathways, in particular using 2-DG to inhibit glycolysis. Regeneration was assayed through transgenic labeling of hair cells and BrdU analysis of cell proliferation.

Results:

Following neomycin damage, 2-DG inhibited hair cell formation suggesting that efficient glycolysis is necessary for regeneration. When both hair cells and supporting cells are ablated, 2-DG blocks regeneration leaving just a cluster of mantle cells. We determined through BrdU analysis that this inhibitory effect is not due to a lack of proliferation and that 2-DG likely has its greatest impact during the differentiation phase. We then tested whether inhibition of specific pathways linked to glycolysis similarly blocks regeneration. Inhibiting N-linked glycosylation with tunicamycin completely blocked regeneration in the neuromast, suggesting this pathway may be one of the reasons for the glycolytic shift during regeneration.

Conclusion:

These results support the importance of a Warburg Effect in neuromast regeneration and indicate that a metabolic shunting toward N-linked glycosylation may be needed for regeneration. This research provides insights for understanding how manipulation of metabolism may contribute to regeneration in this system.

252

Towards more effective Glioblastoma Multiforme therapies: HDAC6 and HDAC8 combined targeting

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background: Glioblastoma multiforme (GBM) is an aggressive primary tumor of the central nervous system, characterized by high recurrence and poor prognosis. Current therapeutic strategies consist of surgical resection of the tumor area followed by radiotherapy and chemotherapy. Temozolomide (TMZ) remains the standard of care for GBM; however, over 50% of patients fail to respond and develop therapeutic resistance. Among the signalling pathways found dysregulated, HDAC6 and HDAC8 have been reported to be overexpressed. We previously demonstrated that selective HDAC6 inhibition impairs GBM progression. In this study, we investigated whether the combined inhibition of HDAC6 and HDAC8 could enhance the antitumor effects compared to single-agent treatments.

Methods: To this end, we performed *in vivo* analyses using the zic:RAS zebrafish GBM model, in which tumor development is induced by specific oncogene expression in neural cells. In parallel, human GBM cell line T98G, characterized by a high resistant phenotype, was used as *in vitro* models. Cell viability, tumor growth, and autophagic flux were assessed.

Results: Our results show that the combined HDAC6 and HDAC8 inhibition reduces cell viability and significantly suppresses tumor growth in zic:RAS zebrafish larvae. Moreover, combined inhibition enhances TMZ sensitivity both *in vitro* and *in vivo*. From a mechanistic perspective, HDAC6 and HDAC8 inhibition leads to impaired autophagic trafficking. To further elucidate the molecular and cellular mechanisms underlying these effects, we performed omics-based analyses to assess transcriptional changes following HDAC6 and HDAC8 inhibition.

Conclusion: Overall, this integrated approach provides a powerful platform to evaluate the therapeutic potential of selective and combined HDAC6/HDAC8 inhibition and to dissect the biological mechanisms relevant to GBM progression and therapy resistance. Our findings support dual HDAC6/HDAC8 inhibition as a

promising therapeutic strategy for GBM and other cancers characterized by HDAC6 and HDAC8 overexpression.

253

Aging Alters the Cellular and Molecular Dynamics of Cutaneous Wound Healing in Zebrafish

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Topic

14 Stem Cells and Regeneration

Abstract Content

Understanding how tissues restore vascular networks after injury is critical for treating chronic wounds, which affect approximately forty million people worldwide, particularly in aging and diabetic individuals. Adult zebrafish provide a powerful model to dissect the cellular and molecular mechanisms of vascular regeneration, combining conserved wound healing processes with optical transparency, extensive transgenic resources, and compatibility with long-term live imaging. We established a cutaneous wound model that pairs precise rotary tool injuries with cellular-resolution confocal imaging, enabling direct, longitudinal visualization of the full sequence of wound repair in living adult fish over weeks to months. Live imaging revealed a defined temporal sequence of wound healing. Re-epithelialization and neutrophil recruitment initiate within hours and peak by one day post injury, macrophage activity and vessel regrowth increase between one and four days, and complete vascular remodeling occurs over several months. Aging fundamentally alters this temporal program of repair, with aged fish exhibiting accelerated neutrophil recruitment but markedly delayed vascular regrowth. To define the molecular programs underlying endothelial responses during repair, we developed 'AngioTag' transgenic zebrafish that enable tissue-specific profiling (TRAP-RNAseq) of the endothelial translatome *in vivo*. Using this system, we generated endothelial translatome datasets across multiple organs as well as across defined timepoints during wound healing. Comparative analyses reveal conserved pan-endothelial signatures alongside organ-specific expression programs that shift with age. Together, these approaches establish an integrated platform to visualize and molecularly profile vascular regeneration in adult zebrafish, providing new insight into how endothelial cells coordinate vascular repair and why this process becomes impaired with age.

254

Generation of a *prop1* knockin zebrafish enables single-cell transcriptomics of early pituitary development

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The pituitary is the master endocrine gland, controlling metabolism, stress, growth, and puberty. Anterior pituitary gland development is highly conserved across vertebrates, with zebrafish possessing the full complement of hormone-producing cell populations found in mammals. The transcription factor PROP1 is the primary regulator defining these lineages, and *PROP1* mutations in mammals result in congenital hypopituitarism. This study aimed to establish a robust transgenic zebrafish line to track *prop1+* cells *in vivo* and elucidate early pituitary organogenesis.

To ensure the reporter accurately recapitulated endogenous expression, we employed a CRISPR-Cas9 knock-in strategy. Using modified double-stranded DNA donors, a P2A-GAL4-VP16 construct was precisely inserted into exon 2 of the *prop1* gene. Following PCR screening, positive founders were crossed with a UAS:mScarlet-CAAX line. We leveraged this newly developed line for high-resolution live imaging and FACS-based cell isolation to generate the first single-cell RNA sequencing (scRNA-seq) atlas of the early zebrafish pituitary.

Our analysis identified *epcam* as an excellent marker of the anterior pituitary. We extended these findings to mice, where EPCAM expression was also highly specific at both mRNA and protein levels. This expression is maintained throughout development and is independent of PROP1 function, making *epcam/Epcam* a robust marker of pituitary tissue regardless of differentiation deficits.

The dataset also allowed us to identify the molecular drivers of endocrine cell differentiation. Early ituitary stem cells are *notch3+*, and express receptors for several other pathways, including Robo, Fgf, Wnt, and Shh. As differentiation progresses, receptor expression for all pathways is lost and cells begin expressing Notch ligands. Finally, differentiating cells start to express conserved transcription factors that delineate each hormone-producing cell type.

Overall, our work provides a novel tool for studying pituitary development and a robust resource to explore the early pituitary transcriptome at single-cell resolution.

255

Zebrafish larval model of diet-induced obesity: integrated metabolomic and behavioural profiling

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Topic

8 Metabolism

Abstract Content

Background: Metabolic syndrome affects 20–25% of adults globally and is increasingly linked to neurocognitive decline. *Danio rerio* shares key metabolic regulators with humans — including SREBF1 and PPARα/γ — and conserved pancreatic endocrine architecture, making it a tractable vertebrate model for diet-induced obesity (DIO) research.

Methods: Larvae (5–10 dpf) were assigned to control (CON; *Brachionus plicatilis* 2×/day) or high-fat diet (HFD; 20 mg egg yolk 2×/day) groups. At 10 dpf, morphometrics (body length, width, abdominal circumference, BMI-like ratio) were recorded. Locomotion and spatial working memory were assessed by free-movement Y-maze (EthoVision XT15, 60 min), with tetragrams analysed for alternation and repetition strategies. Pools of 5 larvae per replicate were homogenised by sonication, extracted in MeOH:EtOH (1:1), and analysed by LC-MS/MS (Orbitrap Exploris 480, C18 RRHD, H-ESI positive mode, *m/z* 100–700). Differential metabolites were identified using Wilcoxon rank-sum test with Benjamini-Hochberg correction ($p<0.01$, $|Log_2FC|>2$) in Compound Discoverer 3.3.

Results: HFD larvae showed reduced locomotor distance (1501±251 vs. 1838±315 cm; p=0.005) and Y-maze alternation count (39.6±20.4 vs. 76.5±36.4; p=0.007), indicating hypoactivity, while alternation percentage was unchanged (p=0.637), suggesting preserved spatial working memory. Metabolomic profiling revealed pathway-level dysregulation: decreased carnitine/acylcarnitines, nicotinamide and pantothenic acid (β-oxidation, CoA biosynthesis); depleted glutathione and taurine (antioxidant capacity); and reduced acetylcholine and sepiapterin (neurotransmitter metabolism). Elevated aromatic and branched-chain amino acids (L-Tyr, L-Phe, Ile) and 5-methylcytosine indicate disrupted proteostasis and epigenetic remodelling; proline-rich dipeptides (Arg-Pro, Lys-Pro, Ala-Tyr) suggest oxidative protein degradation.

Conclusion: A 10 dpf HFD protocol induces a metabolic phenotype in zebrafish larvae encompassing mitochondrial dysfunction, oxidative stress, and impaired neurotransmitter metabolism, coupled with hypoactivity and preserved cognition — supporting its utility as a tractable DIO model with potential for translational studies.

256

An improved toolkit for prolonged transient expression in zebrafish embryos

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Topic

2 Emerging Technologies

Abstract Content

Transient expression of exogenous mRNA in zebrafish embryos is a rapid, high-throughput strategy for testing genetic constructs and observing early developmental gene expression. However, a major limitation of this method is the short stability of exogenous mRNA, which lasts only about two days after injection into the embryo. Additionally, delivering nucleic acids to embryos requires microinjection, which can cause developmental abnormalities and is labor-intensive, limiting experimental throughput. To address both issues, we are implementing methods to stabilize mRNA transcripts and have developed zebrafish embryo transfection as an alternative strategy for delivering nucleic acids. We demonstrate successful delivery of both mRNA and plasmids, enabling the transfection of multiple constructs in parallel and increasing throughput compared to microinjection. However, our results suggest that transfection delivers a lower amount of nucleic acids to each embryo than a standard injection, limiting our ability to generate stable transgenic lines with this approach. As an alternative, we are evaluating approaches to prolonged transient expression that bypass the need to generate transgenic lines. We found that the Chikungunya (ChikV) viral replicon, an engineered, self-amplifying RNA derived from the viral genome, is expressed for at least nine days post-fertilization in injected and transfected embryos, although the activation of innate immunity is a concern. We are also piloting mRNA modifications that improve transcript stability to prolong transient expression. These methods can be used alone or together in a modular fashion to extend the lifespan of mRNA introduced into zebrafish embryos, enabling experimentation and observation beyond early development. This toolkit improves the time frame and scalability of transient zebrafish experiments, enabling faster *in vivo* construct validation.

257

Detecting endogenous transcription factors using CRISPR integration of short tags

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Topic

2 Emerging Technologies

Abstract Content

Endogenous protein tagging enables precise readouts of cell-state information, such as protein localization and transcription factor signaling at the single-cell level *in vivo*. Traditional zebrafish genetic engineering tools, such as the Tol2 transposase, have enabled high-efficiency transgene expression. As powerful as this approach has been for the community, it suffers from many drawbacks, including overexpression phenotypes or failure to recapitulate endogenous protein localization. A potential approach to mitigate these issues is to create a fusion with a fluorescent protein at the endogenous locus using CRISPR-mediated knock-in. However, the efficiency of CRISPR-mediated knock-in of long donor sequences in zebrafish is often very low, preventing widespread adoption of this approach. We found that reducing the size of DNA donors dramatically increases integration efficiency at multiple different loci. ALFA tag is a short (39 bp) epitope tag that can be used to label endogenous proteins *in vivo* by genetically expressing its cognate nanobody, NbALFA. We are adapting the ALFA tag to label transcription factors involved in the Notch and Wnt signaling pathways to probe endogenous signaling. We have achieved high knock-in efficiency, with PCR-confirmed integration in over 90% of injected embryos, and have recovered multiple stable knock-in lines currently undergoing characterization. In parallel, we are capitalizing on the multifunctionality of the ALFA tag system by developing transgenic lines to manipulate signalling pathway activity via optogenetic induction of ALFA-degron expression. Collectively, these tools will establish a valuable platform for detecting and manipulating endogenous signalling dynamics with high spatial and temporal resolution.

258

pbx4 and *pbx1b* control trunk progenitor territories in the lateral plate mesoderm

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Topic

19 Cell fate

Abstract Content

Pre-B cell leukemia (Pbx) proteins bind Hox proteins to confer body segment identity during embryonic development. How and which pbx genes are responsible for the specification, maturation and morphogenesis of which cell types remains poorly understood. In this study, we found expression of *pbx4* and *pbx1b* to be specific to mesodermal cells, specifically those deriving from the lateral plate mesoderm (LPM). To corroborate our wild-type analyses, we injected zebrafish embryos with CRISPR/Cas9 guides targeting either *pbx4*, *pbx1b*, or both *pbx4* and *pbx1b*. Thereafter, we captured single-cell transcriptomes of each embryo. We found that our approach detects both expected and unexpected changes in cell type abundances and gene expression. Of note, we found that loss of *pbx4* and *pbx1b* results in down-regulation of *wt1a*. *wt1a* is an early LPM transcription factor that promotes anterior kidney and trunk smooth muscle development. Interestingly, we found that loss of *pbx4* and *pbx1b* impacted trunk smooth muscle cell abundances. Lastly, we showed that *pbx1b* and *pbx4* crispants have an expanded heart field at the expense of *wt1a* expression. Together, we found that *pbx1b* and *pbx4* are critical regulators of trunk LPM fate and organ territories. Future work will interrogate how other tissues in the trunk region like the gut and migratory muscle precursors are regulated by *pbx4* and *pbx1b*. Funding: Bonny Brewer Fellowship, Seattle Hub for Synthetic Biology

259

Visualization and molecular control of synovial cells in joint development and regeneration

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Topic

19 Cell fate

Abstract Content

Background

Within synovial joints, synoviocytes maintain tissue homeostasis by producing lubricating synovial fluid critical for frictionless movement. Synoviocytes respond to joint cartilage injury and undergo pathological changes during joint disease. Therefore, it is crucial to understand synoviocyte dynamics during development, health, and disease. However, the mechanisms underlying synoviocyte fate specification and maintenance are unknown, partially due to a lack of specific biomarkers for synoviocytes.

Methods

We identified a synoviocyte-specific *fam219aa_p1* enhancer using snATACseq. To characterize *fam219aa_p1:NTR-mCherry+* cell identity, we used whole-mount embryonic imaging and adult tissue-clearing of *lect1_p1:eGFP, sox9a:eGFP,* and *actb2:MA-Citrine* transgenics with HCR, RNAScope, and immunofluorescence. *nkx3-2* knockdown used published morpholino sequence. 3.5mM Ronidazole induced *fam219aa_p1:NTR-mCherry* cell ablation in adult (3-8 mpf) fish.

Results

We find that the *fam219aa_p1* enhancer specifically labels synoviocytes within the zebrafish synovial jaw joint, with no expression in other joint cell populations (e.g., chondrocytes; n=9) or joint types (e.g., fibrous joints; n=5). We characterized the spatiotemporal dynamics of *fam219aa_p1:NTR-mCherry+* cells and found that they first appeared at 6 dpf and extended cell projections into the joint cavity. Knockdown revealed that *nkx3-2* is required for the fate specification of synoviocytes in development (n=5). Ablation of *fam219aa_p1:NTR-mCherry* cells in adults induced proliferation of *cxcl12a+* stromal cells peripheral to the joint after 4 days (n=4), with mCherry+ cells fully regenerating by 8 days (n=4), suggesting that *cxcl12a*+ stromal tissue may provide a cell source for regenerating synoviocytes.

Conclusion

Here we identified a *fam219aa_p1* sequence that can specifically drive gene expression in synoviocytes within the zebrafish synovial jaw joint. Synoviocyte development in the jaw joint requires *nkx3-2* expression, while adult synoviocyte

regeneration involves proliferation of surrounding stromal tissues. This new model enables visualization and manipulation of synoviocytes, enabling future investigation of their role in joint development, homeostasis, and disease.

262

Light intensity and opsin sensitivity shape the morphology of cone photoreceptor outer segments

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Topic

3 Neurobiology

Abstract Content

Regulation of neural cell morphology remains a fundamental question in neuroscience. Photoreceptor cells, a specialized class of neurons capable of initiating the phototransduction cascade, exhibit distinct structural and morphological characteristics. While the structural and morphological differences between rod and cone photoreceptors have been extensively studied, the variability in the morphology of cone outer segments (OS) remains largely unexplored. Zebrafish possess four distinct cone types, each displaying unique OS morphologies. By modulating opsin expression across cone types, we reveal that the morphology of the cone OS correlates directly with the wavelength sensitivity of the expressed opsins, with cones expressing longer wavelength-sensitive opsins exhibiting elongated OS. This regulatory mechanism is conserved across various vertebrates. Furthermore, we show that alterations in light intensity—induced by ectopic lipid droplet formation in the light path or by changing the environment light intensity—can also modulate OS morphology. Notably, this morphological plasticity is not transient, but rather dependent on long-term neural activity. Based on these findings, we propose a model for the regulation of cone OS length. Our data suggest that both opsin sensitivity and light intensity shape cone OS morphology through long-term neural activity, providing critical insights into neural plasticity in these light-sensitive neurons.

263

The role of actinotrichia as a scaffold for osteoblasts in fin morphogenesis

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Fin rays in teleost fish grow by the addition of bone matrix at their distal ends. At these distal regions, large collagenous crystalline structures called actinotrichia are distributed and are thought to support fin growth throughout the life. Osteoblasts and pre-osteoblasts at the distal-most region of the fin rays are positioned in direct contact with the surface of actinotrichia, and mutants with defective actinotrichia exhibit abnormal fin ray morphology (Huang et al., 2009; Tanimoto et al., 2026). However, the relationship between actinotrichia and osteoblasts organization remain poorly understood.

We focus on the Collagen9a1c (Col9a1c) -deficient mutant that displays abnormalities in both fin ray morphology and actinotrichia organization. Our previous study showed that actinotrichia in this mutant are shortened and disorganized accompanied by fin ray curvature (Nakagawa et al., 2022). In this study, we used this mutant to investigate osteoblasts behavior during fin skeletal morphogenesis.

Using immunohistochemistry, transmission electron microscopy, and transgenic reporter lines, we examined osteoblast organization in mutant. We found that osteoblasts at the fin tip exhibit These analyses revealed that osteoblasts at the fin tip exhibit altered cell shape and abnormal layer organization in the mutant. These results suggest that actinotrichia function as a scaffold that enables osteoblasts to maintain a flattened morphology and properly produce bone matrix.

Our findings highlight the importance of interactions between osteoblast behavior and extracellular scaffold structures during fin skeletal morphogenesis.

264

Exploring the effects of two-dimensional MoS_2 nanomaterials in zebrafish embryos development

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Topic

10 Toxicology

Abstract Content

Background. Toxicity tests using fish are widely recognized as effective tools for assessing the impact of chemical compounds by evaluating both lethal and sublethal toxicological endpoints. Zebrafish (*Danio rerio*) is a freshwater species largely used in ecotoxicological research thanks to its small size, rapid growth, high reproduction rate and genetic similarity to humans. Furthermore, the transparency of embryos during early developmental stages allows direct observation of embryogenesis and facilitates the identification of potentially harmful substances.

Among two-dimensional transition metal nanomaterials, molybdenum disulfide (MoS_2) is one of the most investigated and has been proposed for several applications which may involve direct interaction with biological tissues. Therefore, following environmental exposure, MoS_2 may accumulate in organisms, interact with cellular systems, and potentially induce adverse biological effects.

Methods. Our study evaluated the effect of commercial two-dimensional MoS_2 on zebrafish embryos. Nanopowders purchased from Sigma-Aldrich and supplied by CNR-IMM (Catania Unit), have been used to perform acute toxicity test according to EU guidelines. Zebrafish embryos were exposed to three concentrations (0.05, 0.5, 5 mg/L) of MoS_2 nanopowders in order to assess mortality, hatching rate and malformation occurring during embryogenesis. Moreover, heartbeat rate, body length and pericardial area have been measured by DanioScope™ software.

Results. No significant mortality was observed during embryogenesis in treated groups over exposure. However, an early hatching was found at 48 hpf in embryos exposed to the highest concentration (5 mg/L). No morphological abnormalities were detected in the exposed organisms compared to the controls. Significant differences in both heartbeat rate and pericardial area have been shown ($p<0.05$), while body length was not significantly impaired.

Conclusion. Overall, this study aims to provide new insight about the effects of two-dimensional MoS_2 nanomaterials in aquatic organisms and contributes to the assessment of the potential risks associated with these compounds.

266

Analysis of vocalisation and behaviour of Danionella cerebrum under mating context

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Topic

7 Neural Circuit Behavior

Abstract Content

Danionella cerebrum, a miniature fish with one of the smallest vertebrate brains, produces remarkably loud vocalisations and offers unique opportunities for investigating the neural basis of social behaviour and acoustic communication in vertebrates. We aim to investigate the role of acoustic signals in male and female social interactions and its implication for mating behaviour. As only males are vocal, we pair a single male with one or multiple females while acoustic and behavioural outputs are recorded simultaneously using a multi-hydrophone array and videography. Preliminary result has shown that
male *D.cerebrum* possesses individual vocal signatures, and a machine learning classifier (XGboost) can identify male identity from vocalisations with 94% accuracy across seven individuals. We will further analyse the postural and locomotor dynamics during male-female interactions to link specific acoustic features to behavioural states and assess how females respond to male behaviour. To directly assess female responsiveness to male vocal signals, we will conduct sound playback experiments, presenting females with

natural and synthetic male calls toexamining their behavioural responses. Together,
these behavioural findings will lay the groundwork for uncovering neural basis of social decision-making
and advance our understanding of the neural and behavioural basis of vertebrate acoustic communication.

269

Dissecting the role of YOD1 and ATXN3 DUBs in fine-tuning the inflammatory responses to mechanical injury and skeletal integrity

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Topic

15 Infection and Immunity

Abstract Content

Background

YOD1 and ATXN3 are deubiquitinating enzymes (DUBs) involved in the ubiquitin-proteasome system, ER-associated degradation, and inflammatory signalling. Preliminary data show both DUBs are activated in injured and osteoarthritic cartilage, and that pan-DUB inhibition with PR619 reduces neutrophil migration and NFκB activation in zebrafish. Given that mechanical injury drives osteoarthritis (OA), we hypothesise that YOD1 and ATXN3 are critical regulators of OA-associated inflammation and degeneration. This project aims to (1) delineate their in vivo function using zebrafish knockout and active-site knock-in models; (2) characterise their role in injury-induced inflammation and skeletal development; and (3) identify small molecule inhibitors as therapeutic leads.

Methods

CRISPR/Cas9 was used to generate INDEL knockouts and active-site point mutations of YOD1 and ATXN3 in zebrafish. Tail-fin injury was performed at 3 dpf and neutrophil migration quantified at 2h, 4h, and 24h post-injury by fluorescence stereomicroscopy. Cartilage morphology was assessed using the sp7:col2a1 transgenic line and Alcian Blue staining. A 48-compound library was screened via in silico molecular docking using UCSF ChimeraX and AutoDock Vina against YOD1 (PDB: 4BOQ) and ATXN3 (PDB: 2AGA), with hits validated using recombinant proteins.

Results

Both knockouts significantly reduced neutrophil migration to the injury site. ATXN3 mutants showed decreased neutrophil numbers at 4h and 24h, while YOD1 knockouts showed reductions at all three time points, implicating YOD1 in the earliest inflammatory response. YOD1 knockout fish also displayed cartilage structural abnormalities, revealing a novel role in skeletal morphogenesis. Computational screening identified high-affinity ligand candidates currently undergoing biochemical validation.

Conclusions

YOD1 and ATXN3 regulate injury-induced inflammation and skeletal integrity in zebrafish. DUB knockdown attenuates neutrophil recruitment, and YOD1 loss produces skeletal defects suggesting a broader developmental role. Validated inhibitor leads may offer therapeutic avenues for OA and post-traumatic inflammation.

270

Chronic exposure to titanium dioxide nanoparticles alters SHBG gene expression in adult male Danio rerio

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Topic

10 Toxicology

Abstract Content

The environment is a key receptor for nanoparticles (NPs), allowing them to interact with organisms and cause cellular and systemic effects. Over time, titanium dioxide nanoparticles (TiO_2-NPs) have attracted considerable attention due to their unique physicochemical properties, leading to their widespread use and increasing release into the environment, posing potential risks to ecosystems and human health. Following internalization, TiO_2-NPs can be distributed to various organs via the bloodstream, resulting in multiple biological effects. Growing evidence indicates that NPs exert progressively toxic effects on reproductive systems. We investigated the ability of TiO_2-NPs to influence the expression of sex hormone-binding globulin (SHBG) and, consequently, the physiological activity of the endocrine system.

Adult male *Danio rerio* were exposed to TiO_2-NPs (1, 2, 4 mg/L) for 30 days by chronic toxicity test. A control group was included, and all experimental groups were monitored daily. At the end of the exposure, fishes were anesthetized and dissected to collect liver, intestine, and testes to assess TiO_2-NPs accumulation using ICP-MS. Subsequently, SHBG gene expression was evaluated by qRT-PCR and correlated with immunohistochemical analysis. Addition, the morphological organization of these organs was evaluated.

Our results showed a greater accumulation of TiO_2-NPs in the 4 mg/L exposure group compared to the other groups, particularly in the testes and intestine, where SHBG gene expression was upregulated, as supported by immunohistochemical analysis. Structural alterations occurred only in the testes.

Overall, our findings indicate that TiO_2-NPs can deregulate SHBG gene expression patterns, similarly to other environmental contaminants as phthalates and bisphenol A, which are recognized endocrine disruptors. Physiologically, SHBG is produced in the liver of *D. rerio* during development; however, our findings suggest that in adults, SHBG expression can be altered by TiO_2-NPs, implying a potential disruptive effect on the endocrine system.

272

Activating the protein degradation machinery: Molecular mechanisms regulating protein degradation and developmental tempo during early embryogenesis

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Topic

9 Germline and Early Development

Abstract Content

Life begins with a proteostatic switch: in long-lived oocytes, protein degradation is suppressed to preserve proteome integrity, whereas in embryos, it is reactivated to clear maternal proteins and initiate the embryonic program. A key determinant of proteostasis is the ubiquitin-proteasome system (UPS), which is the primary degradative pathway for short-lived regulatory proteins and damaged soluble proteins, responsible for 80-90% of protein degradation. Although UPS function and regulation have been studied for decades, it is still unclear when UPS activity changes at the transition from dormant oocytes to rapidly developing embryos and how this is regulated on a molecular level. Protein turnover has furthermore been implicated as a regulator of developmental tempo in stem cell models, with faster protein turnover rates correlating with faster developmental tempo. However, whether this applies also in developing embryos and how species-specific turnover rates are achieved mechanistically is currently unknown.

To assess the regulation of protein degradation across developmental time and between species, I am using zebrafish and medaka as vertebrate models. These distantly related teleost species develop under the same environmental conditions but at different speeds. To investigate when proteasome and ubiquitination activities are upregulated during early embryogenesis, I employ biochemical assays and photoconvertible reporters, combined with inhibitor studies. This will establish a fine-grained profile of ubiquitination and proteasome activity and reveal whether faster developing zebrafish have a higher protein degradation activity compared to medaka. In addition, mass spectrometry-based approaches detecting actively degraded proteins and measuring global protein turnover rates will provide insights into proteome remodelling and turnover kinetics of dormancy regulators after fertilisation.

Taken together, my study will elucidate conserved and species-specific regulators of the UPS during early development.

274

Beyond the Cable: Mechanisms and Function of Axon Diameter Diversity in vivo

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Topic

7 Neural Circuit Behavior

Abstract Content

Neurons exhibit enormous diversity, but the mechanisms regulating key aspects of their morphology and function remain poorly understood. For example, we have a particularly limited insight into what influences axon diameter in the nervous system. Axon diameters range from 0.1 to over 10 microns in the vertebrate nervous system, directly influencing conduction speed and myelination. Moreover, axon diameters are altered in neurological disease, injury, and ageing, but the underlying mechanisms and consequences of such alterations remain unknown. Although the biophysical relationship between axon diameter and conduction velocity has been well-established for decades, surprisingly, whether axon diameter is a fixed structural program or dynamically regulated in response to physiological and pathological states remains an open question.

To address the biology of axon diameter, we exploit the optical and genetic advantages of zebrafish to study its regulation in vivo. We developed tools to visualise and characterise axon diameter growth at subcellular resolution in vivo across the CNS and PNS. Further, we performed the first large-scale, discovery screen to identify compounds that influence axon diameter growth in the CNS. Screening 880 compounds, we identified 33 hit compounds influencing axon diameter growth and have made our findings available as a resource for the community. Validation experiments revealed that compounds modulating β2-adrenoceptor and dopamine signalling increase axon diameter, suggesting neuromodulatory pathways in the control of axon diameter. Using chemical manipulation and genetic models with impaired axon diameter, combined with functional readouts of conduction velocity and axonal transport, we are now testing the hypothesis that axon diameter growth does not reflect a fixed developmental program, but is adaptable and can be tuned to meet the conduction, transport, and metabolic demands of the nervous system. Understanding this regulation — in health and disease — may reveal axon diameter as a key feature in nervous system plasticity and resilience.

275

A Novel Infection Platform Using Zebrafish Larvae to Investigate Immune Responses in Microbial Keratitis

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Topic

15 Infection and Immunity

Abstract Content

Background: Microbial keratitis (MK) is the second commonest cause of unilateral blindness, yet there remains limited therapeutic interventions for this. While excessive dysregulated inflammation is known to induce bystander tissue injury, immunomodulatory options remain inadequate. This means, patients are left with visually disabling consequences due to corneal scarring.

One of the reasons for this is the scarcity of disease models to study the inflammatory response to the disease. The 2024 UK Clinical Eye Research Strategy recognised MK as a priority area, underscoring the critical need for improved experimental models to elucidate disease mechanisms and identify novel therapeutic targets. Here, we aim to use the zebrafish larvae system to study the immune response to MK.

Methods: Three-day post-fertilisation transgenic zebrafish larvae co-expressing fluorescent reporters in neutrophils, macrophages and basal epithelial cells, *Tg(LysC:mTurquoise; mfap4:tdTomato-CAAX; K19:GFP),* were employed. We used a custom zebrafish injury platform to maintain stable horizontal positioning during corneal injury and imaging, eliminating the need for agarose embedding. Using a standardised mechanical corneal abrasion, larvae were exposed to established chemoattractants - lipopolysaccharide and leukotriene B_4 - or live bacteria. Innate immune cell recruitment and behaviour were quantified using spinning disc confocal and electron microscopy.

Results: Corneal injury elicited rapid immune cell accumulation at the wound site, augmented by pro-inflammatory mediators ($P<0.01$). Electron microscopy confirmed epithelial disruption and neutrophil extracellular trap formation. Live bacterial challenge triggered a 2-fold increase in neutrophil and macrophage recruitment ($P<0.0002$), with time-lapse imaging demonstrating enhanced mean neutrophil velocity and migratory displacement by 1.9- and 1.7-fold respectively ($P<0.001$).

Conclusion: This platform enables real-time, mechanistic interrogation of innate immune dynamics during MK *in vivo*. Its scalability makes it highly advantageous for high-throughput screening of candidate immunomodulatory agents, which are urgently needed. This offers an exciting route towards therapeutic development to temper pathological inflammation for this sight-threatening condition.

276

Conserved non-coding elements dowsntream of *tbx5a* gene show enhancer activity in developing zebrafish hearts

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Topic

4 Cardiovascular

Abstract Content

Background. Heterozygocity for loss-of-function mutations in Tbx5 causes the Holt-Oram syndrome in humans. The affected individuals display congenital defects in hand and heart development. Gain of function mutations in the Tbx5 have been shown to also cause Holt-Oram phenotypes, albeit atypical, indicating that expression of Tbx5 must be very precisely regulated in humans. Its essential role in forelimb and heart development is conserved across vertebrates including the zebrafish ortholog *tbx5a*. Despite biomedical importance and high degree of functional evolutionary conservation, our knowledge of cis-regulatory elements required for proper Tbx5 expression remains scarce.

Methods. Two different miniTol2 vectors were used to clone and test candidate enhancers. We used the *c-fos* minimal promoter driving eGFP as a reporter. As our alternative construct, we cloned the *hsp70* minimal promoter in front of the mRFP reporter. We used CRISPR/Cas9 nuclease to delete a ~15 kb region downstream of *tbx5a* that contains multiple *tbx5a* enhancers.

Results. We identified several conserved non-coding elements capable of driving reporter expression in developing zebrafish hearts using both eGFP and mRFP reporter systems. We deleted the 15-kb enhancer-rich region that contained the identified *tbx5a* enhancers, however the deletion did not lead to overt developmental heart defects.

Conclusion. The deletion of a 15-kb enhancer-rich region downstream of *tbx5a* is not sufficient to disrupt the development of zebrafish heart.

277

A larval zebrafish thrombocytopenia model for assessment of cardiovascular toxicity induced by anti-cancer drugs

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Topic

4 Cardiovascular

Abstract Content

Background
Zebrafish thrombocytes are nucleated cells functionally analogous to mammalian platelets. Beyond their role in coagulation, they are important regulators of vascular integrity and endothelial homeostasis. They contribute to the maintenance of vascular barrier function. While thrombocytopenia models have been described in adult zebrafish, comparable approaches for larval stages are limited. The aim of this study was to develop a zebrafish larval model with reduced thrombocyte number to enable investigations of thrombocyte involvement in cardiovascular toxicity induced by anti-cancer drugs such as tyrosine kinase inhibitors (TKIs).

Methods
Candidate genes involved in thrombocyte development and regulation (*thpo, mpl, brf1b*) were selected based on the literature. For our experiments we used the *Tg(cd41:GFP)* zebrafish line with fluorescently labelled thrombocytes. Gene knockdown was performed using the CRISPR/Cas9 system in zebrafish embryos. Selected genes were tested individually as well as in different combinations to evaluate their effect on thrombocyte depletion. Larvae were subsequently assessed both phenotypically and genotypically to confirm the efficiency of gene targeting and to evaluate thrombocyte levels in circulation.

Results
Targeting selected genes resulted in a reduction of circulating thrombocytes in zebrafish larvae exceeding 50%. Notably, combined targeting of multiple candidate genes did not lead to a significantly greater decrease in thrombocyte numbers compared with targeting the *mpl* gene alone, suggesting that *mpl* disruption plays a dominant role in observed thrombocytopenic phenotype. These findings indicate that CRISPR/Cas9-mediated targeting of thrombocyte-related genes can be used to establish a larval zebrafish model with reduced thrombocyte number.

Conclusion

CRISPR-based *mpl* knockdown approach provides a foundation for establishment a zebrafish larval thrombocytopenia model that is currently being applied in our studies investigating cardiovascular toxicity of TKIs. Given the emerging role of thrombocytes/platelets in

maintaining vascular integrity and contributing to vascular disease progression, thrombocytes may represent important effectors in cardiovascular alterations triggered by TKIs.

278

Endothelial profiling in zebrafish: focus on tyrosine kinase inhibitors

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Topic

4 Cardiovascular

Abstract Content

Background:
Tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1 have revolutionized the treatment of chronic myeloid leukaemia, but their use is associated with cardiovascular toxicity, recently linked to activation of the innate immune response. Although zebrafish are widely used to study drug mechanisms, they are rarely applied to assess endothelial function. Here, we developed a methodology to evaluate TKI-induced endothelial dysfunction in zebrafish larvae.

Methods:
Endothelial effects of TKIs were examined in zebrafish larvae by measuring vascular permeability and vasoconstriction in the dorsal aorta. Cardiac toxicity was evaluated through assessment of pericardial oedema and cardiac performance (fractional shortening and fractional area change). Expression of inflammatory and endothelial activation markers was analysed, and the contribution of innate immune cells was tested using genetic ablation of leukocytes (neutrophils and macrophages).

Results:
Ponatinib (third-generation TKI) induced vascular hyperpermeability and vasoconstriction in the dorsal aorta, accompanied by pericardial oedema and reduced cardiac fractional shortening and fractional area change. Nilotinib (second-generation TKI) also increased vascular permeability and caused vasoconstriction and pericardial oedema, but without cardiac dysfunction. Imatinib (first-generation TKI) had minimal effects on vascular tone. However, imatinib and asciminib were not effectively absorbed by static immersion in zebrafish larvae. Ponatinib-induced endothelial effects were associated with increased *vcam* and *icam* expression and slightly elevated *il-1β* and *tnfa* levels. Genetic ablation of leukocytes did not modify ponatinib-induced vasculotoxicity.

Conclusion:
Endothelial profiling in zebrafish larvae provides a quantitative *in vivo* approach to predict vascular toxicity of anti-cancer drugs. Ponatinib showed the strongest detrimental effects on endothelial function, independent of innate immune cell–driven inflammation.

279

Cre at Work: Expanding Conditional Zebrafish Lines for Visualization and Functional Studies

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Topic

2 Emerging Technologies

Abstract Content

Strong promoters with broad activity across developmental stages and in adult organisms are crucial for robust, consistent transgene expression. In the context of conditional gene inactivation or reporter assays, such promoters are required to drive sufficient recombinase expression for complete and reproducible excision or inactivation, as well as to ensure strong reporter expression for reliable detection and quantification.

In our lab, we developed a hybrid ubiquitous promoter, *ubb^{R}* (R for recombinant), by combining the native zebrafish *ubiquitin* promoter with an enhancer element from the *beta-actin 2* gene of common carp. Using a targeted PhiC31-mediated integration system to ensure identical genomic context, we found that *ubb^{R}* drives substantially more efficient Cre-mediated recombination of the *Tg(-3.5ubb:loxP-EGFP-loxP-mCherry)**cz1701*** (*ubi:Switch*) reporter than either parental promoter, especially at later stages of development. Subsequent removal of the vector backbone further improved the recombination efficiency, allowing us to establish a strong Cre driver line, *Tg(tpl102.ubb^{R}:CreER^{T2}*)**vln2***, which reliably induces recombination of the *ubi:Switch* reporter across later developmental stages and in various tissues of adult zebrafish. This line also efficiently induces a complete knockout of the *tbx20* and *tcf21* genes in both developing embryos and the adult zebrafish heart. Additionally, three randomly integrated Cre-driver lines generated with the same promoter all exhibit distinctly high recombination efficiency at later embryonic stages.

To further facilitate functional studies and expand experimental capabilities, we generated a versatile suite of single-copy transgenic zebrafish lines, including reporter lines for lineage tracing (*ubb^{R}:loxP-STOP-loxP-sfGFP* and *ubb^{R}:loxP-mRFP-loxP-sfGFP*) and lines for Cre-inducible visualization or manipulation of intracellular processes (*ubb^{R}:loxP-mRFP-loxP-GCaMP6; ubb^{R}:loxP-mRFP-loxP-LifeAct; ubb^{R}:loxP-mRFP-loxP-UtrCH*, and *ubb^{R}:loxP-mRFP-loxP-dnATG5K130R*). Validated single-copy transgenics will serve as a versatile platform for in vivo studies of cell behaviour and function during zebrafish development and regeneration.

280

Ancestral functions of genes regulated by imprinting in mammals: Characterising the role of *grb10* genes in Zebrafish

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Genomic imprinting is an epigenetic form of gene expression that affects a group of ~100 genes in mammals. This involves silencing of one of the parental alleles and results in parent-of-origin dependent monoallelic expression. Imprinting has been extensively studied in mammals, yet the origins of imprinting remain uncertain. The prevalent parental conflict hypothesis suggests that genomic imprinting evolved to act on genes that already had established roles in growth regulation, as for IGF2, yet very few genes have been investigated across vertebrate species.

Grb10 is an adaptor protein and imprinted gene involved in growth, cell proliferation and insulin/mTORC1 signalling. It is a known growth inhibitor, with maternal *Grb10KO* mice being 30% larger than their WT siblings at birth.

To test the hypothesis that imprinting evolved to act on genes involved in growth, there is a need to investigate the role of orthologous genes in species that lack imprinting mechanisms.

For both zebrafish *grb10* orthologues, WT expression patterns have been established using RNAScope multiplex fluorescence assay, showing they are distinct. We have developed CRISPR knockout lines and have characterised the growth phenotypes of single and double homozygotes using light microscopy, Fiji Image J analysis, various staining techniques, and RNA transcriptomics.

Single homozygous mutants show normal morphology and are viable to adulthood. Double homozygous mutants have normal morphology for the first 5 days post fertilisation. Thus far, there does not seem to be an obvious growth phenotype in *grb10* mutant zebrafish. We hypothesise that other roles for Grb10 identified in mammals, including insulin signalling, may be conserved in zebrafish. We propose that the ancestral function of grb10 may not be related to growth or development and that imprinted expression evolved together with a novel growth regulatory role directly in mammals.

281

SNUPN Loss Disrupts the Muscle Stem Cell Niche and Regeneration in Zebrafish

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Topic

1 Disease Models

Abstract Content

We recently identified *SNUPN* loss-of-function variants as the genetic cause of a novel form of muscular dystrophy (MD), expanding the spectrum of limb-girdle MDs (MIM#620793). Snurportin-1(SPN1), encoded by *SNUPN*, is an adaptor protein required for the nuclear import of spliceosomal small nuclear ribonucleoproteins (snRNPs). Despite its established role in RNA processing, its physiological function in muscle homeostasis remains poorly understood, partly due to the lack of a suitable in vivo model. To address this gap, we generated a *snupn* mutant zebrafish model.

snupn mutant zebrafish line was generated using CRISPR/Cas9. Validation and characterization were performed using Sanger sequencing, quantitative PCR (qPCR), immunofluorescence, histology/ultrastructural analyses, confocal microscopy, birefringence assays, behavioral analyses, and omics approaches.

The *snupn* mutant line carries an 8-bp insertion resulting in a premature stop codon (p.Ser116Ter) and significantly reduced *snupn* transcript levels. Mutant larvae displayed muscle fiber abnormalities and subcellular organelle alterations accompanied by reduced birefringence and impaired locomotor activity. Transcriptomic analyses revealed dysregulation of genes involved in muscle morphogenesis, extracellular matrix (ECM) organization and basement membrane composition, consistent with ECM disorganization observed in patient muscle biopsies. Pathway enrichment indicated perturbations in metabolic pathways, niche disruption, inflammatory responses, and impaired regenerative processes. In line with these alterations, *snupn* mutants exhibited early defects in muscle progenitor function, characterized by a significant reduction in satellite cell markers, impaired activation of key myogenic regulators, and defective muscle fiber differentiation.

Together our findings demonstrate that SPN1 is essential for early muscle fiber stabilization, ECM organization, and regenerative capacity. The Spn1-deficient zebrafish

model recapitulates key pathological features of the *SNUPN*-related muscular dystrophy and provides a robust platform to investigate disease mechanisms and therapeutic strategies.

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282

Zebrafish meningeal fibroblasts repress the chondrogenic program via *Osr1*

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Topic

19 Cell fate

Abstract Content

In early vertebrate development, meningeal fibroblasts have a critical role in secreting bone morphogenetic proteins (BMPs) which facilitate cortical neuronal migration and cranial suture closure, but the genetic requirements of meningeal fibroblasts have yet to be fully catalogued. Single-cell RNA-seq datasets sampled throughout a developmental time-course posit the potential regulators required for the specialization of meningeal fibroblasts. Using a single-cell RNA-seq dataset generated from whole zebrafish embryos sampled throughout the first 96 hours of development, I found that *Osr1*, a transcription factor known to repress downstream effectors of BMP signaling, was specifically upregulated in meningeal fibroblasts secreting BMPs. This was noteworthy, as *Osr1* is known to mark cells which should not undergo chondrogenesis in several vertebrate contexts such as the mammalian tongue mesenchyme and synovial joint, by directly inhibiting the transcription of the BMP-induced master regulator of chondrogenesis, *Sox9.* I hypothesized that *Osr1* was necessary for the specialization of meningeal fibroblasts, and loss of function of *Osr1* would result in the induction of chondrocyte markers via ectopic BMP effector activity. To test my hypothesis, we utilized the F0 CRISPR knockout strategy to introduce mosaic, frame-shift mutations to *Osr1*, and I applied single-cell RNA-seq to whole, perturbed zebrafish embryos to survey the cell-type resolved shifts in gene expression throughout the embryo. As expected, in the absence of *Osr1* function, meningeal fibroblasts significantly upregulate a slew of chondrocyte markers such as the Sox trio, and collagens canonically induced during chondrogenesis. Satisfyingly, this result supported my model that meningeal *Osr1* reprises its role in regulating chondrogenesis likely through a shared inhibitory mechanism previously characterized in the mammalian tongue and limb mesenchyme.

Future work will validate these differentially expressed chondrocyte genes through whole-mount confocal microscopy. Funding: NIH (5R01HG012761-02), Chan Zuckerberg Initiative (CZIF2023-008738)

283

Extensive transcriptomic response and sustained *ankrd1a* activation in cryoinjured adult zebrafish skeletal muscle

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Topic

14 Stem Cells and Regeneration

Abstract Content

Skeletal muscle repair relies on conserved cellular and molecular programs that scale with the severity of injury. Cryoinjury has recently been established as a skeletal muscle injury model that induces extensive tissue degeneration resembling volumetric muscle loss, but its transcriptomic response remains unexplored. The stress-responsive gene *ankrd1a*, associated with tissue remodeling during muscle healing, is activated during repair of stab-wounded skeletal muscle, a model of localized injury, and during regeneration of the cryoinjured heart, suggesting that it may participate in repair responses across injuries of different severity, including cryoinjury.

To characterize the transcriptomic response to extensive muscle damage, we performed RNA sequencing of adult zebrafish skeletal muscle at 7 days post-cryoinjury (dpci), when damaged muscle is partially restored, and newly formed fibers emerged. Differential expression and functional enrichment analyses were conducted. Gene expression was quantified by qPCR, and spatiotemporal activation of *ankrd1a* was assessed using a transgenic reporter line expressing EGFP under the *ankrd1a* promoter.

RNA sequencing analysis revealed pronounced transcriptional remodeling of cryoinjured muscle. Principal component analysis showed clear separation between the injured and control samples. A total of 5,330 genes were differentially expressed, the vast majority being upregulated (5,077 vs. 253 downregulated). Upregulated genes were enriched in processes related to cell adhesion, mitotic activity, extracellular matrix remodeling, and skeletal/connective tissue development. Downregulated genes were primarily associated with metabolic processes. Comparison with transcriptomic changes reported after stab injury revealed that cryoinjury elicits a substantially broader transcriptional response while engaging overlapping regenerative pathways. Among the strongly induced genes, *ankrd1a* showed robust and sustained upregulation, with persistent activation observed from 4 to 50 dpci.

Extensive skeletal muscle injury in zebrafish triggers a broad transcriptional response and prolonged *ankrd1a* activation, further supporting *ankrd1a* as a marker of adaptive muscle remodeling across different injury paradigms.

285

Retinoic acid signaling from pericytes is required for the development of zebrafish gill filaments

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The development of the teleost gills is a fascinating process that involves all three germ layers and the neural crest. Gill filaments in zebrafish emerge from the distal surface epithelium of the first four branchial arches and develop numerous lamellae on their surfaces. The core of each filament is formed by a looped artery deriving from the branchial arch vasculature. Gill filaments provide structural support for important physiological processes, such as gas exchange, oxygen uptake, osmo- and pH-regulation, and for maintaining the nitrogen equilibrium.The molecular mechanisms of gill formation so far have remained largely unexplored.

Here we show that gill filaments develop under the control of retinoic acid (RA) signaling. Prior to the formation of each filament, a tip cell sprouts from the branchial artery to initiate the angiogenic process that eventually forms an arterial loop within each filament. We first establish that RA-generating and RA-degrading enzymatic activities are expressed in gill arches, but not in filaments. *aldh1a2*, encoding the enzyme producing RA, is expressed in neural crest-derived pericytes, a type of mural cells that stabilize newly formed vasculature by wrapping around smaller blood vessels. Because GFP-fluorescence remains high in migrating cells in filaments, we conclude that pericytes down-regulate *aldh1a2* expression before migrating into the forming filament, where they accompany the newly forming microvasculature. Blocking RA signaling inhibits the formation of new gill filaments prior to or during their emergence from the branchial arches, while increasing endogenous RA levels results in up to 25% more gill filaments by 5 dpf. These findings support a model in which gill arch pericytes control a sprouting rate-limiting early step during angiogenesis in developing gill filaments and identify RA as a new signal through which pericytes control vascular development.

289

Jam2 Signaling Functions Downstream of Hand2 To Initiate The Formation Of Organ-Specific Vascular Progenitors

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Topic

4 Cardiovascular

Abstract Content

Background: The mechanisms regulating the formation of organ-specific vasculature are still poorly understood. We have previously identified a population of late-forming endothelial progenitor cells in zebrafish embryos (termed secondary vascular field, SVF), which emerge from the lateral plate mesoderm after 24 hpf stage, after blood circulation has been initiated.

Methods: Here we investigate the functional role of SVF cells and the molecular mechanisms that govern the emergence of these SVF cells and their contribution to vasculature in zebrafish embryos.

Results: We identified that the bHLH transcription factor Hand2 and Junctional Adhesion Molecule Jam2b are expressed in the SVF-forming region and are required for the emergence of SVF cells in zebrafish embryos. Time-lapse imaging and *jam2b:Cre* based lineage tracing showed that SVF cells serve as the major source of the intestinal vasculature, including the supraintestinal artery (SIA) and subintestinal vein (SIV), which are subsequently remodeled to provide the blood flow to many internal organs. To analyze the functional role of *jam2b* and a related *jam2a* gene in vascular development, we generated double maternal-zygotic *jam2a; jam2b* mutants, which display a greatly reduced number of SVF cells and show defects in the intestinal vasculature development. Further analysis showed that *hand2* functions in the SVF-forming region upstream of *jam2b* and is required to induce expression of transcription factor *etv2/etsrp,* a known master regulator of vasculogenesis.

Conclusion: Our results identify new roles for Jam2 signaling and Hand2 function in the emergence of organ-specific vascular progenitors. The presence of similar progenitors in mammalian embryos suggests that this mechanism is evolutionarily conserved.

292

Role of the transcription factors Onecut1 and Pou4f1 in the differentiation of the neurons of the statoacoustic ganglion.

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Topic

3 Neurobiology

Abstract Content

The inner ear is a complex sensory organ responsible for the senses of hearing and balance. Bipolar neurons of the Statoacoustic Ganglion (SAG) are in charge in transmitting auditory and vestibular information received by hair cells to the brainstem. ATAC-seq and RNA-seq data was performed and identified time-specific open chromatin regions associated with gene activation of the transcription factors, *pou4f1, onecut1, cux2a,* and *mef2aa*. Here we present detailed characterization of the dynamical expression of *pou4f1* and *onecut1* using new knock-in transgenics, while their functional roles in SAG development was assessed in crispant embryos. Phenotypes were analyzed from 24–96 hpf via lightsheet microscopy, HuC immunostaining (post-mitotic neurons), and functional flotation assays.

We found that *pou4f1* is expressed throughout the development of the SAG when neuroblasts mature. Interestingly, *pou4f1* also expressed in one or two hair cells (HCs) of the otic vesicle at 24hpf that correspond to first born HCs, this expression is maintained while is excluded from new HC. *pou4f1/pou4f2* double-crispants exhibit failed SAG growth and reduced otic vesicle size from 24 hpf; notably, the most severe phenotypes decrease by 96 hpf, suggesting larval lethality. Onceut1 expression is only detected in the SAG and notably only in a subpopulation of neurons corresponding to vestibular neurons. In *onecut1* crispants, there is severe SAG volume reduction, loss of posterior SAG development, and diminished *islet2b* expression from 24 hpf. This was accompanied by a loss of HuC+ neurons, indicating decrease of differentiated post-mitotic neurons. Functionally, *onecut1* crispants show impaired buoyancy, sinking immediately in flotation assays.

Our results identify *onecut1* and *pou4f1/pou4f2* as critical SAG regulators. The loss of *islet2b* and HuC+ cells in *onecut1* crispants suggests a requirement for maintaining the *islet2b* program or sensory neuron survival, rather than initial specification. Furthermore, localized *pou4f1* expression suggests a specialized role in tether cell patterning.

294

V-ATPase activity promotes hepatocyte polarity and directs hepatocyte positioning in the liver architecture during liver development in zebrafish.

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Topic

5 Tissue Formation

Abstract Content

Impaired liver tissue integrity is a hallmark of liver pathogenesis and frequently leads to loss of liver function. To advance therapeutic approache it is of significance to unravel the developmental processes orchestrating hepatocyte polarization and the stereotypic positioning of the different cell types, with respect to each other, in the tissue. There is emerging evidence that endothelial cells play a pivotal role in modulating hepatocyte polarity in early stages of liver development. However, the nature of the signals and how they are integrated by hepatocytes remains largely unknown. Utilizing high resolution imaging in *cloche* mutants, lacking endothelial cells, we showed that hepatocytes exhibit unusually widened ZO-1 and aPKC-positive apical domains at potential connection sites to the billary system, and frequently ectopic apical cysts. This suggests endothelial cells direct hepatocyte polarity and connectivity of the apical, bile secreting domain of hepatocytes to the bile ducts. Based on a proteomic screen performed on *cloche* livers, we investigated the vacuolar H^+-ATPase, a regulator of protein trafficking, as a potential intracellular mediator. Genetic and pharmacological perturbation of the v-ATPase, utilizing *atp6ap1b*^{pd1209} mutants and Bafilomycin treatment respectively, revealed aberrant canaliculi morphologies, reduced canaliculi number and increased formation of intersections between the vascular and biliary networks, suggesting contacts normally not observed in the healthy liver. These results suggest that the v-ATPase, a protein trafficking component, promotes hepatocyte polarity and maturation of apical canalicular domain. Thereby, it contributes to establishing the liver´s functional architecture by directing hepatocyte positioning essential for correct spacing of the vascular and biliary networks during liver development.

296

Slit1a in retinogenesis: effect of its mutation on developing retinal ganglion cells

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Topic

5 Tissue Formation

Abstract Content

Background. Retinal ganglion cells (RGCs) extend axons that reach the tectum, growing through the optic nerve, chiasm and tract. This process is regulated by axon guidance cues such as the Slit factors, acting on Robo receptors. We demonstrated a role for Slit2 and Slit3 in RGC axon guidance around the optic chiasm. Mutations/knockdown in these genes individually showed a similar phenotype, where axons from one optic nerve eventually split in two, surrounding the contralateral nerve. *slit2* mutants, in addition, showed defects in axon segregation at the optic tract. Double mutants presented a more severe axon guidance phenotype, comparable to that seen in the *robo2* mutant, *astray*. We have now analyzed the role of Slit1a using a similar approach.

Methods. We generated *slit1a* crispants by injecting Cas9 + 4 gRNAs in one-cell embryos. Effects on RGC integrity and axon guidance were assessed using immunofluorescence and confocal microscopy. Image processing and analysis was made with Fiji.

Results. Upon *slit1a* mutation, no axon guidance defects in the optic pathway were evident, but we observed a significant reduction of the optic nerve diameter. We wondered if this was due to lower RGC numbers, and decided to quantify this by measuring the volume of retinal layers. We found a proportionally smaller RGC layer in *slit1a* crispants compared to controls. These embryos were not affected in other aspects, and all the staging parameters showed a normal general development. This indicated that *slit1a* mutation might be causing a reduction in the number of RGCs, which could be due to a combination of affected cell proliferation and cell death.

Conclusion. Slit1a does not appear to be essential for axon guidance in the optic pathway, but could regulate RGC numbers inside the retina, opening new doors for understanding the role of Slit factors during retinogenesis.

297

Molecular-scale analysis of the cellular behavior and plasticity of donor and host cells revealed by inter-species chimeras in fish

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Topic

9 Germline and Early Development

Abstract Content

Embryonic development reflects an interplay of diverse intrinsic and extrinsic factors that shape cell fate decisions and tissue formation. To disentangle the intrinsic role of developmental modules at the cellular level and to discover the effect of their interaction during embryogenesis, we performed inter-species blastomere transplants in zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). Building on our previous findings that this inter-species transplantation strategy has a strong tendency to generate ectopic (zebrafish) retinae in medaka hosts, we sought to uncover mechanisms of donor cell fate preference by combining genetic perturbations with a single-cell transcriptomic readout. By perturbing rx3, a transcription factor required for retinal cell fate choice, we assessed alternative differentiation paths for zebrafish donor cells. To overcome the challenge of visual readouts for cell fate choice, we profiled whole chimeric embryos and to account for embryo-specific variability, we performed embryo-resolved single-cell RNA-seq on 20 chimeras, coupled to imaging readouts. Our analyses demonstrate a wide-ranging differentiation potential of donor cells, and we characterize similarities and differences of zebrafish and medaka retinal development in these diverse extrinsic contexts. By combining molecular and cellular readouts of how cells develop in diverse external contexts, we shed light on how the cell-intrinsic genetic programs are altered by the species-specific and spatial environmental signals. Overall, this study uncovered new avenues about the molecular and cellular basis of development, evolution, and regulation of cellular traits.

298

E-cigarette condensate impairs early neutrophil recruitment during zebrafish tail fin regeneration

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Topic

10 Toxicology

Abstract Content

Electronic cigarettes (e-cigarettes) are widely advertised as safer alternative to conventional ones. However, increasing evidence suggests exposure to e-cigarettes can negatively affect wound healing and immune system. Previous studies have reported altered cytokine production in macrophages and changes in protease levels following exposure to e-cigarettes. However, effects of e-cigarettes on early immune responses during tissue regeneration remain poorly understood. In this study, we examined effects of e-cigarette condensate on neutrophil recruitment during tail fin regeneration in zebrafish larvae, a well-established in vivo model for studying inflammation and tissue repair.

Two-day-old Tg(mpx:GFP) larvae, in which neutrophils are fluorescently labeled with GFP, underwent amputation of tail fin, and immediately exposed to home-made e-cigarette condensates containing nicotine (ECN; 0.1%) or watermelon ice flavor (ECW; 0.01% and 0.02%). The concentrations used were tested and shown to be subtoxic for larval development. Each group consisted of at least 10 larvae. Larvae were imaged 4 and 24 hours post amputation (hpa), on Zeiss fluorescent microscope. Neutrophils were counted 300µm region of interest proximally from wound plane, using ZEN software. Statistical analysis was performed using GraphPad Prism 8.4.2. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test.

The average neutrophil count in the control group was 30.9±9.2. At 4 hpa, exposure to ECN significantly reduced neutrophil numbers (18.6±11.7, p=0.019). Larvae treated with 0.01% ECW showed a modest decrease (26.3±11.9), whereas 0.02% ECW resulted in a marked reduction (14.2±5.1, p=0.0015). At 24 hpa, neutrophil numbers remained lower in treated groups but differences were not statistically significant, suggesting partial recovery of the inflammatory response.

These findings indicate that e-cigarette condensate disrupts early neutrophil recruitment during regeneration, which can impair this process. Future studies will investigate macrophage recruitment and TNF-α signaling to further elucidate mechanisms underlying e-cigarette–induced immune modulation.

299

Morphogenesis and cell fate during olfactory epithelium formation

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Topic

19 Cell fate

Abstract Content

Morphogenesis and cell-type differentiation are highly coordinated in sensory organs to ensure their function. Morphogenesis of the olfactory epithelium (OE) in zebrafish provides a unique model to study this process as undifferentiated cells aligned around the anterior neural plate mature into clusters of early olfactory cells across a short time scale. While the Cxcl12a/Cxcr4b signaling pathway drives this process, what constraints on pathway activation apply during morphogenesis are unclear.

We developed a mathematical model recapitulating Cxcl12a-mediated OE morphogenesis. Restoring Cxcl12a expression in mutants for the ligand rescues correct morphogenesis both in silico and in vivo. However, where expression of the ligand is restored is crucial for rescue, a point not predicted by our model and suggesting an unexpected level of pathway activation control. Analysis of a Cxcr4b activation reporter supports this idea. We concluded that mosaic and heterochronic Cxcl12a activation along the anteroposterior axis sculpts the olfactory epithelium. However, we lack proper tools to compare our 4-D samples and track olfactory cells over long periods of time. To quantify spatial organization in developing embryos and understand morphogenetic processes, we are developing a semi-automated approach implemented as a Napari plug-in, ImaRef. Our method allows users to generate a reproducible reference frame by manually placing a minimal set of identifiable key points in the zebrafish olfactory organ.

Through single-cell transcriptomic analyses of olfactory cells at different stages of morphogenesis and cell identity acquisition in zebrafish, we have identified the signature of ciliated olfactory sensory neurons over time. Our preliminary data suggest that there is a population of olfactory cells already committed to this cellular fate.

300

Nr2f2 impacts brain asymmetry through the regulation of parapineal cell fate

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Topic

19 Cell fate

Abstract Content

Background: Brain asymmetries are fundamental to vertebrate nervous system organisation. In zebrafish an unidentified signal from the left-sided parapineal promotes left-sided habenular character and consequently habenular asymmetry. The transcription factor encoding gene *tbx2b* is required for the specification of parapineal cells from the anterior domain of the pineal complex, but as *tbx2b* is expressed throughout the forming pineal and parapineal, it is likely that other genes have activity restricted to the nascent parapineal precursors.

Methods: We conducted targeted CRISPR/Cas9 screening of genes expressed in dorsal habenular progenitors. Mutant phenotypes were assessed for expression of left-right asymmetry markers, using both HCR in situ hybridisation and immunohistochemistry. Parapineal development was analysed using live imaging with transgenic lines labelling the pineal complex and a transgenic *nr2f2* reporter line. Further analysis included multi-gene spatial expression mapping using HCR.

Results: *nr2f2* mutants show complete parapineal absence with bilateral right-symmetric habenulae, while visceral asymmetries remain normal. Unlike *sox1a* mutants where parapineal cells develop but cannot signal, *nr2f2* mutants lack parapineal cells entirely, indicating an earlier developmental requirement. *nr2f2* mRNA and reporter transgene expression are restricted to parapineal precursors in the anterior pineal complex with expression maintained during later parapineal migration. Current work is examining whether Nr2f2 establishes the anterior subdivision of the pineal complex to distinguish parapineal from pineal fates.

Conclusions: Nr2f2 is required for parapineal cell development from within the anterior domain of the pineal complex. This work is providing new insight into the genetic control of brain asymmetry development.

301

Rapid and functional intestinal regeneration after full-thickness transection in zebrafish is differentially regulated by IL-22 and IL-26

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Topic

14 Stem Cells and Regeneration

Abstract Content

Zebrafish are widely used to study organ regeneration after severe injury, yet the gut remains largely unexplored in this context. In mammals, complete transection of the intestinal tube, disrupting all layers, does not result in regeneration. This raises the question: can a highly regenerative vertebrate such as zebrafish spontaneously restore a fully functional gut tube after full transection?

Here, we establish a zebrafish larval model of complete intestinal transection using mechanical injury. We then use live imaging, cellular analysis, and transcriptomic profiling to characterize and validate our model.

We demonstrate that the zebrafish gut can quickly restore epithelial continuity, re-establish a functional lumen, and resume food transit within 48 hours post-injury. We show that regeneration involves coordinated contributions from epithelial, immune, and mesenchymal compartments. Neutrophils and macrophages are recruited early to the injury site, while mesenchymal cells rapidly reposition. We identify a transient wave of epithelial proliferation during repair, accompanied by STAT3 signaling activation. Pharmacological inhibition of the JAK/STAT pathway impairs epithelial reattachment and decreases injury-induced proliferation, supporting its role in repair. Injury also induces *il22* expression in epithelial cells at the lesion site, with the cellular source shifting from mostly *neurod1*$^{+}$ cells under homeostatic conditions to a mainly *neurod1*$^{-}$ population during regeneration. Functional studies show that IL-22 promotes epithelial reattachment and regenerative proliferation via STAT3, while IL-26 restrains proliferation, revealing opposing cytokine functions during intestinal regeneration.

Together, our work establishes a tractable in vivo model to study complex intestinal repair and reveals how cytokines of the IL-10 family orchestrate distinct phases of epithelial regeneration.

303

Investigating the role of Her4.1 gene expression dynamics in spinal cord regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

Spinal cord injuries in humans are incompletely repaired due to a lack of neuronal regeneration and axonal regrowth. In species that successfully repair spinal cord lesions, developmental signals such as Notch, Wnt, and BMP, are reactivated, promoting neurogenesis and the formation of axonal bridges. However, these same signalling pathways are also upregulated in response to injury in non-regenerative species, suggesting further requirements for the reactivation of neuronal differentiation.

The downstream effectors of the Notch signalling pathway, Hes/Her transcription factors, show ultradian oscillations in gene expression (i.e. fluctuations in protein concentration in a periodic manner), and changes in their oscillatory behaviour can influence cell fate decisions, including successful exit of neural progenitors from quiescence. Therefore, we hypothesise that different Hes/Her expression dynamics lead to divergent cell fate outcomes following spinal cord lesion. To test this hypothesis, we aim to live image Hes/Her protein dynamics following spinal lesion in zebrafish and perturb expression dynamics to assess the function of dynamics during regeneration. To this end, we have tagged the endogenous *her4.1* coding sequence, using CRISPR/Cas9-mediated homology-directed recombination, generating a Her4.1::mNeongreen knock-in fusion protein reporter. Her4.1::mNeonGreen is expressed in cells lining the central canal of the postembryonic spinal cord and preliminary live imaging experiments suggest dynamic and pulsatile Her4.1 protein expression in the uninjured and regenerating spinal cord.

Regulation of gene transcripts by microRNAs is known to modulate protein expression dynamics. We identified the microRNA miR-219, which is upregulated in animals undergoing spinal cord regeneration, as a putative regulator of Her4.1. We then specifically deleted the predicted miR-219 binding site within the *her4.1* 3'UTR to determine whether regulation by miRNAs modulates Her4.1 dynamics and to assess the functional role of gene expression dynamics in spinal cord repair.

304

Cell-type-specific adaptations of clathrin-mediated endocytosis in living zebrafish embryos

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Topic

2 Emerging Technologies

Abstract Content

A long-held ambition of biologists is to understand how subcellular processes scale to complex multicellular behaviours such as development. These behaviours rely heavily on communication between cells and their environment. Clathrin-mediated endocytosis (CME) is a major pathway that regulates how cells receive, process, and respond to extracellular signals, making it a key mechanism underlying this communication.

Despite its central role, CME has been studied primarily in cell culture, where artificial conditions fail to capture the complex biochemical and mechanical environment of living tissues. To understand how CME integrates these diverse cues to support cell-type-specific functions, it must be investigated in the intact organism, where these signals arise naturally.

Studying CME in living tissues is technically challenging, as observing subcellular processes require high spatiotemporal resolution and imaging depth. Here, we address this challenge by combining recent advances in genome engineering and light-sheet microscopy to directly visualize CME dynamics in zebrafish.

Our imaging of the pectoral fin at 3 days post-fertilization revealed striking tissue-specific adaptations of Ap2s1, a core endocytic protein. We resolved individual endocytic events and quantitatively distinguish between highly dynamic, motile processes in the periderm and long-lived, tethered Ap2s1 structures in the mesenchyme. By generating mosaically labeled embryos through cell transplantation, we obtained precise measurements of cell size and shape and correlated these features with CME dynamics. Furthermore, tissue wounding induced a reprogramming of these cell-type-specific adaptations, likely driven by changes in mechanical stress and ionic conditions.

Our findings place the molecular dynamics of CME in a physiologically relevant context within living zebrafish. Next, we aim to uncover the mechanisms linking membrane trafficking to cell and tissue mechanics during wound responses under varying osmotic conditions. This work establishes a technical framework for the systematic and quantitative study of subcellular processes in living animals, bridging cell biology, physiology, and developmental biology.

306

Stage-Dependent Notch Control of Epithelial Stem Cells in Zebrafish Tooth Development and Replacement

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Topic

14 Stem Cells and Regeneration

Abstract Content

Regeneration of functional teeth remains a major goal in regenerative dentistry, yet research on epithelial-derived ameloblast stem cells, which are essential for enamel formation and tooth regeneration, remains limited. Zebrafish continuously generate replacement teeth from epithelial and mesenchymal stem cell populations, offering a unique in vivo system to investigate the regulation of dental stem cells. Among key regulatory pathways, Notch signaling is widely known to maintain stem and progenitor cell populations during tissue development and regeneration. However, its role in dental epithelial stem cells during tooth regeneration remains poorly understood.

In this study, we investigated the role of Notch signaling during zebrafish tooth replacement. Using Notch reporter transgenic lines, TP1:EGFP, we identified Notch^{+} epithelial progenitor cells associated with the successional dental lamina. Pharmacological modulation of Notch signaling revealed that early inhibition depleted TP1^{+} epithelial progenitors and blocked replacement tooth formation, whereas early activation expanded progenitor populations and enhanced mineralization after washout. Late activation increased Notch^{+} cells with modest mineralization effects, while late inhibition disrupted tooth morphology. In addition, prolonged modulation of Notch signaling impaired mineralization across conditions. Together, these findings demonstrate that Notch signaling regulates epithelial progenitor cell dynamics during zebrafish tooth replacement in a timing-dependent manner, indicating that precise regulation of Notch activity is essential for epithelial stem cell maintenance and differentiation during tooth regeneration.

307

Genome-wide association study of ornamental common carp (Nishikigoi) elucidates the molecular basis of body coloration and morphological traits

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background

Nishikigoi, the ornamental strains of common carp (*Cyprinus carpio*) developed in Japan, have a history of more than 250 years. They exhibit remarkable phenotypic diversity, including a wide range of body colors and complex color patterns such as red, white, and yellow. In addition to variation in body coloration, several morphological variations are observed in scales and fins. However, the genetic and molecular mechanisms underlying these phenotypic characteristics remain largely unknown.

Methods

In this study, we conducted whole-genome resequencing of 50 individuals to generate a comprehensive catalog of genomic variation. We further performed genome-wide association studies (GWAS) using approximately 117 individuals representing about 40 strains to detect loci associated with ornamental traits.

Results

GWAS analyses were performed for 21 phenotypes, including the Hikari phenotype related to iridophore development and reflective scale formation. These analyses identified multiple significant candidate loci potentially involved in pigmentation patterns and skeletal morphology. Furtheremore, transcriptome analyses of 22 individuals provided insights into gene expression paternes and gene networks involved in body color determination and skeletal development.

Conclusion

Our results provide a genomic framework for understanding the genetic basis of phenotypic diversification in Nishikigoi. We also discuss how these findings compare with previous genomic studies of other ornamental teleost species, such as medaka and goldfish to highlight both shared and lineage-specific mechanisms of phenotypic evolution.

308

Developing zebrafish cell culture toolkits to bridge the dish to fish

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Topic

15 Infection and Immunity

Abstract Content

Although zebrafish are a great model for tissue and organism scale infectious disease research, the scale of such studies make it difficult to measure phenomena with molecular and subcellular precision. Alternatively, while many highly controlled and detailed studies of infection biology are possible in mammalian cell lines, these methods often don't capture the full complexity of infection and host-virus interactions. This is especially important in cross-species infections, where zebrafish are used to model the progression of viruses that infect humans, because it's unclear how the innate immune mechanisms present in mammalian cells will translate to a zebrafish background. Therefore, zebrafish cell culture studies are necessary to bridge mammalian cell culture and whole organismal zebrafish studies of viral infection. However, tools to genetically modify zebrafish cell lines lag far behind both those available in zebrafish and those available in mammalian cell lines.

We have optimized both DNA and RNA transfections in three different zebrafish cell lines. We also compared multiple methods for creating stable cell lines in zebrafish cells. Through these optimizations, we've been able to develop a zebrafish cell line expressing a split fluorescent reporter for both zebrafish and human viruses, allowing us to track viral replication by microscopy in these cells. We have also tested multiple human viruses for their ability to infect zebrafish cell lines and quantified their patterns of innate immune response. This will allow us to establish candidates that are most translatable between zebrafish and human cell culture system studies, opening the door to a more complete picture of virus-host dynamics in the zebrafish model.

309

Natural Otoprotectant: Corchorus capsularis Leaf Extract Mitigates Cisplatin-Induced Hair Cell Damage in Zebrafish

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Topic

1 Disease Models

Abstract Content

Background Cisplatin is a widely used platinum-based chemotherapeutic agent that often causes irreversible hair cell loss, leading to hearing impairment. To date, effective strategies for preventing cisplatin-induced ototoxicity remain limited. Corchorus capsularis L. (CCL) is rich in bioactive phytochemicals with antioxidant and anti-inflammatory properties; however, the protective role of CCL stem against cisplatin-induced hearing loss has not been explored.

Methods Herein, we use 7-dpf transgenic zebrafish larvae as a high-throughput screening platform to assessed CCL stem extract against cisplatin-induced hair cell injury. Endpoints included mechanotransduction (MET) function, ROS production, apoptotic and inflammatory responses, and locomotor behavior. Antioxidant capacity and acute toxicity were also evaluated.

Results Antioxidant capacity and acute toxicity were also evaluated. Pretreatment with CCL stem extract preserved hair cell viability, restored MET function, reduced ROS accumulation, upregulated Nrf-2-dependent cytoprotective genes, suppressed apoptosis, and attenuated macrophage infiltration. Recovery of swimming behavior correlated with hair cell protection, confirming the phenotypic relevance. This study demonstrates, for the first time, that CCL stem exerts potent otoprotective effects through antioxidative, anti-apoptotic, and anti-inflammatory mechanisms, contributes to maintain mechanosensory function and swimming behavior.

Conclusion The findings support CCL stem as a promising candidate for otoprotection and validate zebrafish-based high-throughput screening for novel therapeutic discovery.

310

Valorisation of aflatoxin-B1-contaminated corn in freshwater aquaculture via yellow mealworm larvae and assessing frass toxicity by zebrafish embryo microinjection

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Topic

10 Toxicology

Abstract Content

Mycotoxin contamination, particularly by aflatoxin B1 (AfB1), poses a significant challenge to food safety and the circular economy. This study investigates the potential of utilizing AfB1-contaminated corn as a substrate for yellow mealworm (*Tenebrio molitor*) larvae to produce safe and sustainable feed components for aquaculture.

Mealworm larvae were reared on four corn samples with varying AfB1 concentrations, ranging from below the European Union livestock feed threshold (20 µg/kg) to a twenty times higher (415 µg/kg). Following a five-week feeding period, larval mortality and mycotoxin accumulation in the insects were analyzed. The resulting insect meal was then incorporated into the diet of common carp (*Cyprinus carpio*) fries to assess its physiological impact. In addition, the toxicity of the residual insect by-product (frass) was evaluated using a microinjection model in zebrafish (*Danio rerio*) embryos.

Aflatoxin exposure did not significantly increase larval (carp) mortality, even at highest concentration. Mycotoxin accumulation in the dried insect meal was minimal, with a maximum of only 2.2 µg/kg detected only in the highest-exposure group. Feeding trials with common carp fries indicated that insect meal derived from contaminated corn had no significant adverse effects on fish health or physiology compared to control groups. The reduction of AfB1 in residual frass had a significant level (57.5%-82.8%), but it was still 157.6 µg/kg in the highly case. Microinjection of these frass extracts into zebrafish embryos resulted in increased mortality (in line with the concentration) and various phenotypic abnormalities.

The findings demonstrate that yellow mealworms can effectively bioconvert AfB1-contaminated corn into safe fish feed without detrimental effects on the insects or the consuming fish. However, the toxicity observed in the resulting frass highlights the necessity for responsible waste management of this by-product to mitigate environmental risks.

311

Disruption of YY1AP1-like region in Gon4lb causes arterial stenosis via misregulation in Gon4lb(YY1AP1)/Tp53/VEGF signaling axis

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Topic

1 Disease Models

Abstract Content

Background

Loss-of-function mutations in human YY1AP1, derived from an incomplete segmental duplication (SD) of GON4L, cause vascular stenosis in patients with Grange syndrome. Due to the lack of suitable animal models, the investigation of the underlying molecular mechanism is challenging. Here, we would like to explore the possibility of utilizing species, which carry additional homologous genes, to elucidate the molecular mechanism of how YY1AP1 regulates arterial function.

Methods

We used bioinformatic tools to systemically analyze the Gon4l/YY1AP1 homologs and identify species harboring additional gene duplications, selected zebrafish *gon4lb* as a functional surrogate for human YY1AP1, and subsequently generated and used mutants to mimic the presence and loss of YY1AP1 function.

Results

We identified teleosts with *gon4l* paralogs (*gon4la* and *gon4lb*), including zebrafish. Similar to human YY1AP1, C-terminal PAH/SANT domains of zebrafish Gon4lb are dispensable for vascular development. Disruption of the YY1AP1-like region in Gon4lb caused arterial stenosis/occlusion comparable to that found in patients with Grange syndrome. Furthermore, we revealed that Gon4lb transcriptionally regulates *tp53* through its YY1AP1-like region, impacting arterial specification via *vegfc*, a signaling axis essential for vascular integrity not reported previously. Knockdown of *tp53* partially rescued this phenotype. Additionally, qPCR analysis revealed distinctive roles for the YY1AP1-like region and the PAH/SANT domains in the transcriptional regulation of Gon4lb.

Conclusion

This study demonstrates that species harboring additional gene duplications, such as zebrafish, could be valuable models for elucidating the function of novel genes that emerged during human evolution. Our findings reveal that YY1AP1-region in Gon4lb can modulate the transcription of *tp53*, which subsequently impacts the development and function of the artery via *vegf* signaling

315

Emilin-1 deficiency impairs cardiovascular development and function in zebrafish

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Topic

1 Disease Models

Abstract Content

Background

Emilin-1 is an ECM glycoprotein of the EMILIN/Multimerin family, abundant in heart, blood vessels and lungs. It plays a key role in elastogenesis, cell adhesion, vascular morphology maintenance and modulation of blood pressure homeostasis. Despite its relevance, the in vivo functions of Emilin-1 in cardiovascular development remain insufficiently explored.

Methods

We employed *Danio rerio* as an in vivo model, chosen for its genetic tractability and similarity to human cardiovascular physiology. Because the zebrafish genome contains two Emilin-1 paralogs, we generated *emilin-1a* and *emilin-1b* single knockout lines using CRISPR-Cas9 and crossed them to obtain a double knockout. Gene disruption was confirmed by immunofluorescence and Western blot analyses. To assess cardiovascular development, we employed transgenic fluorescent reporter lines labelling endothelial and smooth muscle cells and evaluated cardiac function using pyHeart4Fish.

Results

Unexpectedly, double knockout fish exhibited significantly reduced reproductive success and decreased survival rates during early development. This phenotype, absent in our Emilin-1 knockout mouse model, suggests an essential role for Emilin-1 in embryonic viability. Vascular analyses revealed abnormalities in double knockout larvae, including increased diameters of the dorsal aorta and posterior cardinal vein, as well as a significantly enlarged aortic bulb. The latter resembles aneurysmal features, indicating a potential role for Emilin-1 in maintaining aortic integrity. Cardiac function analysis showed increased cardiac size and reduced circulatory efficiency, suggesting compromised hemodynamics.

Conclusion

Our study provides in vivo evidence for the essential role of Emilin-1 in cardiovascular development and function. The zebrafish model we generated offers a solid platform to dissect Emilin-1-dependent mechanisms. Building on these findings, future research will integrate reporter lines for TGF-β signalling, inflammatory cells to clarify pathway dysregulation, and will evaluate blood flow and potential extravasation to elucidate mechanisms underlying Emilin-1 deficiency in cardiovascular disease.

316

Structure-Dependent Toxicity of Acyl-Fumonisin B1 Derivatives in Zebrafish Embryos

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Topic

10 Toxicology

Abstract Content

Acylated derivatives of fumonisin B1 (FB1) represent a poorly characterized group of mycotoxin metabolites whose toxic effects may differ substantially from those of the parent compound. These derivatives can occur in naturally infected grains and may also arise during food processing of cereal-based products and therefore represent a potential food-safety concern.

To clarify the corresponding *in vivo* hazard, the structure- and time-dependent toxicity of six acyl-FB1 analogues was examined in zebrafish embryos: *N*-palmitoyl-, *N*-oleoyl-, *N*-linoleoyl-, 5-*O*-palmitoyl-, 5-*O*-oleoyl-, and 5-*O*-linoleoyl-FB1. To assess these effects, zebrafish embryos were exposed to each derivative and toxicity was evaluated over different exposure periods up to 120 h, enabling comparison of time-dependent responses as well as determination of LC_{50} values.

Acylation markedly increased the toxic potency of FB1, but the extent of this effect strongly depended on both the position and the structure of fatty acid substituents. Among the compounds tested, *N*-acyl-FB1 derivatives were consistently the most toxic, with 120 h LC_{50} values in the 4–18 µM range; whereas 5-*O*-acyl-FB1 derivatives displayed lower but still pronounced toxicity, with 120 h LC_{50} values between 22–38 µM. Considerable differences were also observed in the temporal pattern of toxic actions: besides the overall potency, the time-dependent development of toxic responses strongly varied among the derivatives. N-palmitoyl-FB1 proved to be the most toxic analogue, inducing substantial embryo mortality at low micromolar concentrations already after 24 h exposure. These findings demonstrate that relatively small structural differences among acyl-FB1 metabolites can lead to major changes in their embryotoxic potential in zebrafish.

Overall, this study highlights the suitability of zebrafish embryo model for the sensitive *in vivo* evaluation of structure-related toxicity of FB1 derivatives. In addition, we also emphasize here that certain modified forms of FB1 (especially *N*-acyl analogues) pose significantly higher toxicological risk vs. the parent mycotoxin.

317

Beyond Bone: Neuronal Insights into Hypophosphatasia from a Zebrafish Model

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Topic

1 Disease Models

Abstract Content

Background: Hypophosphatasia (HPP) is a rare inherited metabolic bone disorder caused by pathogenic variants in *ALPL*, encoding for tissue-nonspecific alkaline phosphatase (TNAP). TNAP deficiency disrupts phosphate homeostasis and impairs bone and tooth mineralization. Patients with severe HPP exhibit a markedly increased risk of neuropsychiatric symptoms, suggesting additional roles of TNAP in the central nervous system. This study investigated the systemic and neuronal consequences of Tnap deficiency using three independent *alpl* knockout zebrafish models.

Methods: Stable *alpl* knockout zebrafish lines were generated using CRISPR/Cas9 genome editing. Skeletal, neuronal, and other developmental abnormalities were assessed using histological and molecular approaches, including transmission electron microscopy and whole-transcriptome sequencing.

Results: Tnap deficiency significantly reduced larval survival and impaired early skeletal development through decreased bone mineralization. Ultrastructural analyses revealed early cellular abnormalities in the forebrain and midbrain. Transcriptome analysis showed downregulation of pathways involved in stimulus detection and response, along with altered expression of neuronal activity–associated genes, including deregulation of immediate-early genes and upregulation of apoptosis-related markers. Together, these findings suggest impaired neuronal function and increased cell death.

Conclusion: We established transgenic *alpl* knockout zebrafish lines that recapitulate main features of severe HPP and provide a valuable vertebrate model for studying Tnap deficiency. Our findings extend the role of Tnap beyond bone mineralization and demonstrate its critical involvement in early brain development and vertebrate metabolism. These processes remain difficult to investigate in mammalian models.

318

Simultaneous dual-fate mapping to resolve convergent differentiation in vivo

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Topic

19 Cell fate

Abstract Content

Throughout embryogenesis, multiple tissues are formed via the complex interplay of distinct embryonic lineages. And in some cases, multiple lineages converge to produce the same cell type. For example, the vertebrate skull is comprised of endochondral bone from neural crest (NC) and mesodermal origins. The majority of evidence for dual-lineage contributions to the same cell population is based on inferences from fate mapping one lineage at a time. Moreover, previous studies in our lab have revealed that the gene expression programs of cranial chondrocytes reflect their embryonic origin, suggesting lineage-specific mechanisms of fate specification.

To systematically characterize the dynamics of dual-lineage convergence *in vivo*, I am generating a simultaneous, dual-recombinase system in zebrafish. First, we are driving recombinase enzymes (Cre and flipase) under endogenous control of lineage-specific transcription factors using CRISPR-Cas9 knock-in approaches. In combination with the zebrafish pIGLET safe-harbor system, we will label NC and mesoderm lineages with unique fluorophores downstream of a recombination cassette.

We aim to resolve the cellular and molecular dynamics of cell fate convergence, both in our main tissue of interest, the embryonic skull, and embryo wide by combining live imaging and single cell transcriptomic readouts. We anticipate that in addition to resolving key questions about when and how fate convergence is achieved in the embryo, these tools will be broadly useful to the zebrafish community.

320

Concentration-dependent effects of silver nanoparticles on caudal fin regeneration in zebrafish larvae

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background

Silver nanoparticles (AgNPs) are increasingly used in biomedical, industrial and environmental applications, raising concerns about their potential impact on tissue repair and regeneration. Zebrafish larvae provide an excellent model for studying regenerative biology and immune responses, however, the effects of AgNPs exposure during caudal fin regeneration remain insufficiently characterised. This study investigated how chitosan-stabilised silver nanoparticles (CS-AgNPs; ~6 nm) influence caudal fin regeneration in zebrafish larvae.

Methods

Toxicity assays across a range of nanoparticle concentrations were first performed to select non-lethal doses for regeneration experiments. Based on these results, three concentrations (0.05, 0.1 and 1 µg/ml) were selected for regeneration experiments. Following caudal fin amputation, larvae were exposed to CS-AgNPs throughout the regenerative period. Regrowth was quantified morphometrically. To assess cellular responses, transgenic zebrafish lines with fluorescently labelled macrophages- Tg(mpeg1:mCherry), and neutrophils- Tg(mpx:eGFP) were used to observe migration of these cells to the site of amputation at 4, 6, 24 and 48 hours post-amputation. Gene expression changes were further analysed using RT-qPCR at 4, 6 and 48 hours post-amputation, focusing on genes involved in inflammation and tissue remodelling.

Results

Fin regrowth was more efficient in larvae exposed to lower concentrations of AgNPs than in those exposed to higher concentrations. RT-qPCR analysis revealed changes in the expression of inflammation-related genes in the early stages following injury. Furthermore, fluorescence microscopy indicated differences in the dynamics of neutrophil and macrophage recruitment to the wound site between the experimental groups

Conclusion

Overall, the results suggest that exposure to silver nanoparticles can impact immune cell recruitment, expression of genes involved in inflammation and tissue remodelling, and

regenerative processes following caudal fin injury in zebrafish larvae. These findings provide additional insight into the interactions between nanoparticle exposure and the biological mechanisms involved in wound healing.

321

A genetically encoded sensor for real-time imaging of mitochondrial membrane potential *in vivo*

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Topic

2 Emerging Technologies

Abstract Content

Mitochondrial membrane potential (MMP, ΔΨm) is a fundamental electrochemical gradient across the inner mitochondrial membrane, essential for ATP synthesis, calcium regulation, and reactive oxygen species (ROS) control. Loss of MMP is a hallmark of neurodegenerative diseases and cancer, leading to impaired energy metabolism, oxidative stress, and apoptosis. Existing therapeutic candidates, such as coenzyme Q10 and resveratrol, show limited clinical efficacy due to poor bioavailability and lack of mitochondrial specificity. Conventional MMP assays using fluorescent dyes, including JC-1 and TMRE, suffer from instability and variability, restricting their use in real-time in vivo studies. To overcome these limitations, we generated a transgenic zebrafish line expressing a mitochondria-localized, destabilized GFP reporter under a ubiquitous promoter. This model enables dynamic, organism-wide monitoring of MMP in real time. Validation experiments demonstrated that the reporter sensitively responded to MMP inhibitors(FCCP, rotenone) and activator(coenzyme Q10, resveratrol). The reporter line represents a powerful in vivo platform for evaluating drug efficacy and toxicity, ultimately facilitating the development of next-generation therapeutics for MMP-related diseases.

322

Discovery of candidate therapeutics for early infantile epileptic encephalopathy 3 (EIEE3) using a slc25a22a mutant zebrafish model

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Topic

1 Disease Models

Abstract Content

Early Infantile Epileptic Encephalopathy 3 (EIEE3) is a rare and severe neurodevelopmental disorder characterized by early-onset, refractory seizures and global developmental delay. Mutations in SLC25A22, which encodes a mitochondrial glutamate transporter, have been implicated as a genetic cause of EIEE3. To investigate the pathophysiology of SLC25A22-related epilepsy and identify potential therapeutics, we generated a zebrafish mutant of *slc25a22a*, the ortholog of human SLC25A22.

The *slc25a22a* mutant zebrafish exhibited spontaneous seizures, epileptiform discharges in local field potential recordings, and aberrant calcium propagation from the forebrain to the spinal cord. Among nine clinically used anti-seizure medications (ASMs) tested, only valproic acid (VPA) significantly reduced seizure phenotypes in mutant larvae, suggesting a specific response pattern relevant to EIEE3.

To explore novel therapeutic candidates, we conducted a high-throughput screening of 7,040 small molecules from the Korean Chemical Bank using this zebrafish model. Two hit compounds were identified that significantly suppressed seizure-like activity. Docking simulations revealed potential interactions of these compounds with neuronal sodium channels, indicating a mechanistically distinct anti-epileptic effect.

This study highlights the *slc25a22a* mutant zebrafish as a valuable in vivo model for EIEE3, enabling both mechanistic insight and therapeutic discovery. Further optimization and derivative synthesis of the hit compounds are underway to develop effective lead compounds for SLC25A22-related epileptic encephalopathy.

323

Phototoxicity of BODIPY Photocage in Zebrafish Larvae

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Topic

10 Toxicology

Abstract Content

Background: Photopharmacology enables precise, non-invasive control of drug activity using light. BODIPY-based photocages are particularly useful in this field due to their tunable photophysical properties, high quantum yields, and biocompatibility. Zebrafish embryos, due to their transparency, serve as an excellent in vivo model for assessing the phototoxicity and biological effects of light-responsive compounds during early development. Objective: To assess the phototoxicity of BODIPY photocages in zebrafish larvae. Methods: Zebrafish embryos were injected into the yolk up to 30 minutes post-fertilization with 1 nL of BODIPY photocage solutions (0, 1.25, 2.5, 5, 10 and 20 µM) prepared in 0.4% DMSO and 0.05% Tween-80. Immediately after injection, embryos were exposed/or not to 525 nm light for 15 minutes and subsequently monitored for survival over a 5-day period. Results: BODIPY-treated animals exhibited a concentration-dependent decrease in survival beginning at 5 µM compared with control. Irradiation further decreased survival in photocage-treated embryos, demonstrating phototoxicity. Control groups injected with vehicle alone maintained high survival rates regardless of irradiation, demonstrating that neither the solvent system nor the light exposure protocol caused detectable toxicity. Conclusion: The observed photoxicity of the BODIPY photocages confirms that zebrafish larvae are a highly sensitive model for detecting phototoxic effects, supporting their utility for early-stage evaluation of photopharmacological tools. Funding: CNPq (Grants 307825/2022-1 and 403468/2024-8).

324

Role of ext1 in zebrafish pectoral fish development.

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Topic

1 Disease Models

Abstract Content

Osteochondromas are the most common benign bone tumors and are characterized by cartilage and bone overgrowth near the growth plates. Mutations in the EXT1 gene are associated with hereditary multiple osteochondromas (HMO), also known as hereditary multiple exostoses type 1, in humans. Despite its clinical significance, the precise role of EXT1 in appendicular skeletal development remains incompletely understood. EXT1 encodes a glycosyltransferase that is essential for the polymerization of heparan sulfate, a critical component of the extracellular matrix that regulates the distribution and signaling of morphogens such as FGF, Wnt, and Hedgehog. Proper heparan sulfate synthesis is therefore crucial for the patterning and outgrowth of pectoral fins during development.

To investigate the role of EXT1 in appendicular skeletal development, we used the zebrafish model system, which shares conserved appendicular skeletal features with humans, including pectoral and pelvic girdles and their associated fins or limbs. Using CRISPR–Cas9 genome editing, we generated zebrafish lines carrying single and double homozygous mutations in ext1a and ext1b, the zebrafish orthologs of human EXT1. Interestingly, single homozygous mutants of either ext1a or ext1b exhibited normal pectoral fin development, whereas double homozygous mutants completely lacked pectoral fins. These findings demonstrate the redundant yet essential roles of ext1a and ext1b in zebrafish pectoral fin development and establish a valuable model for studying EXT1-related skeletal disorders.

Future studies will focus on identifying the signaling pathways and molecular mechanisms underlying EXT1-mediated regulation of pectoral fin development.

327

Generation of zebrafish models to visualise lymphatic vasculature by utilising enhancer sequences

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Lymphatic vessels, predominately, develop from pre-existing venous vasculature in zebrafish. Most of the available tools to visualise lymphatic vessels are expressed in both vascular beds, limiting the possibility to distinguish between veins and lymphatics in tissues and organs where the vessel morphology is not well documented.

Using a mulit-omic (HiC, ATAC-sequencing and scRNA-sequencing) approach we identified enhancer elements around genes whose expression is enriched in lymphatic endothelial cells. By overlaying HiC and ATAC-seq we have selected enhancers surrounding 4 genes, *mafba*, *nr2f2*, *tbx1* and *lgmn*. Using the enhancer sequence inserted into a pDestTol2ACryGRP backbone we generated mosaic transgenic lines and imaged embryos at F0. To further assess the enhancer activity, we injected the construct into *prox1RFP* zebrafish and assessed the GFP expression driven by the enhancers in F1 embryos.

We found that the enhancers showed a broad range of specificity, with some enhancers being enriched in the facial or trunk lymphatics while others showed expression in both the lymphatics as well as the arterial and venous vasculature. Together we have generated two transgenic lines that drive expression in lymphatic vasculature and not in the other endothelial cell compartments. We believe these tools serve as an important resource to the zebrafish community.

328

Mechanisms underlying SUPT16H deficiency-associated neurodevelopmental disorder and neurocristopathy

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Topic

1 Disease Models

Abstract Content

SUPT16H encodes a subunit of the FACT (Facilitates Chromatin Transcription) complex, a chromatin-specific histone chaperone that regulates DNA replication, transcription, and DNA repair. Variants in *SUPT16H* are associated with intellectual disability, autism spectrum disorder, hypotonia, and characteristic craniofacial dysmorphism. Previous studies in model organisms have established that *SUPT16H* is essential for neuronal and glial maintenance; however, the early developmental processes by which its dysfunction contributes to neurodevelopmental disorders remain largely unexplored. To investigate the in vivo consequences of *SUPT16H* dysfunction, we generated a *supt16h* knockout zebrafish using CRISPR/Cas9 genome editing. Morphological development, locomotor behavior, and early embryonic phenotypes associated with *supt16h* loss-of-function were systematically analyzed. In addition, selected patient-derived *SUPT16H* variants were evaluated for their ability to rescue developmental defects in mutant embryos. The *supt16h* loss-of-function model recapitulated key phenotypes observed in patients. *supt16h* deficiency induced p53-dependent apoptosis in the central nervous system and pharyngeal arches and disrupted neural crest development, which may underlie the microcephaly and craniofacial abnormalities observed in individuals carrying *SUPT16H* variants. Furthermore, loss of *supt16h* impaired oligodendrocyte specification and myelination, indicating defects in glial lineage development within the central nervous system. Functional analysis of *SUPT16H* variants demonstrated variant-specific rescue of developmental defects, revealing differential functional consequences of disease-associated variants in vivo. Together, these findings identify previously unrecognized roles of *SUPT16H* in neural crest and glial development and provide mechanistic insight into *SUPT16H*-associated neurodevelopmental disorders and neurocristopathy. This study also establishes a functional framework for interpreting the pathogenicity of disease-associated *SUPT16H* variants.

329

Investigating the role of the neuropeptide galanin in pancreatic β-cell regeneration

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Topic

1 Disease Models

Abstract Content

Maintenance of blood glucose levels relies on the release of hormones from pancreatic islet cells, including β- and α-cells. Pathogenesis of diabetes is associated with the impairment of β-cell output, caused by the depletion and/or the secretary dysfunction of β-cells.

Neuropeptides released from pancreatic nerve endings have an impact on islet hormone release. Galanin nerve fibres are found within the pancreas of several species, and preliminary studies from our group indicate that galanin varicosities are in close proximity to all of the islet cell types. Galanin is reported to lower insulin secretion by β-cells and our data reveal that zebrafish galanin mutants are hypoglycaemic, indicating the involvement of galanin in glucose regulation. As galanin can promote regeneration in other contexts, we aimed to investigate the role of galanin in β-cell regeneration.

The Tg(*ins*:NTR);Tg(*gcga*:GFP);Tg(*sst2*:RFP) fish were used to ablate β-cells, and visualise α- and δ-cells, respectively, in galanin mutants and wildtypes. β-cells were ablated by treating embryos at 75 hpf with nifurpirinol for 25 h, and assessed 20 h post recovery. Embryos were immunostained with insulin antibody and imaged using confocal microscopy.

Analysis of preliminary data on pancreatic islet morphology in galanin mutants suggests there are no significant changes in comparison to wildtypes. The number of the β-cells was comparable in the short regeneration window tested, suggesting the initial β-cell regeneration was not dependent on galanin signalling.

While early β-cell regeneration rates in galanin mutants are similar to controls, we plan to next extend the regeneration window to assess the long-term impact of galanin signalling on the functional recovery of the β-cells. These analyses on β-cell function will shed light on the importance of galanin in β-cell regulation, potentially leading to better understanding of diabetes pathogenesis.

331

Spatial transcriptomics identifies candidate molecular markers of the developing zebrafish swim bladder

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The zebrafish swim bladder is a relatively understudied organ that shares evolutionary and developmental similarities with the mammalian lung. Spatial transcriptomics enables visualization of gene expression within intact tissues, facilitating identification of novel gene markers. In this study, we used Stereo-seq Transcriptomics to resolve the spatial gene expression landscape and identify candidate molecular markers of developing zebrafish swim bladder.

Spatial transcriptomic analysis of 3-day-old larvae was performed using BGI Stereo-seq technology and DNBSEQ sequencing. Stereo-seq Analysis Workflow and StereoMap were used for quality control, preprocessing, cell and tissue segmentation, generating a feature expression matrix. This matrix was subsequently preprocessed, clustered and annotated using StereoPy. Genes from the annotated swim bladder cluster exhibiting a log fold change ≥2 and exceeding 200 transcripts were selected and manually checked in StereoMap, to confirm their unique spatial distribution in the swim bladder.

Using this workflow, we identified 191 candidate genes associated with the swim bladder, including 21 with highly restricted spatial expression. Previously reported swim bladder markers at this developmental stage (*anxa5b, acta2, elovl1a,* and *aqp1a.1*) were among them. Comparative analysis using the Bgee expression database further highlighted *apodb*, *fn1b*, *fbp2*, *cygb1*, and *si:ch211-256m1.8* as novel marker genes with the highest expression in the swim bladder relative to other anatomical structures. *crip1*, *ctrl*, *myo5b*, *pgm2*, and *uraha* showed the highest expression scores in digestive organs, while remaining enriched in the swim bladder. Several newly identified genes are linked to biological processes relevant to lung development and function, reinforcing the evolutionary relationship between the swim bladder and mammalian lung.

Together, these results demonstrate the potential of spatial transcriptomics to uncover organ-specific gene expression patterns during early vertebrate organogenesis, identify novel candidate markers of the developing zebrafish swim bladder and provide new insights into the molecular programs underlying swim bladder development.

337

DSPE Is essential for brain development: functional analysis using a CRISPR/Cas9 zebrafish model

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Topic

1 Disease Models

Abstract Content

Rare diseases are often difficult to diagnose, and patients frequently travel between hospitals to undergo numerous tests and treatments. Even after a diagnosis is made, the psychosocial and financial burden on patients and their families can be substantial. Therefore, understanding the pathophysiological roles of gene variants associated with rare diseases is essential for developing improved diagnostic and therapeutic strategies. DSPE is one of the enzymes that play a role in the dolichol synthesis pathway. However, the pathogenic mechanisms underlying DSPE variants remain poorly understood. Genetic defects in dolichol pathway have been associated with the accumulation of misfolded glycoproteins in the endoplasmic reticulum. In this study, we aimed to investigate the pathophysiological functions and molecular mechanisms associated with DSPE variants. We generated a DSPE knockout zebrafish model using CRISPR/Cas9-mediated genome editing and characterized the resulting phenotypes. Homozygous DSPE mutant embryos exhibited developmental delay and abnormal brain development, recapitulating key clinical features observed in affected patients. These findings suggest that DSPE plays a critical role in normal brain development and embryonic survival. To further explore the molecular mechanisms underlying DSPE deficiency, we performed transcriptomic analysis in mutant zebrafish embryos. Ongoing studies will provide deeper insights into the biological functions of DSPE and the molecular mechanisms underlying DSPE-associated rare disease.

338

Fipronil Toxicity in Zebrafish Larvae: Evidence from Oxidative Stress Biomarkers

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Topic

10 Toxicology

Abstract Content

Fipronil (FP) is a broad-spectrum insecticide widely used as a veterinary ectoparasiticide and for urban pest control due to its effectiveness against invertebrates. However, it poses potential toxic risks to non-target organisms. This study aimed to assess toxicity by evaluating oxidative stress through the expression of the genes SOD1, SOD2, CAT, GPX, and GSR in zebrafish larvae. Following the guidelines of OECD 236 and approved by the CEUA (No. 5216030425), zebrafish embryos (TU strain; ~3 hpf) were exposed for 120 h to different environmentally relevant concentrations of fipronil (50, 100, and 200 µg/L). For gene expression analysis, total RNA was extracted from zebrafish larvae at 120 hpf, reverse-transcribed into complementary DNA (cDNA), and analyzed by quantitative real-time PCR (qPCR) targeting oxidative stress–related genes (SOD1, SOD2, CAT, GPX, and GSR). No significant increase in the expression levels of SOD1, SOD2, and GSR was observed at the evaluated concentrations (One-way ANOVA, $p > 0.05$). Significant alterations were observed only at the lowest evaluated concentration (50 µg/L) in the CAT and GPX genes (One-way ANOVA, $p < 0.05$), characterized by increased gene expression. These findings suggest that exposure to the insecticide may interfere with molecular pathways involved in oxidative stress, indicating an imbalance in cellular antioxidant defense systems. Such responses may reflect an adaptive mechanism to chemical stress, highlighting the compound's potential to induce sublethal effects in zebrafish larvae.

339

Decoding the Axonal Transcriptome Across ALS-Causative Mutations to Reveal Early Mechanisms of Motor Neuron Pathology

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Topic

3 Neurobiology

Abstract Content

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of both upper and lower motor neurons. This degeneration leads to a progressive decline in voluntary muscle control, ultimately resulting in severe paralysis and a short life expectancy of approximately 2 to 5 years after diagnosis. Both familial and sporadic forms of ALS are associated with degeneration of the neuromuscular junction (NMJ), followed by the peripheral axon, often described as a "dying-back" pathology of the motor neuron (MN). To understand this pathological event, we have investigated how ALS causative gene mutations in *FUS* and *TARDBP* perturb the transcriptome of MN somas as well as the repertoire of RNAs localised in motor axons. Transcriptomic analysis of MNs from patient-derived human induced pluripotent stem cells (hiPSC) revealed both shared and unique gene dysregulation (Schweingruber et al., *Nat Commun*, 2025). Furthermore, our team has developed an RNA-sequencing method, called Axon-Seq (Nijssen et al., *Stem Cell Reports*, 2018) where MNs are grown in microfluidic devices allowing for separate harvesting and sequencing of axons. Using Axon-seq, we found that a unique repertoire of mRNAs were dysregulated in axons compared to somas, and that each mutation resulted in distinct response.

Our approach revealed multiple axon targets, which were further evaluated *in vivo* in *Danio rerio*. Here, microinjections were conducted at the one-cell stage with target genes conjugated to a fluorophore, allowing us to assess the impact on axonal connectivity, as well as stability and maintenance of the NMJ in a live imaging-based assay.

Collectively, our results reveal promising molecular candidates that may serve as potential therapeutic targets in ALS.

341

Promoting Best Practices and Collaboration in Zebrafish Husbandry: Insights from the Polish Zebrafish Society

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Topic

21 Husbandry

Abstract Content

Over the past decade, the zebrafish has become an increasingly popular model organism in Poland. The Polish Zebrafish Society (PTZ) was founded in 2016, bringing together researchers working with this model organism in Poland. Over time, the increasing number of zebrafish husbandry facilities highlighted the need to create a communication platform specifically for personnel responsible for animal care and husbandry. In response, the Zebrafish Husbandry and Welfare Section was established within the PTZ in 2023.

The Section aims to connect and support professionals involved in zebrafish breeding, enable the exchange of experience and addressing everyday husbandry challenges. Over more than two years of activity, it has developed into a well-integrated community with regular monthly online meetings, providing a forum to discuss ongoing challenges, share technical solutions, and exchange best practices. Communication between meetings is maintained through an active mailing list. Two in-person meetings have also been organized, including lectures given by international speakers. The Section promotes inter-institutional collaboration through on-site visits and training sessions.

Regular interactions allow the community to respond efficiently to regulatory changes and promote harmonized approaches to zebrafish husbandry. This is particularly important in the context of increasing emphasis on the standardization of breeding practices to ensure comparability of scientific results. The Zebrafish Core Facility at the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw plays a key role in this process as the largest zebrafish breeding facility in Poland and a potential reference center for the national community, undertaking these efforts in cooperation with other zebrafish facilities.

Together, these initiatives contribute to strengthening collaboration, improving husbandry standards, and supporting the continued development of the zebrafish research community in Poland.

342

Activation of mfap5 in cardiac fibroblasts is essential for heart regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

Zebrafish can regenerate their hearts in a process that involves an early inflammatory phase [NM1] [Id2] followed by the activation of fibroblasts and subsequent revascularization and replacement of the lost myocardium by newly formed cardiomyocytes. A transient “scarring-like” response, including shedding of extracellular matrix components is thus not inhibiting regeneration, but the current view is that it might actually contribute to generate a scaffold necessary for its correct progression. Here we explored the role of *myofibrillar associated protein 5* (*mfap5*) during zebrafish heart regeneration, a gene shown to be involved in skin wound repair in mammals, and upregulated in human failing hearts. We found that *mfap5* is expressed in the epicardium and a subset of fibroblasts in the regenerating heart. To study *mfap5* in zebrafish heart regeneration we generated a loss of function model using CRISPR/Cas9. In the absence of *mfap5*, cardiomyocyte proliferation was increased at 7 dpi, concomitant with alterations in pathways associated to muscle cell differentiation and cell metabolism. This did however, not lead to precocious regeneration and at 30 dpi, we observed an impaired scar resolution that correlated with an upregulation of extracellular matrix associated pathways. scRNAseq analysis indicated that *mfap5* loss of function led to a compensatory shift in fibroblast populations and this might be the cause of altered ECM resolution.

Together our data highlights the importance of *mfap5* throughout the different steps of heart regeneration.

344

Zebrafish Embryos as an Alternative for Assessing Wastewater Effluents via Mortality and Behavioral Analysis

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Topic

10 Toxicology

Abstract Content

BCKGROUND: Environmental safety has gained attention due to increasing pollutants threatening ecosystems and human health. Currently, cell culture-based *in vitro* toxicity tests are widely used for their speed and convenience, but they often fail to replicate *in vivo* effects, which are critical for assessing environmental relevance. Traditional fish acute toxicity tests (FAT) are costly, complex, and require sacrificing fish. Zebrafish embryos present a practical alternative, being transparent, rapidly developing, and readily available.

METHODS: In 2017, the Taiwanese EPA (Environmental Protection Administration) introduced the zebrafish embryonic toxicity test (ZFET, NIEA B909.10C) as an alternative to FAT (NIEA B902.13B, B904.13B) using native species *Pseudorasbora parva* and *Cyprinus carpio*. In this study we used these two methods parallelly.

RESULTS: We tested 39 effluents from Taiwanese Science Parks using both ZFET and FAT and observed high correlation between the methods. Mass spectrometry detected 177, 82, and 76 and 174 compounds in effluents that induced embryonic abnormalities despite viability. Of these, 24 common chemicals with distinct properties were identified. Using a zebrafish toxicity prediction model (TOX_SCO, MOV21 and AUC21) further discovered 10 out of 24 substances predicted by at least one predictive model to have the potential to induce deformities in zebrafish embryos. Zebrafish behavioral tests indicated that certain effluent-derived chemicals altered zebrafish movement patterns.

CONCLUSION: Our findings indicate that ZFET is not only a viable alternative to FAT but also offers deeper insight into wastewater impacts on aquatic ecosystems

348

Investigating the role of AP-1 transcription factors during zebrafish ligament regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background

Mammalian ligaments have a limited capacity to regenerate and heal with a fibrotic scar. Adult zebrafish regenerate their craniofacial interopercular mandibular (IOM) ligament following transection without scar. After injury, ligamentocytes downregulate expression of the mature ligament cell fate marker, *scxa:mCherry,* and undergo dedifferentiation. Dedifferentiated ligamentocytes re-enter the cell cycle to proliferate and give rise to regenerative mesenchymal cells; however, it is unclear what transcription factors regulate these early steps during zebrafish ligament regeneration.

Methods

To explore what transcriptional mechanisms promote ligamentocyte dedifferentiation, we performed unilateral ligament transection surgery in adults and used snATACseq to profile chromatin accessibility. To investigate AP-1 activity, we examined Jun phosphorylation by Western blot. We tested the requirement of Jun phosphorylation for dedifferentiation by treating *scxa:mCherry* fish with a JNK inhibitor (JNKi) to prevent Jun phosphorylation and live imaged fish. To assess cell cycle re-entry, we analyzed proliferation by PCNA staining.

Results

From snATACseq, we identified accessible regions in regenerative mesenchymal cells after injury and found significant enrichment of AP-1 (Fos:Jun) motifs. We find that Jun phosphorylation increases after injury indicating that Jun is transcriptionally active (n= 6-10 per timepoint, $p<0.0001$). In JNKi-treated fish, we observed a failure to downregulate *scxa:mCherry* expression in ligament stubs after injury (n=5/8) suggesting that Jun phosphorylation is required for ligamentocyte dedifferentiation. JNKi-treated fish have a 20% decrease in proliferation after injury suggesting that Jun phosphorylation is required for cell cycle re-entry (Ligament stub: $p<0.0001$, regenerative mesenchyme: $p<0.0004$). Finally, we identified putative AP-1 target genes upregulated after injury including both known pro-regenerative genes and novel targets.

Conclusion

Our study demonstrates an important role of AP-1 transcription factors during the early stages of ligament regeneration. This study will impact our understanding of

transcriptional networks governing ligament regeneration and offer insights to improve scarred connective tissue healing.

349

Studying the effects of type V collagen in zebrafish: classical Ehlers-Danlos syndrome as a model

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Topic

1 Disease Models

Abstract Content

Background

Classical Ehlers-Danlos syndrome (cEDS) is a rare hereditary connective tissue disorder caused by pathogenic variants in *COL5A1* or *COL5A2*, which encode type V collagen, a quantitatively minor but structurally essential fibrillar collagen that regulates type I collagen fibrillogenesis. Haploinsufficiency of type V collagen leads to hallmark cEDS features, including skin hyperextensibility, atrophic scarring, joint hypermobility, and chronic pain. We previously demonstrated pain and altered dermal nociceptive innervation in both cEDS patients and *Col5a1*$^{+/-}$ mice. To expand available model systems and enable high-throughput drug screening, we aimed to generate and characterize zebrafish models of *col5a1*haploinsufficiency.

Methods

CRISPR/Cas9 genome editing was used to introduce out-of-frame variants in zebrafish *col5a1* to establish two stable lines: a 5 bp deletion in exon 8 and a 1-bp duplication in exon 10. *col5a1* transcript levels were quantified using RT-qPCR. Behavioral assays were conducted at larval (5 dpf) and adult stages, and survival was monitored up to 28 dpf. Additional phenotyping included whole-mount phalloidin staining of muscle (5 dpf) and cardiac functional analysis (3 dpf).

Results

Both *col5a1* mutant lines showed significantly reduced *col5a1* expression, consistent with haploinsufficiency. Heterozygous larvae (*col5a1*$^{+/-}$) exhibited normal movement at 5 dpf, whereas homozygous mutants (*col5a1*$^{-/-}$) displayed significantly reduced locomotion resembling pain-like behavior. Survival analysis revealed Mendelian ratios at 1 dpf but progressive loss of homozygous animals, with complete absence around 20 dpf. Bulk RNA sequencing at 5 dpf is ongoing to pinpoint molecular changes associated with the different col5a1 genotypes. Muscle morphology and early cardiac function appeared unaffected. Adult *col5a1*$^{+/-}$ zebrafish showed no behavioral alterations.

Conclusion

We established two *col5a1* zebrafish models that provide a promising platform for future studies on the role of type V collagen, connective tissue pathology, nociception, and therapeutic compound screening.

354

Neuroinflammation and the first cues for injury-induced Muller Glia reprogramming in Zebrafish retina

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Topic

3 Neurobiology

Abstract Content

Background: Unlike mammals, Zebrafish retina possesses the remarkable ability to regenerate the lost neurons following injury. This is largely endowed by the resident Müller Glia (MG) which actively re-enter the cell cycle to produce multi-potent progenitors that ultimately repopulate the retinal architecture and restore vision in Zebrafish. While several signaling cascades are known to be inherently pivotal, it is largely believed that the humoral signals, secreted by the dying cells and the inflammatory immune cells at the site of injury, may be sufficient to drive the downstream neurogenic reprogramming of the Müller Glia. Elucidating these “sufficient” signals in Zebrafish would go a long way to toggle the switch against regenerative barriers in the mammalian retina.

Methodology: We employed scRNA-seq to obtain the selective transcriptomic landscape of pro-inflammatory immune cells and the responding Müller Glia during the inflammatory phase of retinal regeneration, followed by manual curation of potential ligand-receptor interactome to capture the intricate crosstalk between immune cells and Müller Glia. We further validated these selected interactions using publicly available RNA-seq data and functional in-vivo assays.

Results: Our dataset included a total of 111,391 cells, including distinct clusters corresponding to neutrophils, microglia and macrophages. Selective enrichment of inflammatory cytokines and receptors revealed factors like *tnfa*, *il1b*, *il10* and *il6*, which are already proven in the literature to be indispensable for retinal regeneration, corroborating the strength of our dataset. Additionally, we uncovered several candidate factors which may potentially be required for MG activation and subsequent pro-neurogenic reprogramming.

Conclusion: This study is among the first to focus exclusively on inflammatory immune cell subtypes at a single-cell level during retinal regeneration. By uncovering a variety of previously unexplored signaling molecules, we aim to provide new insights into the potential mechanisms driving injury-induced retinal regeneration in Zebrafish.

355

Nervous System Mediated Cardiac Arrest in the Mangrove Killifish

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Topic

6 Evolution and Alternative Fish Models

Abstract Content

Background

The mangrove killifish, *Kryptolebias marmoratus*, is one of two known self-fertilising, simultaneous hermaphroditic vertebrates and inhabits harsh, fluctuating environments. Embryos can enter diapause, a state of arrested development, that delays hatching until conditions improve. We have discovered a remarkable behaviour in which embryos transiently stop their heartbeat in response to physical stimulation, termed heart stopping in response to physical disturbance (HSPD). Understanding the mechanisms underlying HSPD may provide insight into neural regulation of cardiac activity during development.

Methods

Mechanical stimulation assays were performed across developmental stages to quantify cardiac responses to varying stimulus strengths and repeated stimulation. Pharmacological treatments, myocardial calcium imaging and immunohistochemistry were used to characterise the involvement of autonomic pathways and pacemaker activity in HSPD, and cardiac innervation throughout development.

Results

Embryos were highly sensitive to physical stimulation, which frequently induced prolonged heart stops (>60s). The behaviour first appeared late in embryogenesis, and heart stop durations increased throughout development, persisting in diapause but become highly variable. Heart stop duration was dependent on both stimulus strength and number, with stronger or repeated stimuli causing longer stops. Repeated stimulation also resulted in habituation (heart stops becoming progressively shorter). After hatching, prolonged heart-stops became absent, stimulation instead causing a brief increase in inter-beat interval before returning to baseline. Pharmacological experiments showed that general anaesthesia inhibited HSPD, indicating a neural mechanism. Inhibition of either sympathetic or parasympathetic signalling significantly reduced HSPD, suggesting a requirement of coordinated activity of both branches. Consistent with this, cardiac innervation increased as HSPD developed, particularly around the pacemaker region.

Conclusion

HSPD is a neurally mediated, developmentally regulated cardiac response. Stimulus dependence and habituation suggest an adaptive function, potentially related to predator avoidance. These findings support *K. marmoratus* as a valuable model for investigating interactions between the nervous system and cardiac function during development.

359

Mechanical profiling and regulators of egg activation in zebrafish

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Topic

9 Germline and Early Development

Abstract Content

Fertilization, the fusion of egg and sperm cell, marks the beginning of new life. Despite its fundamental importance, the role of the egg's actin cytoskeleton and mechanical forces during fertilization remain incompletely understood. During egg activation, a dramatic remodelling of the cell surface and intracellular reorganisation of the egg take place.

To study the mechanobiological dynamics in the activating egg we use *Danio rerio* as an externally fertilizing species. Zebrafish eggs are an ideal model system to study egg-intrinsic mechanisms versus sperm-driven processes during fertilization since egg activation can be decoupled from sperm fusion.

Profiling of mechanical changes during egg activation support an increase in egg stiffness. High-resolution live imaging together with pharmacological perturbation assays allowed us to directly visualize the actomyosin cortex and cortical granules during the early stages of activation. We found that the actin cytoskeleton regulates cortical granule exocytosis and is important to maintain the egg's morphology and surface integrity. Mechanical measurements further support that the actin cortex regulates egg stiffness and chorion expansion, which establishes a physical barrier to protect the fertilized egg.

These findings highlight an important role of mechanical forces in egg activation and fertilization.

360

Dynamic Gene Expression and microRNA Interplay Control Neural Progenitor Fate During Spinal Cord Regeneration

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Topic

14 Stem Cells and Regeneration

Abstract Content

The spinal cord (SC) has minimal regenerative capacity in humans, and no treatments exist to restore functionality after SC injury (SCI), resulting in reduced quality of life and life expectancy. In non-regenerative species, damaged axons fail to regrow, and neural progenitor cell (NPC) proliferation primarily forms glial scars. Conversely, regenerative species exhibit robust axonal regeneration with rapid NPC proliferation that promotes neurogenesis and functional repair.

NPC maintenance, proliferation, and cell-fate decisions are regulated by signalling pathways (ie. Notch, BMP, Wnt) and post-transcriptional control via microRNAs. During developmental neurogenesis, expression of Notch downstream effectors, Her/Hes, oscillates dynamically rather than simply switching on or off. These microRNA-modulated oscillations critically determine whether NPCs exit quiescence, proliferate, or differentiate.

Following SCI, NPCs in both regenerative and non-regenerative species reactivate developmental signalling pathways but produce divergent outcomes: neurons or glial cells, respectively. The mechanisms underlying these differences remain unclear, particularly whether injury reactivates developmental genes oscillatory and whether such dynamics influence NPC fate during SC regeneration (SCR).

Using zebrafish, we investigate how dynamic gene expression and microRNA-mediated regulation fine-tune NPC fate decisions during SCR. Endogenously tagged fluorescent reporters (her6::Venus and her4.1::mNeonGreen) enable in vivo analysis of Notch signalling during regeneration. Preliminary live-imaging data reveal oscillatory Her/Hes expression following SCI, suggesting pulsatile Notch activity may drive regenerative processes.

To identify regulatory microRNAs, we performed high-throughput microRNA sequencing across multiple SCR stages, revealing candidates with predicted binding sites in Her/Hes transcripts. Using CRISPR-Cas9 genome editing, we selectively disrupted these microRNA–mRNA interactions to assess their impact on Her/Hes expression dynamics and regenerative outcomes.

Our findings provide new insights into how microRNA mediated regulation of pulsatile gene expression influences neural progenitor behaviour during SCR.

363

Epithelial fusion and perforation in zebrafish semicircular canal formation

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Epithelial fusion and perforation events are critical for the generation of shape and form during organogenesis in the developing embryo. Examples include formation of the neural tube, heart, soft palate, oesophagus/trachea and eye. Failure of epithelial fusion can result in congenital anomalies such as spina bifida, cleft palate and ocular coloboma.

We are interested in the epithelial remodelling events that occur during early stages of semicircular canal morphogenesis in the zebrafish inner ear. These events involve the meeting, fusing and perforation of pairs of epithelial projections to form continuous pillars of tissue, which become the hubs of the developing semicircular canal ducts. We have used live imaging of different transgenic lines to measure changes in cell shape, motility and cytoskeletal organisation where epithelia meet. We are identifying the genetic requirements for fusion plate formation through analysis of zebrafish mutants with defects in different aspects of semicircular canal formation.

Cells at the projection tips increase in motility, break filopodial tethers (cytocinches), and exchange neighbours prior to fusion, aligning to form the fusion plate, a flat transient epithelial bilayer. Perforation (epithelial fracturing) of this structure is achieved through rearrangement of cell junctions, with occasional cell death. Cell mixing across the fusion plate is rare: some cells crossing the boundary are eliminated through apoptosis. Dynamic cytoskeletal changes include the assembly of a supracellular actin cable that persists for several weeks following perforation.

Epithelial fusion in the ear is critically dependent on the adhesion GPCR Adgrg6. Signalling through this receptor is required and sufficient both for the down-regulation of extracellular matrix genes associated with projection outgrowth, and for the up-regulation of *nr4a3* expression, tightly localised to the fusion plate. Our data illuminate both generic and inner-ear-specific requirements for epithelial fusion and fracture during morphogenesis of a sensory organ.

366

De novo induction of chiral morphogenesis in a vertebrate embryo

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The establishment of animal Left-Right (LR) asymmetry has long been thought to rely on species-specific mechanisms that range from cilia-driven fluid flows to chiral cell rearrangements. Only recently has work form a number of groups including ours identified the non-muscle Myosin Myo1D, which was initially identified as a Master regulator of Drosophila Left-Right asymmetry, as an evolutionarily conserved regulator of animal LR asymmetry. Zebrafish Myo1D controls the orientation of motile cilia and the establishment of a symmetry-breaking fluid flow in Kupffer's Vesicle, which acts as a central LR Organizer. In addition to Myo1D, the zebrafish genome encodes the closely related protein Myo1G that controls embryonic LR asymmetry through a different mechanism, by regulating the Nodal-mediated transfer of laterality information.

Although our observations established Myosin1 proteins as central regulators of zebrafish LR asymmetry, the fact that neither cilia nor Nodal ligands are involved in Drosophila LR asymmetry suggests that a conserved cellular mechanism controlling chiral morphogenesis in different animal species still remains to be identified.

Independently of the species and organ under consideration, all instances of lateralized morphogenesis ultimately require chiral cell and tissue deformations. Of particular interest, studies in *Drosophila* revealed that - in addition to being required for endogenous LR asymmetry - Myosin1 proteins possess the unique capacity to induce chiral morphogenesis *de novo.*The evolutionary conservation of this ability to actively induce lateralized morphogenesis has however not yet been investigated. Here we will present the results of our current work that aims at performing a first functional analysis of the asymmetry-inducing activity of Myosin1 proteins in a vertebrate embryo.

369

A dual-phase enhancer couples progenitor maintenance and pancreatic lineage stability

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background
Enhancers orchestrate transcriptional programs that control organ development and maintain differentiated cell states, yet how individual enhancers integrate developmental processes with long-term tissue maintenance remains poorly understood. Here, we investigate the function of a distal enhancer downstream of *ptf1a* (z3'-DpE) and its role in coordinating pancreatic development and acinar cell homeostasis in zebrafish.

Methods
We generated zebrafish mutants lacking the z3'-DpE enhancer and examined its effects on pancreatic development and tissue maintenance. Transcriptomic analyses were performed to assess gene expression changes in pancreatic progenitor cells, while chromatin accessibility profiling of purified acinar cells was used to evaluate alterations in regulatory landscapes. Histological and morphological analyses were conducted to assess pancreatic architecture.

Results

Deletion of z3'-DpE reduces *ptf1a* expression in pancreatic multipotent progenitor cells, resulting in depletion of the progenitor pool, altered morphogenesis, and premature exocrine differentiation. Transcriptomic profiling reveals broad repression of genes involved in proliferation and morphogenesis. Following differentiation, loss of z3'-DpE leads to acinar cell loss, expansion of ductal and endocrine compartments, and disruption of pancreatic architecture. Chromatin accessibility profiling further reveals extensive remodeling of the acinar regulatory landscapes, with reduced accessibility at loci associated with acinar identity and developmental programs, and increased accessibility at regions linked to inflammation, epithelial plasticity, and pancreatic cancer susceptibility. Histopathological examination confirms severe acinar disorganization, with increased duct-like structures and mucinous lesions reminiscent of early pancreatic neoplasia.

Conclusion

Our findings demonstrate that the z3'-DpE enhancer sustains *ptf1a* expression to safeguard acinar cell identity and maintain a chromatin landscape that restricts pathological plasticity. This work highlights how a single enhancer can coordinate progenitor expansion with long-term lineage stabilization, providing insight into how developmental regulatory elements preserve tissue integrity and suppress disease-associated states.

371

Comparative effect assessment of 200 chemicals using high content screening in zebrafish embryos

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Topic

10 Toxicology

Abstract Content

The zebrafish embryo (*Danio rerio*) has been extensively used as an alternative model for hazard characterization and to explore the mechanistic basis of chemical toxicity. Traditionally, these assessments relied on scoring microscopic observations, which were prone to observer bias and had limited capacity for deriving concentration-response information of specific endpoints. Consequently it was not possible to use the effect data for a comparative assessment of effect patterns. Within the EU project *PrecisionTox*, we utilized the zebrafish embryos alongside other alternative organismal models to characterize 200 chemicals spanning diverse modes of action and use categories. Our approach involved an automated, unbiased morphological phenotype assessments, including also cardiovascular and behavioral effects. Morphological phenotypes were evaluated using automated positioning and imaging of embryos. Subtraction of video frames was used to analyze heart rate and cardiovascular effects. Behavioral endpoints such as spontaneous tail contractions, photomotor responses, and locomotor responses were systematically assessed using video analysis. From over 30 endpoints, concentration-response curves and corresponding EC50 and/or point of departure values were derived. To estimate the specificity of the observed effects, we compared the them with the predicted baseline toxicity and observed mortality of zebrafish embryos. For pharmaceuticals and pesticides with known specific modes of action, high sensitivity ratios (predicted baseline toxicity LC50 / observed EC50) confirmed that our approach could detect specific effects. We used various approaches of hierarchical cluster and correlation analysis to group chemicals according to their effect patterns. The approach demonstrated that a quantitative assessment of phenotypes considerably improves the diagnostic capacity and sensitivity based on a non-invasive analysis of chemicals effects.

372

LDexplore: A Shiny application for multivariate analysis of zebrafish larval locomotion data

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Topic

2 Emerging Technologies

Abstract Content

Background: Larval locomotion assays provide quantitative time- and condition-dependent data for high throughput analysis of effects of treatments in zebrafish. There are multiple paradigms and associated analytical tools already developed yet there is a need for integration of regression as well as multivariate statistics to analyze whole plate locomotion data.

Methods: Herein, we developed an online application called LDexplore using R Shiny for the dark:light alternating cycle experimental paradigm in the Zantiks MPW platform. In LDexplore, user needs to upload an output file that contains the time points, conditions and motility data obtained in each well in pixels, of which are parsed automatically. Visualization options include heatmaps that allow for tracking of each larva/well across treatments and clustering based on the selected variables/columns. Moreover, LDexplore performs and reports regression analyses for the transitions of user-defined dark:light transitions as well as across a selected dark and/or light period.

Results: We used LDexplore in two different contexts to demonstrate its use in testing effects of a) high fat diet and b) injected xenografts on locomotory behavior of zebrafish larvae. Regression analyses incorporated in LDexplore enabled us to assess the differences in the slopes and intercepts of treatment effects in an automated manner during different dark:light:dark transitions. Heatmaps and distances travelled were plotted across the time points for the selected groups and annotated. We found that high fat diet as well as xenografting had significant effects on larval motility and dark:light transition statistics.

Conclusion: LDexplore with a user-friendly interface helps users to analyze larval locomotion data without the need to write any code and can be applied in contexts other than dark:light paradigm in the future.

374

Identifying Regulators of Hepatocyte Polarity During Zebrafish Liver Development

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Topic

5 Tissue Formation

Abstract Content

The liver is an essential organ with a unique function, such as detoxification and bile production. These essential functions are mediated by its characteristic tissue architecture. The establishment of hepatocyte polarity is an important factor in livers tissue architecture and is crucial for organ function, which is frequently compromised in chronic liver disease.

Hepatocyte polarity is characterized by apical formation of bile canaliculi connecting to adjacent bile ducts and basal orientation towards sinusoidal blood vessels. A correct organization of liver architecture is crucial for bile secretion and metabolite exchange. However, the molecular mechanisms regulating hepatocyte polarity and the establishment of liver architecture are poorly understood.

Endothelial cells, which line blood vessels are thought to play crucial roles in regulating hepatocyte polarity. Zebrafish mutants lacking blood vessels, exhibit severe hepatocyte polarity defects including missing or reduced canaliculi and defective connectivity to the biliary system. Using these mutants, we conducted a proteomic screen by label-free mass spectrometry, identifying candidate genes with potential roles in polarity regulation, including cytoskeletal regulators, cell adhesion molecules, and instructive signals.

We are following two candidates: *mcamb* (*melanoma cell adhesion molecule b*) a known cell adhesion gene located in vasculature and a novel, liver-specific zona-pellucida domain-containing gene. To investigate how these candidates contribute to hepatic polarity, we first analyse their spatiotemporal expression in zebrafish livers by whole mount in situ hybridization. For this we combine cell-type specific transgenes with HCRs to achieve high sensitivity and cellular resolution. To assess gene function, we perform CRISPR/Cas9-mediated F0 genome editing, targeting promoter region to disrupt gene transcription. In parallel, we follow complementary gain-of-function strategy overexpressing candidates throughout the vasculature with an endothelial-specific Gal4/UAS approach. Together, these experiments aim to determine the role of candidates in hepatocyte-endothelial crosstalk and the establishment of hepatocyte polarity, providing insights into fundamental mechanisms of liver development.

375

GABRA1 is required for visual experience dependent plasticity in the zebrafish

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Topic

3 Neurobiology

Abstract Content

Critical periods (CP) are developmental windows where the environment has profound impact on structural and functional changes to neural circuits, a form of experience dependent plasticity. The visual CP is a well-established window where visual experience is required for normal visual function. Impaired visual experience during the CP can lead to amblyopia and potentially lifelong vision impairment. Cortical mechanisms instructing visual CP plasticity are well established. Conversely, subcortical mechanisms have more recently been described and remain incompletely understood. We investigate thalamic mechanisms underlying visual CP plasticity using a zebrafish model that provides a thalamus-centric model to address gaps in knowledge about CP plasticity. Using zebrafish we connect experience, neurophysiology, and behavior to understand how visual input modulates visual processing in the brain. We found two functionally distinct thalamic neuron populations that encode visual experience. Within a hemisphere these populations display different magnitudes of photo-responsiveness, which display reciprocal asymmetric patterns across hemispheres. These thalamic asymmetries correlate to visual experience and match visuomotor behavioral performance. We also show that the AMNs are inhibitory, and inhibitory signaling controlled the timing of visual plasticity and thalamic neuron encoding of visual experience. To find specific inhibitory molecular mechanisms instructing this plasticity, we performed FACS RNAseq. We identified that the GABRA1 subunit of the GABAergic receptor complex was upregulated, which we confirmed expression in the zebrafish thalamus. Using a combination of CRISPR knockdown and a stable GABRA1 mutant line, we show visual plasticity is dependent on GABRA1 mediated inhibitory signaling. In addition, experience dependent thalamic changes are also lost. These data suggest that inhibitory signaling mediated by GABRA1 is necessary for visual experience dependent modulation of thalamic physiology. This work has identified a specific molecular mechanism for thalamus plasticity and establishes a vertebrate model to understand neuron level subcortical changes imposed by visual experience during development.

378

Investigating the molecular mechanisms of cardiac chamber coverage by epicardial cells

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

The epicardium forms the outermost layer of the vertebrate heart. It is crucial to maintain the integrity of the heart by serving as a progenitor population for different cardiac cell types and as a source of paracrine signalling factors. Epicardial cells are derived from a transient cluster of cells, the proepicardium, which are released into the pericardial cavity and attach to the myocardium.

Unpublished work from the lab has found that epicardial cells cover the ventricular surface through dynamic cell shape changes, migration, and proliferation in a cardiac contraction-dependent manner (manuscript in preparation). Moreover, we also observed that these cells further extend to cover the atrial surface over time. However, how epicardial cells sense and transduce the signals that drive chamber coverage remains unknown.

In this study, we investigate the molecular and biophysical mechanisms that control dynamic behaviour of epicardial cells by focusing on the cell-extracellular matrix (ECM) and cell-cell interactions. We are taking a multimodal approach by combining genetics, subcellular-resolution live imaging, and Brillouin microscopy to measure the mechanical properties of living cells/tissue. Our preliminary data indicate that as the epicardium covers the ventricle, a new layer of ECM forms between the epicardium and the myocardium. We plan to modulate ECM components and mechanical properties of the myocardium to study their roles during the epicardial coverage. Moreover, we will visualise and study the functions of junctional proteins that mediate epicardial-epicardial and epicardial-myocardial cell interactions using various transgenic and mutant and lines. We further plan to extend these studies to the atrium to investigate whether the coverage mechanisms are conserved. Overall, this study aims to reveal precise mechanisms of epicardial morphogenesis during cardiac development.

380

Single-cell characterization of cell fate decisions in a ribosomopathy model

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Topic

19 Cell fate

Abstract Content

Ribosome biogenesis is essential for all proliferative cells, yet mutations in ribosome production factors often produce strikingly tissue-specific developmental phenotypes. In vertebrates, craniofacial structures are particularly sensitive to disruptions in ribosome biogenesis, but the mechanisms underlying this lineage specificity remain unclear. Using zebrafish mutants we investigate how impaired rRNA transcription impacts craniofacial cartilage development.

Embryos carrying mutated alleles of polr1a, the catalytic subunit of RNA Pol I, exhibit severe craniofacial defects, failing to generate both cranial neural crest cell (cNCC)-derived and mesoderm-derived cartilage elements. Removal of tp53 restores overall morphological growth and rescues mesoderm-derived cartilage structures, demonstrating that a component of the growth defect is mediated by canonical cell cycle impairment and apoptosis. Unexpectedly, cNCC-derived cartilage fails to recover in the polr1a; tp53 double mutant background, revealing a tp53-independent requirement for Pol I activity during cNCC differentiation into cartilage.

To resolve lineage-specific responses at cellular resolution, we performed single-cell RNA sequencing on polr1a CRISPant embryos. While global activation of the tp53 and integrated stress responses is observed across lineages, cNCC-derived mesenchyme uniquely exhibits disruption of differentiation programs. These findings indicate that neural crest cells are not merely dying, but are failing to execute lineage-specific differentiation under conditions of rRNA insufficiency.

Consistent with Pol I's role in nucleolar assembly, polr1a mutants display altered nucleolar architecture, suggesting that disruption of nucleolar organization – rather than simple loss of translational output – may underlie the lineage-specific phenotype. We propose that impaired nucleolar integrity, defective ribosomal protein incorporation, and consequent proteotoxic stress may contribute to neural crest-specific vulnerability. Together, this work provides a mechanistic framework for understanding the tissue specificity of ribosomopathies and supports a model in which ribosome biogenesis governs cell fate decisions not only through translational capacity, but also through maintenance of nucleolar structure and proteostasis.

381

Basement membrane integrity governs tissue morphogenesis and biomechanical stabilization of developing cardiac valves

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Topic

4 Cardiovascular

Abstract Content

Background: Organs rely on tissue folding to establish spatial organization and patterning. In the developing heart, folded valve leaflets arise from endocardial cells and must achieve precise size, shape and cell-state organization to ensure unidirectional blood flow. However, the mechanisms that coordinate valve size, shape, and function remain poorly understood.

Methods: Combining zebrafish genetics, quantitative confocal and Brillouin microscopy, single-cell transcriptomics and pharmacological interventions, we investigated the roles of the Laminin-containing basement membrane network in shaping valve morphology, mechanics and function. We focused our study on *laminin alpha5*, which encodes a core Laminin subunit enriched in endocardial cells and whose loss disrupts basement membrane deposition in the cardiac valve.

Results: We found that the basement membrane (BM) constrains valve leaflet growth while promoting valve morphogenesis and function. Disruption of this matrix compartment in *lama5* mutants led to enlargement of the valve tissue and extracellular space, accompanied by markedly reduced tissue stiffness and cellular tension in the valve. *lama5* mutant valve endocardial cells exhibited excessive proliferation but failed to fully differentiate into valve fibroblasts/interstitial cells, indicating that the BM restricts valve overgrowth while promoting endothelial-to-fibroblastic differentiation. Through single-cell transcriptomics and biosensor imaging, we found overactivation of the oncogenic ERK/MAPK pathway in *lama5* mutants. Pharmacological inhibition of ERK/MAPK signaling rescued valve overgrowth but did not restore valve function or interstitial cell differentiation, suggesting that BM-dependent mechanical cues act in parallel with signaling control.

Conclusion: Our findings support the requirements for the BM during cardiac valve morphogenesis, limiting ERK/MAPK-dependent valve tissue growth and providing mechanical cues required for valve function and interstitial cell fate. More broadly, this work identifies basement membrane–mediated tissue folding as a fundamental mechanism that integrates extracellular mechanics, cell fate specification, and morphogenetic signaling to shape organ architecture during development.

383

Investigating the importance of niche diversity in hematopoietic stem cell function during development and stress

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Topic

14 Stem Cells and Regeneration

Abstract Content

Background
During development, hematopoietic stem and progenitor cells (HSPCs) reside in diverse niches with distinct microenvironmental properties. Under stress, HSPCs can relocate between niches to adopt different fates. We investigate the role of microenvironmental niches in regulation of HSPCs during development, particularly how the cardiac endothelium_endocardium and the intermediate niche, caudal hematopoietic tissue (CHT) regulate the HSPC population, their adhesion to these niches and their fate under physiological conditions.

Methods
High-resolution imaging was used to examine localization of HSPCs in different niches, including cell tracing using the photoconvertible Kaede protein and time-lapse of beating hearts. To compare niche-dependent transcriptional states, FACS-purified HSPCs from the heart and CHT were analysed by single-cell RNA sequencing in control and under reduced blood flow. Different physiological stress conditions using hypoxia and *Staphylococcus aureus* infection were established.

Results
Our imaging uncovered stable integration of HSPCs within the endocardium, which originate de novo from the endocardium and the dorsal aorta. Comparative single-cell transcriptomics of heart- and CHT-resident HSPCs identified a higher proportion of metabolically and transcriptionally active HSPCs in the heart. These “transitional” HSPCs appeared to be more ready to differentiate compared with bona fide HSPCs in the CHT. Reduced blood flow increased HSPC numbers in the heart but decreased those in the CHT. Transcriptomic analysis indicated a shift away from quiescent niche states toward heart-like HSPC states in both tissues. Hypoxia promoted redistribution of HSPCs from the endocardium to the CHT. Following infection, HSPCS relocalized to the affected region in the heart.

Conclusion

These findings identify the heart as a resident tissue containing metabolically active and transitioning HSPC population that persist under altered hemodynamic conditions and respond to stress. Broadly, our findings will uncover the contributions

of niche-extrinsic cues vs. cell-intrinsic mechanisms that confer HSPC programming and resilience throughout development.

384

Loss of mitochondrial protein Atad3 disrupts retinal development and function in zebrafish

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Topic

3 Neurobiology

Abstract Content

Background
The visual system is highly sensitive to reduced mitochondrial function due to its exceptional energy demands. Consequently, mitochondrial diseases frequently lead to visual impairment. However, how mitochondrial dysfunction affects retinal development and early visual function remains poorly understood. *Atad3* is a nuclear-encoded mitochondrial protein critical for mitochondrial dynamics and function. Mutations in human *ATAD3* cause childhood-onset developmental delay, neurological deficits, and impaired visual function.

Methods
To elucidate the role of *Atad3* in visual development and function, we generated an *atad3*-deficient zebrafish model. We analyzed retinal morphology, gene expression, and visual behavior using fluorescent in situ hybridization (FISH), histology, optokinetic response (OKR) assays, electroretinogram (ERG) recordings, and transmission electron microscopy (TEM).

Results
atad3$^{-/-}$ zebrafish larvae exhibited microphthalmia and reduced visual function from 4 days post-fertilization (dpf). FISH analyses of the ciliary marginal zone (CMZ) showed decreased expression of proliferative genes, possibly explaining the smaller eye size. Mutant larvae displayed impaired OKR, consistent with reduced acuity, progressing to apparent blindness by 7 dpf. Histology revealed delayed cone photoreceptor development at 3 dpf that recovered by 5 dpf, though UV cones retained shorter outer segments and reduced ellipsoid mitochondria. Despite these structural abnormalities, ERG recordings showed normal photoreceptor responses but absent bipolar cell activity, suggesting impaired synaptic transmission. TEM demonstrated altered synaptic vesicle density at photoreceptor ribbon synapses, potentially underlying the observed functional deficits.

Conclusion
Our findings demonstrate that Atad3 is essential for maintaining photoreceptor and mitochondrial integrity and for enabling effective synaptic transmission between photoreceptors and bipolar cells. Loss of Atad3 disrupts photoreceptor structure,

CMZ progenitor dynamics, and neuronal signaling pathways, culminating in severe visual impairment.

388

Role of Histone acetyltransferase 4 in Muscle Deformities

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background:
Histone acetyltransferase 4 (HAT4) is a Golgi-localized N-terminal acetyltransferase responsible for modifying membrane-associated proteins and is involved in maintaining the structure of organelles. However, its developmental functions need to be understood. Preliminary evidence suggested that the loss of histone acetylation can disrupt transcriptional programs essential for muscle differentiation. Disruption of acetylation patterns has been found to be associated with congenital myopathies, impaired muscle regeneration, and developmental deformities. In zebrafish HAT4$^{C\text{-}\Delta 126/183aa}$ model we observed reduction in intensity of light with mild birefringence. Furthermore, Myocyte enhancer factor 2C (MEF2C) which is a key regulator of myofibrillar organization, sarcomere assembly, and muscle fiber maturation was observed to be reduced in HAT4$^{C\text{-}\Delta 126/183aa}$ mutants. The reduction in MEF2C in the mutants suggests that the loss of histone acetylation may be essential for proper muscle structure and musculoskeletal development. No previous studies have described how HAT4 regulates tissue architecture nor weather its loss leads to muscular deformities. Thus, investigating HAT4 in zebrafish model provides a unique opportunity to identify unknown regulator of muscle growth and its link to epigenetic signaling networks.

Methodology:

HAT4$^{C\text{-}\Delta 126/183aa}$ mutants were analyzed by birefringence assay.

Western blot was performed to quantify MEF2C levels.

Phalloidin and H&E staining for disrupted muscle fiber structure.

Results:

Preliminary findings show reduced light intensity through birefringence in HAT4$^{C\text{-}\Delta 126/183aa}$ mutants. The mutants exhibit myofibrillar disorganization and musculoskeletal deformities. Mef2c protein levels in HAT4$^{C\text{-}\Delta 126/183aa}$ mutants are significantly reduced indicating a transcriptional link between HAT4 and MEF2C.

Conclusions:

These findings indicate that HAT4 plays an important role in maintaining muscle structure and proper musculoskeletal development. Our study suggests that HAT4-mediated acetylation may influence transcriptional programs involved in muscle formation, potentially through regulation of MEF2C. This work highlights a previously unrecognized connection between organelle-associated acetylation and nuclear transcriptional pathways involved in tissue morphogenesis tissue morphogenesis.

391

Interferon-phi4 Signaling Drives Receptor-Mediated Patterns of Tissue-Specific Immune Activation in Live Zebrafish

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Topic

15 Infection and Immunity

Abstract Content

Interferons (IFNs) are a group of secreted cytokines that play key roles in activating the antiviral immune response. The optical transparency and genetic tractability of the zebrafish model offer a unique lens to deeply interrogate immune dynamics across biological scales through the integration of molecular analysis with spatiotemporal resolution of live dynamics. To date, however, only Type I (systemic) and Type II IFN signaling have been corroborated in teleosts, which are thought to lack the Type III (tissue-specific) signaling present in mammals, and the activation, functionality, and receptor usage of the four known zebrafish interferons (IFNϕ1, IFNϕ2,IFNϕ3, and IFNϕ4) are yet to be fully characterized.

Seeking to parse the specific contributions of individual interferons to antiviral and innate immunity, we drove ubiquitous overexpression of each interferon in Tg(*isg15:GFP*) zebrafish larvae, which express green fluorescent protein under the control of a critical interferon-stimulated gene (ISG) promoter. We obtained visual and molecular profiles of subsequent immune activation using confocal microscopy and scRNAseq. In contrast to the anticipated systemic (Type I-like) immune response, we identified a unique tissue-restricted pattern of immune activation driven by IFNϕ4 overexpression.

Hypothesizing that this tissue-restricted immune response is mediated by differential expression of IFNϕ4 receptors on immune and mucosal cells, we identified five key candidate IFNϕ4 receptors by integrating DEG analysis of responding (ISG+) versus non-responding (ISG−) cells from our libraries with canonical IFN receptors described in prior literature and mammalian homologs associated with Type III IFN signaling.

Necessity experiments in Tg(*isg15:GFP*) immune reporter zebrafish were performed using CRISPR-Cas9 to knock out each candidate receptor and identify those required for this pattern of tissue-restricted IFNϕ4–stimulated immune activation. Ongoing work will interrogate the role of these receptors during zebrafish picornavirus infection to better understand host–viral conflict across biological scales, from cellular signaling to organismal physiology.

392

Testing a valproic acid –induced ASD model in zebrafish larvae

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Topic

3 Neurobiology

Abstract Content

Background

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition associated with motor, cognitive, and social impairments. Prenatal exposure to valproic acid (VPA) is a widely used method for inducing ASD-like phenotypes in both rodents and zebrafish. To further refine the zebrafish VPA model, we examined the effects of four different VPA concentrations.

Methods

Zebrafish embryos were exposed to 12.5, 25, 37.5, or 50 µM VPA. Mortality, teratogenic effects (yolk and pericardial edema, swim bladder development), and behavioral outcomes were assessed. ASD-like symptoms were evaluated in 5-day-old larvae using the open-field test (locomotion) and the light–dark test (anxiety-related behavior).

Results

Increasing VPA concentrations led to higher mortality and more pronounced teratogenic effects, including edema formation and underdeveloped swim bladders. Larvae treated with 37.5 and 50 µM VPA showed significantly increased locomotor activity in the open-field test. In the light–dark test, these groups spent markedly less time in the illuminated zone compared with controls, indicating elevated anxiety-like behavior. Even the lowest concentration (12.5 µM) produced detectable ASD-like alterations.

Conclusion

All tested VPA concentrations induced ASD-like behavioral changes, but 37.5 µM emerged as the most suitable dose for future studies due to its balance between lower mortality and robust behavioral effects. To further validate ASD-like phenotypes at this concentration, additional assays, such as social interaction testing in older larvae, are planned.

395

A pigment-deficient transgenic zebrafish line for in vivo visualization of gonadal morphogenesis during sexual development

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Topic

9 Germline and Early Development

Abstract Content

The primary goal of our research is to understand sexual development of cyprinid teleosts using zebrafish model. During gonad development, a so-called 'juvenile ovary' forms in most zebrafish individuals. In males, an apoptosis-driven process transforms it into a testis. This process is not fully understood yet. We are using the *tg{ddx4::egpf}* (earlier *tg{vas::egfp}*) transgenic line to visualize the gonads. However, pigments interfere with *in vivo* observation of Egfp signals derived from ovaries. For this reason, we introgressed the transgene into the double pigment-mutant transparent zebrafish line, *casper*. The new homozygous transgenic line on *casper* background was named *gáspár* (pronounce: ghaashpar).

Typically, formation of such line would take at least three generations. To accelerate this, we developed an efficient selection protocol that allows the identification of *gáspár* individuals in slightly more than two generations. This procedure includes early pigment-based detection and PCR-based genotyping.

We determined the insertion site of the transgene by re-sequencing the genome of the transgenic line. Detailed analysis of the integration site revealed a 3 kb deletion. Primers designed to the deleted region allow for a direct PCR-based selection of homozygous individuals without their propagation. Bioinformatic analysis of the sequencing data indicated multiple transgene copies: the three tandemly integrated copies were verified experimentally.

We intend to improve the *gáspár* line further by introducing a testis-specific transgene with a different fluorescent tag for enhanced visualization of early testis development. We tested the expression of five testis markers in pooled samples and validated them on individuals. We hope that this new, double-transgenic and double pigment-mutant line will help to reveal details of testis formation that follows the apoptotic phase of juvenile ovary in males.

This work was supported a Frontline Research Excellence Grant of the National Research, Development and Innovation Office of Hungary (KKP 140353 to LO).

396

Context Scripts the Drama: Turning Silent Mosaicism into Disease

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Topic

8 Metabolism

Abstract Content

Somatic mutations are inevitable, yet their consequences are not. Human tissues are naturally occurring mosaics where most genetic variants remain clinically silent throughout life. A striking breakdown of this stability is Proteus syndrome—a mosaic disorder famously associated with Joseph Merrick ("The Elephant Man")—characterized by progressive focal bone overgrowths. Although patients harbor clones with an activating *AKT1* mutation, these mutant clones also reside in unaffected bones, suggesting the mutation itself is not the sole driver of disease.

We leverage the unique advantages of zebrafish to unveil the determinants that transition these "silent" clones into pathological phenotypes. We have developed the first animal model for Proteus syndrome that successfully recapitulates the focal bone overgrowths observed in patients. Our experiments reveal divergent growth dynamics: while all *akt1* mutant clones trigger an initial burst of bone formation, only a small subset results in rapidly progressive overgrowth. Unexpectedly, a fraction of these clones transition into an osteosarcoma-like state.

These divergent outcomes demonstrate that the same somatic *akt1* mutation can remain silent, drive benign overgrowth, or lead to a cancer-like state. Comparative transcriptomics and in vivo imaging reveal distinct microenvironmental signals that fuel lesion growth and transformation. We are currently testing whether modulating these pathways can serve as a strategy for therapeutic interception.

Our work provides a conceptual framework for understanding phenotypic variance of somatic *AKT1* mutations and, more broadly, demonstrates how zebrafish can be used to identify the "scripts" that turn silent somatic mutations into overt disease.

398

A novel *fmr1* zebrafish model reveals ASD-associated behavioral and molecular alterations

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Topic

1 Disease Models

Abstract Content

Autism spectrum disorder (ASD) comprises a group of early-onset neurodevelopmental conditions characterized by impairments in social interaction, repetitive behaviors, and altered sensory processing. Additional symptoms frequently associated with ASD include hyperactivity, anxiety, and epilepsy. Fragile X syndrome (FXS), caused by mutations in the fragile X mental retardation 1 (FMR1) gene, is the most common inherited form of intellectual disability and the most prevalent syndromic form of ASD.

We generated a novel zebrafish mutant line carrying a frameshift mutation in the *fmr1* gene using CRISPR/Cas9 genome editing to model ASD-related behavioral alterations. Gene disruption was confirmed by Western blot analysis, which showed the absence of fragile X mental retardation protein (FMRP) in the adult mutant brain.

Larvae were assessed for developmental morphology and basal locomotor activity using a light–dark transition assay. Seizure susceptibility was tested using stroboscopic light stimulation to induce epileptiform-like responses. Sensorimotor adaptation was evaluated using a closed-loop optomotor response (OMR) paradigm, in which larvae experienced altered sensory feedback during visually guided swimming. Social behavior was measured at 28 days post-fertilization by tracking interactions between freely swimming fish.

fmr1 mutants displayed increased swimming velocity during light–dark transitions, suggesting hyperactivity, and showed a marked decrease in motor activity after flashing-light stimulation, resembling a postictal-like state. In the visuomotor adaptation task, mutants adapted their motor output but exhibited altered dynamics and differences in bout structure, indicating impairments in motor execution. Alterations in social interactions were also observed. Transcriptomic analysis revealed significant changes in genes associated with synaptic machinery and GABAergic and glutamatergic receptor subunits. Preliminary whole-brain neural activity recordings were conducted in larvae.

In conclusion, this novel *fmr*1 mutant zebrafish line exhibits behavioral alterations consistent with ASD-like phenotypes and represents a promising model for investigating the molecular mechanisms and neural circuit alterations associated with ASD risk genes.

401

CRISPR/Cas9 mediated *grb10a* Knockout Zebrafish Reveals a Key Role for Grb10 as a Modulator of Cardiac Development, Function, Growth and Metabolism.

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Topic

4 Cardiovascular

Abstract Content

Growth Factor Receptor-Bound Protein 10 (Grb10), initially recognised for its contributions as a negative regulator of insulin signalling pathways, has recently emerged as a significant player with diverse roles in cellular processes that influence the growth, differentiation, and maturation of cardiac tissues. As the heart undergoes sequential stages of development, Grb10 appears to exert regulatory influences on critical signalling cascades, contributing to the intricate choreography of cardiac organogenesis. Here, we use a *grb10a* CRISPR/Cas9 knock-out (KO) zebrafish model to observe developmental alterations across physiological systems and the impacts on growth and metabolism.

In vivo early cardiac measurements were taken to assess heart rates (HR), cardiac output (CO), fractional area change (FAC), blood flow (BL) and vessel size of the dorsal aorta (DA) and posterior cardinal vein (PCV). To investigate the later life impact on cardiac phenotype, histological sections of the ventricle were taken alongside echocardiograms.Body morphology and length was measured to investigate the impact of *grb10a* KO on growth trajectories, and metabolic rates was measured using embryonic real time oxygen consumption and stop-flow respirometry in adult zebrafish.

KO zebrafish exhibited a persistent early cardiac phenotype characterised by reduced HR. Although CO and FAC showed no differences between groups, further investigation showed altered BL as well as evidence of vascular remodelling in both the DA and PCV. At later stages, the total size of the ventricle and function differed significantly between KO and WT, with thicker compact myocardial layers seen in the KO. Additionally, KO individuals displayed accelerated growth trajectories during early development, followed by a normalisation of body length during later stages. Metabolic rate was also elevated at stages coinciding with the accelerated growth observed.

Overall, we show that Grb10 is a key contributor to cardiac development and highlights how dysregulation of Grb10 significantly alters growth, metabolism and cardiac phenotypes.

403

Whole mount semi-Automated Single Cell Quantitation of RNAscope signals in neural crest and neural tube development.

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Topic

19 Cell fate

Abstract Content

The neural crest (NC) is a transient, multipotent cell population that generates a wide variety of cell types during vertebrate development. How individual NC cells integrate extracellular signals with transcriptional programmes to adopt specific fates remains a central question. Wnt signalling is known to control early NC induction and melanophore specification, yet the relevant Frizzled (Fzd) receptors in zebrafish NC cells have remained elusive as conventional *in situ* hybridisation in zebrafish has failed to detect clear expression patterns.

Recent studies suggest Wnt signals can be delivered directly between cells by cytonemes – specialised actin-based protrusions that extend from a signalling cell to a receiving cell. Besides ligands, cytonemes can also transport preformed, active Wnt ligand-receptor complexes to target cells (1). We hypothesise that dorsal neural tube cells extend cytonemes that transport active Wnt ligand receptor complexes, activating Wnt signalling in a subset of NC cells.

To test this, we combine highly sensitive whole-mount RNAscope with fluorescent genetic labelling in zebrafish NC cells and roofplate-derived signalling cells. This method enables detection of low-abundance transcripts, potentially down to single mRNA molecules in individually resolved cells *in vivo*. Because quantitative single-cell analysis in complex embryonic tissues is limited by cell shape heterogeneity and labour-intensive manual scoring, we developed an algorithm for semi-automated segmentation and quantification of single-cell RNAscope signals in zebrafish embryos imaged as confocal z-stacks.

Using this platform, we aim to generate a single-cell map for Fzd and Lrp co-receptor transcripts in NC cells during melanophore specification. This work will reveal how the spatially restricted Wnt signalling landscape is achieved *in vivo* and establish a new framework to study low-level signalling receptor expression during cell fate acquisition

1 Cytoneme-mediated transport of active Wnt5b-Ror2 complexes in zebrafish. Zhang C, Brunt L, Ono Y, Rogers S, Scholpp S. Nature. 2024 Jan;625(7993):126-133. doi: 10.1038/s41586-023-06850-7.

405

A zebrafish xenograft model for Myxoid liposarcoma

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Topic

1 Disease Models

Abstract Content

Myxoid liposarcoma (MLS) is a distinct subtype of liposarcoma defined by a prominent myxoid extracellular matrix and the characteristic FUS–DDIT3 gene fusion. It is a rare disease, occurs most commonly in young adults and often presents as a deep, painless soft-tissue mass in the extremities. In this study we attempted to establish a novel in vivo model for MLS that is also suitable for therapeutic compound testing. Several cell lines for MLS are well defined and commonly used for in vitro studies.

Following our established xenograft protocol for another sarcoma type, we could inject 4 different MLS cell lines into the perivitelline space of 2dpf zebrafish larvae. We monitored tumor growth on our therefore optimized high content imaging system, a part of the ZANDR platform.

Most of the MLS cell lines persisted as tumor mass over drug treatment time in zebrafish until 5dpf, however showed particularly different engraftment patterns. The subtle behavior differences of the cells during engraftment in zebrafish reflect distinct variances in the underlying genetics of the cells but also in their differentiation status.

406

The E3 ligase Traf7 drives cardiomyocyte proliferation

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Topic

4 Cardiovascular

Abstract Content

Despite cardiovascular diseases remaining the leading cause of global mortality, the molecular mechanisms governing homeostasis and proliferation in cardiomyocytes are still not well understood. Understanding the fine tuning of cardiomyocytes proliferation is crucial for deciphering the molecular mechanisms underlying normal development and disease susceptibility.

Here we describe the recessive, embryonic lethal zebrafish (*Danio rerio*) mutant *herzbuckel*. Mutants show an impaired cardiac growth due to decreased cardiomyocyte proliferation. Through positional cloning we have identified a missense mutation in the zebrafish tumor necrosis factor receptor associated factor 7 (*traf7*) gene resulting in a premature stop codon and termination of protein translation at position 519 and subsequent loss of Traf7 function *in vivo*. To verify *traf7* as the underlying mutation, Morpholino mediated knockdown, as well as CRISPR/Cas9-mediated knockout of *traf7* was conducted, both of which show reduced ventricular cardiomyocyte proliferation, thereby phenocopying the herzbuckel phenotype.

To assess whether the role of Traf7 during cardiomyocyte proliferation is conserved, we analyzed TRAF7 knockdown by AAV-mediated siRNA transfection in neonatal rat cardiomyocytes. Here, cardiomyocyte proliferation was significantly reduced as observed in the developing zebrafish heart. Therefore, the role of *Traf7* appears to be conserved among vertebrates.

In the context of proliferation, the E3 ligase Traf7, is described to be involved in both the activation and repression of the NF-κB pathway. This pathway is known to influence various cellular processes, including the cell cycle and autophagic activity. We were able to show, that loss of TRAF7 in rat cardiomyocytes leads to changes in NF-κB phosphorylation status. In summary, our findings suggest an important and evolutionary conserved role for Traf7 in cardiomyocyte proliferation, putatively via the NfkB signaling pathway.

407

OptoFish: A modular toolkit for multi-scale optogenetic control of signaling pathway activity in zebrafish.

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Topic

16 Morphogenesis and Organogenesis

Abstract Content

Signaling pathways are tightly controlled, intricate systems that exhibit distinct patterns depending on location, time, and target processes. Traditional perturbation methods do not permit experimentalists to resolve this complexity – promoter-driven expression induces genes of interest in specific cell types but lacks temporal control, whereas small-molecule treatments provide temporality but act throughout the whole organism. Optogenetic tools can bridge this gap; however, previous approaches show limited generalisability, restricted quantitative control, or implementation challenges. Here we present OptoFish, a modular molecular toolkit for optogenetic activation of diverse signaling pathway components across spatial and temporal scales in zebrafish. OptoFish is a two-part system consisting of (1) blue light-dependent recombinase expression induced by TAEL-N/c120 and (2) inducible expression of the protein of interest upon recombination, coupled to a fluorescent reporter. To this end, we generated stable lines expressing the light-dependent component, including dose-dependent variants. We quantitatively characterise the leakiness and the light-induced activation efficiency across spatial and temporal scales using a positive control. Future developments will expand this system to the modulation of signaling pathway components. Our toolkit enables simple modification and facilitates cross-platform integration by replacing only a single plasmid containing the protein of interest. Moreover, OptoFish activation ranges from the single-cell level to the whole organism and spans larval development. Using OptoFish, we investigate diverse applications, such as organ development, gastrulation, and immunology.

408

Mechanisms of Wnt and S1P Signaling Pathway Interactions during Brain Angiogenesis

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Topic

4 Cardiovascular

Abstract Content

The brain is a heavily protected site with the brain vasculature contributing to formation of the blood-brain barrier. Therefore brain vessels have specialized properties which also affect their angiogenesis. While multiple signaling pathways are involved in generating and maintaining the BBB, we could show that Wnt and Sphingosine 1-phosphate (S1p) signaling functionally oppose each other by direct interaction during BBB development (Hübner et al 2018). Yet the downstream mechanisms of how these pathways fine tune barrier tightness remain elusive. We found that interrupting Wnt signaling results in pre-mature S1p receptor 1 (S1pr1) and downstream of it Rac1 activation and in turn severe defects in vascular development. However, we are focussing on how the interaction is mediated and which downstream components will balance it. Using comparative transcriptomics on Kaede converted FACS isolated central arteries with and without Wnt-Signaling inhibition, we isolated Arhgap28 as a RAC1 activity regulating factor downstream of Wnt signaling.

We can show that Arhgap28 deficiency phenocopies loss of Wnt signaling and that it is sufficient to mediate the effect of Wnt-signaling on the S1p-signaling pathway.

We can further show how fine-tuning the S1p signaling response is mediated

by regulation of other Rac1 interactors.

Our data provide novel insight into the molecular interaction of the Wnt- and S1p Signaling pathways and the balancing of BBB tightness versus brain vessel angiogenesis.

410

In vivo chemogenetic ablation of TH neurons in zebrafish using the QF3/QUAS system and NTR 2.0

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Topic

2 Emerging Technologies

Abstract Content

In vivo cell ablation technologies are indispensable tools for dissecting intricate biological processes and specific cellular roles within living animal models. Among the most effective methodologies, the nitroreductase (NTR)/metronidazole (MTZ) system is prominent for its capacity to provide precise spatiotemporal control over cell death. This chemogenetic system functions by enzymatically converting the non-toxic prodrug MTZ into a DNA-damaging cytotoxic metabolite, thereby inducing targeted apoptosis specifically in NTR-expressing cells. In this study, a transgenic zebrafish integrating the NTR 2.0 and QF3/QUAS systems was established to achieve the complete ablation of tyrosine hydroxylase (TH) neurons. Utilizing a CRISPR-Cas9-mediated non-homologous end joining (NHEJ) knock-in strategy, a *Tg(TH:QF3)* driver line was developed to specifically express the transcriptional activator QF3 within TH neurons. Furthermore, *a Tg(13xQUAS:YFP-2A-NTR 2.0)* effector line was generated to express NTR 2.0, which exhibits approximately 100-fold greater ablation efficiency than the original NTR variant. By combining high sensitivity with minimal off-target effects, this system enables the precise targeting and removal of TH neurons in the brain. Ultimately, this system facilitates the investigation of developmental processes and regeneration, as well as disease modeling and drug discovery.

411

Modeling human FAM177A1 deficiency in zebrafish elucidates its genetic and biological roles

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Topic

1 Disease Models

Abstract Content

Our previous Undiagnosed Diseases Network study defined *FAM177A1*-related recessive neurodevelopmental disorder characterized by global developmental delay, macrocephaly, diffuse hypotonia, intellectual disability, seizures, behavioral abnormalities, and gait disturbance. *FAM177A*1 encodes an evolutionarily conserved, ubiquitously expressed protein that localizes predominantly to the Golgi complex, though its function is poorly understood. Zebrafish has two functionally redundant homologs, *fam177a1*a and *fam177a1b*. Single mutants show transiently shorter body length during embryogenesis, and double mutants exhibit an additive phenotype,but are viable and fertile . To explore Fam177a1 function, we examined Golgi dynamics during cell division in *fam177a1* double mutants. In WT metaphase cells, GalT-mCherry-labeled Golgi apparatus undergoes extensive fragmentation into smaller clusters and vesicles that dispersed throughout cytoplasm. In contrast, double mutants displayed impaired Golgi dissociation, with persistent GalT-mCherry-positive clumps, suggesting Fam177a1 is essential for Golgi remodeling during mitosis. Additionally, we identified a genetic interaction between *fam177a1* and *vps13b,* encoding a member of a protein family implicated in lipid transport and associated with Cohen syndrome (a lipid transport). In human cells, VPS13B is localized at the interface between proximal and distal Golgi subcompartments in the close proximity of FAM177A1. Reduction of zygotic *vps13b* function in zebrafish using a crispant approach, exacerbated transient body shortening of MZ*fam1a/b* double mutants. Strikingly, MZ*fam177a1*a/b;*vps13b* triple mutants exhibited synergistic defects in chorion elevation, indicating that Fam177a1 and Vps13b cooperatively support early egg activation. Collectively, these finding implicate Fam177a1 in Golgi dynamics, and early developmental processes. These results provide important insights and open new experimental avenues to understanding FAM177A1 deficiency in human.

413

Deleterious mutations in the NR6A1 gene lead to the MRKH syndrome

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Topic

1 Disease Models

Abstract Content

The genetic cause of the Mayer-Rotikansky-Kuster-Hauser (MRKH) syndrome, characterized by malformations in several organs including the female genital tract, the kidney and axial skeleton, are still largely unknown. In this study, we identified 5 MRKH patients from three distinct families harbouring heterozygous deleterious sequence variants in the *NR6A1* gene coding for an orphan nuclear receptor. To verify the implication of *NR6A1* in the MRKH syndrome and investigate its role in development, we generated zebrafish mutant lines by CRISPR/Cas9 in the orthologous genes nr6a1a and nr6a1b. While the *nr6a1b* mutants have no apparent defects, the *nr6a1a* homozygous mutants display several malformations including abnormal patterning of pronephros and axial skeleton, cloacal malformations and lack of anal fin, showing thus some phenotypic similarity with MRKH patients and thus supporting the involvement of NR6A1 mutations in this syndrome. The double nr6a1a;nr6a1b mutants are more affected dying around 10-14 dpf and lacking pectoral fins and posterior pharyngeal arches. Further analyses of these mutants reveal an anterior shift in the expression of some posterior hox genes. As several defects of the double mutants are observed in the aldh1a2 mutants, we are investigating the link between nr6a1 genes and the retinoic acid pathway.

415

Myocardial Notch signalling is required for the morphogenesis of trabecular cells in the zebrafish heart.

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Topic

4 Cardiovascular

Abstract Content

During zebrafish heart development, a subset of ventricular cardiomyocytes differentiates into specialized trabecular cells (TCs), which are essential for optimal cardiac function. These cells coalesce to form ridges that eventually organize into a complex, sponge-like network within the ventricular cavity. The mechanisms governing this myocardial arrangement remain poorly understood.

Previous studies have established that endocardium-derived Notch signalling is required for TC development. However, the contribution of myocardial Notch signalling towards TC development and organisation remains unclear.

We find that in the zebrafish heart, myocardial notch activity is synchronous with the initiation of trabecular ridge formation. As systemic inhibition of Notch signalling affects both the endocardium and myocardium, we sought to isolate the myocardial-specific role of this pathway. Initial analysis of *jag2b* mutants—a myocardium specific ligand for Notch signalling—revealed significant disruptions in TC arrangement. To further investigate, we used the "Cardiodeleter" system to achieve myocardium-specific CRISPR/Cas9 knockdown. After generating and validating our own Cardiodeleter line, we targeted the *notch1b* receptor. Preliminary findings indicate that myocardial *notch1b* crispants replicate some aspects of the *jag2b* mutant phenotype, specifically regarding disordered TC organization.

In conclusion, our study demonstrates the contribution of myocardial Notch signalling towards TC development and the structural organization of the trabecular network. Furthermore, this work underscores the necessity of tissue-specific knockdown tools in dissecting the complex regulatory factors that drive organ development.

417

In vivo estrogenicity of glyphosate, its formulations, and AMPA on transgenic zebrafish embryos

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Topic

10 Toxicology

Abstract Content

Glyphosate-based herbicides (GBHs) are widely used worldwide for weed control. Due to their extensive application, they frequently occur in aquatic ecosystems and may affect various organisms. The active substance glyphosate (GLY) and its major by-product, AMPA, are the most thoroughly studied chemicals; however, the adverse effects of GBHs with complex formulations and diverse, unknown co-formulant contents remain insufficiently researched. This study focused on the embryotoxicity, sublethal malformations, and estrogenic potency of GLY, AMPA, and four commonly used GBHs on zebrafish embryos using a wild-type and an estrogen-sensitive, transgenic zebrafish line (*Tg(vtg1:mCherry)*).

After 120 h of exposition, AMPA did not cause acute toxicity, while the LC_{50} of GLY was 160 mg/L. The GBHs were more toxic with LC_{50} values ranging from 31 to 111 GLY active equivalent (a.e.) mg/L. Exposure to 0.35–2.8 mg/L GBHs resulted in sublethal abnormalities: typical symptoms included structural deformation of the lower jaw and anomalies in the olfactory region. Deformity rates were 10–30% in the treated groups. *In vivo*, fluorescently expressed *vtg1*-mCherry protein in the embryonic liver was detected using a noninvasive microscopy method, indicating estrogenic action via vitellogenin production by GLY, AMPA, and GBHs. To confirm the *in vivo* findings, RT-qPCR was performed to quantify the levels of the estrogenicity-related *vtg1* mRNA. After 120 h of exposure to GLY, AMPA, and three GBHs at a concentration of 0.35 mg/L, the expression of the *vtg1* gene was significantly up-regulated.

Our results highlight the risk that, at environmentally relevant concentrations, short-term GLY and GBH exposure can cause developmental malformations and disrupt hormonal balance in zebrafish embryos in a non-concentration-dependent manner.

418

Comparative analysis of epitranscriptomic profiles in maternal and somatic ribosomes of zebrafish

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Topic

9 Germline and Early Development

Abstract Content

In animal embryos activation of zygotic gene expression coincides not only with a dramatic and efficient overhaul of the chromatin organization and transcriptome composition, but also with significant changes in the composition of the translational machinery. The maternal-zygotic-transition (MZT) of the zebrafish (*Danio rerio*) embryo has been the subject of intensive research over the past years and several characteristics of this process have been recently elucidated.

Earlier studies have demonstrated the presence of different maternal and somatic rDNA clusters the zebrafish genome. The two rRNA types, while mostly similar, contain significant differences and during early stages of development rRNA encoded by the maternal cluster (fem-rDNA) is replaced by RNAs encoded in the somatic cluster.

Using a combination of RNA bisulfite sequencing (RBS-Seq) protocols and nanopore-based detection of epitranscriptomic modifications we provide a comparative analysis of RNA modifications present in these two rRNA types. We also took advantage of a *dkc1* mutant zebrafish line, impaired in the production of dyskerin, the major pseudouridine synthetase involved in rRNA pseudouridylation, and show that when somatic rRNA production is defective, the turnover of the maternal rRNA component is also slower. Interestingly, the persistence of maternal rRNA apparently cannot compensate for the absence of the somatic type, suggesting differential translational efficiencies for certain zygotic mRNAs by the maternal and zygotic ribosomes.

420

Genetic Compensation Secures Embryonic Axis Formation Directed by the Maternal Determinant Huluwa

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Background: In vertebrates, the maternal factor Huluwa functions as a dorsal determinant by specifically activating β-catenin signaling on the future dorsal side, thereby directing embryonic axis formation. However, the mechanisms underlying the genetic robustness of this critical process remain poorly understood.

Methods: We generated a maternal mutant line, *tsu01sm*, in which the transcriptional activity of the *huluwa* gene is abolished due to an upstream retrotransposon insertion. In addition, we isolated a second mutant line, designated as the "reverse mutant" (*rev*), derived from *tsu01sm*, that partially restores both *huluwa* transcriptional activity and axis formation. To elucidate the molecular basis of this rescue, we performed whole-genome sequencing (WGS), RNA-seq, RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE), Cleavage Under Targets and Tagmentation (Cut&Tag), and Hi-C analysis.

Results: Transcriptomic profiling and RLM-RACE revealed that the *rev* mutant likely restores huluwa transcription through the utilization of alternative transcription start sites. Furthermore, integrated analysis of WGS and histone modification mapping identified a mutation-dense region approximately 0.5 Mb downstream of the *huluwa* locus. Cut&Tag analysis of stage I oocytes showed that this region is specifically enriched for H3K27me3 in the *tsu01sm* mutant, suggesting that mutations in this region may alter local chromatin state or higher-order chromatin architecture, thereby influencing *huluwa* gene transcription, which were further confirmed by Hi-C analysis.

Conclusion: These findings uncover a compensatory mechanism underlying maternal gene regulation and provide new insights into how early embryonic systems maintain developmental robustness in response to transcriptional disruption. This study advances our understanding of the genetic robustness and regulatory plasticity governing embryonic axis formation as directed by *huluwa*.

421

Elevated Temperature Alters Proliferation Dynamics in the Developing Medaka Ciliary Marginal Zone

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Topic

9 Germline and Early Development

Abstract Content

Adaption to ambient temperature changes is a necessity for robust development during ectodermic embryogenesis. The bounds of these temperature limitations are species-specific, and different tissues exhibit distinct responses to thermal stress. In teleost models, the retinal stem cell niche (ciliary marginal zone, CMZ) is a prominent integrator of environmental cues and, as such, provides a valuable model system for understanding general developmental robustness in ectothermic vertebrates.

The stem cell niche in the CMZ of medaka and other teleost fish persists into adulthood, indicating the development of a state of homeostatic cell division in stem cell and progenitor cell populations. However, the cell cycle, and division rate are known to increase in rate with temperature, potentially disrupting during specific developmental windows. To examine how proliferative capacity of these cell populations changes over time as a result of elevated temperatures in the medaka CMZ, we optimized a microinjection-based metabolic labelling protocol using EdU to examine cell division.

By integrating EdU incorporation results with a sci-RNA-seq dataset acquired in parallel, we can validate expression-based readouts, CMZ proliferation, and spatial context to these results. Specifically, we compared proliferation rates in CMZ progenitor populations across three temperatures (26°C, 34 °C, 36 °C) and developmental stages (52, 121, 216 hours post fertilisation), revealing a temperature-dependent disruption in the establishment of proliferative homeostasis, with the potential to exhaust growth and regenerative capacity of the tissue.

423

Functional Interpretation of a Patient-Derived BCOR Variant Through Zebrafish Genome Editing

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Topic

1 Disease Models

Abstract Content

A 6-year-old male patient, enrolled through the UCLA Clinical Site of the Undiagnosed Diseases Network (UDN), presented with global developmental delay, possible cerebral palsy, right sided weakness, and hearing loss in the right ear. The variant is a novel 18-bp in-frame p.Glu1464_Arg1469del deletion in *BCOR*(BCL6 corepressor), located on Chromosome X. To evaluate the pathogenic potential of this variant in vivo, we introduced targeted mutations in the endogenous zebrafish *bcor* homolog using CRISPR/Cas9 genome editing. We generated three independent alleles: one in-frame 21-bp deletion removing seven amino acids, *stl735*, corresponding to the human patient variant, and two frameshift indel alleles (*stl704*, Δ10bp and *stl736*, +10bp). Homozygous animals for all alleles are adult viable. However, animals homozygous for the two frameshift alleles displayed pronounced morphological defects, including loss of dorsal, pelvic, and/or anal fins. In contrast, *bcor*$^{stl735/stl735}$ mutants developed all major fins and fin ray numbers were comparable to wild-type siblings. To assess skeletal phenotypes in adults, we performed micro-computed tomography (µCT) analysis on mutants alongside age- and size-matched control animals. Both mutant alleles displayed craniofacial abnormalities, including malformation along the sutures and blebbing of the bone. Additionally, the lower jaw showed considerable malformations in homozygous animals for both alleles. Reduced bone density and fewer pharyngeal teeth were observed exclusively in the frameshift mutant. To assess neuronal defects, we performed stimulus-evoked locomotor assays in larvae. *bcor*$^{stl704/stl704}$ showed an increased locomotor response compared to controls, whereas larvae homozygous for *stl735* in-frame deletion exhibited no significant difference from sibling wild-type controls. Taken together, these results indicate that the UDN variant-like in-frame deletion allele (*bcor*$^{stl735/stl735}$) is pathogenic but not a complete loss-of-function allele. This zebrafish model provides an *in vivo* system for studying BCOR dysfunction and supports functional interpretation of patient-derived BCOR variants.

424

Zebrafish xenograft modeling reveals SIRT1 inhibition as a strategy to enhance 5-fluorouracil efficacy in HNSCC

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Topic

8 Metabolism

Abstract Content

Background:
Resistance to chemotherapy remains a critical barrier in the treatment of head and neck squamous cell carcinoma (HNSCC). SIRT1, a NAD^+-dependent deacetylase, has emerged as a key regulator of tumor cell survival, DNA damage response, and therapeutic resistance. While in vitro studies suggest that SIRT1 inhibition may sensitize cancer cells to chemotherapy, rapid and predictive in vivo validation remains essential for translational application.

Methods:
We investigated the combined effects of 5-fluorouracil (5-FU) and the selective SIRT1 inhibitor EX527 (Selisistat) using an integrated in vitro–in vivo approach. HNSCC cell lines were analyzed for proliferation, apoptosis, and cell cycle alterations, and drug interactions were assessed using isobolographic analysis. To bridge the gap between in vitro findings and clinical relevance, zebrafish (Danio rerio) xenograft models were employed as a high-resolution, real-time in vivo platform for tumor growth assessment, drug response evaluation, and survival analysis.

Results:
Combination treatment with EX527 and 5-FU resulted in a synergistic reduction in cell viability and enhanced induction of apoptosis in HNSCC cell lines. Crucially, these effects were robustly recapitulated in the zebrafish xenograft model. Live imaging enabled dynamic visualization of tumor progression, revealing a significant reduction in tumor burden following combination therapy. Moreover, zebrafish-based survival assays confirmed improved outcomes compared to monotherapies. The zebrafish model proved highly sensitive in detecting treatment response, supporting its utility as a rapid and predictive platform for preclinical drug evaluation.

Conclusion:
Our study demonstrates that SIRT1 inhibition potentiates the anticancer effects of 5-FU in HNSCC and highlights zebrafish xenografts as a powerful translational tool for validating therapeutic strategies. These findings underscore the potential of zebrafish-based models for accelerating drug screening and improving the predictive value of preclinical cancer research.

426

Laminin and fibronectin constrain endothelial self-organization to guide stereotyped zebrafish ISV sprouting

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Topic

4 Cardiovascular

Abstract Content

During zebrafish trunk angiogenesis, endothelial tip cells sprout dorsally from the dorsal aorta, elongate between somites, and connect to form the dorsal longitudinal anastomotic vessel (DLAV). While vascular endothelial growth factor (VEGF), semaphorin signaling, and integrin-mediated adhesion regulate intersegmental vessel (ISV) pathfinding, how extracellular matrix (ECM) organization and mechanics contribute to this process remains incompletely understood. Here, we combined in vivo time-lapse imaging, perturbation experiments, rescue, and mathematical modeling to study how laminin and fibronectin guide endothelial migration during ISV formation.

Using fluorescent ECM reporter lines, we found that laminin and fibronectin are enriched along intersomitic regions during sprout extension, where endothelial tip cells closely interact with the matrix. Morpholino single knockdowns of laminin or fibronectin slowed ISV elongation, but most vessels eventually reached the DLAV, indicating that partial loss of either ECM system reduces migration efficiency without fully disrupting vascular patterning. To analyze how ECM mechanics shape sprout progression, we developed a hybrid mathematical model integrating endothelial self-organization, ECM mechanics, and guidance cues. In this model, endothelial cells intrinsically form vascular network-like patterns, but a pre-patterned ECM confines this behavior to the intersegmental space and channels it into organized sprouts. Reducing ECM stiffness slowed sprout progression and promoted fusion between adjacent ISVs. In agreement with these predictions, combined knockdown of laminin and fibronectin produced errant ISV pathfinding, ectopic branching, fusion events, and network-like endothelial arrangements. Re-expression of fibronectin using chimeric fibronectin mRNA restored ISV organization in the knockdown background, supporting that the severe disorganization reflects loss of fibronectin-dependent function rather than nonspecific injection effects. Together, our results identify laminin and fibronectin as cooperative guidance cues during angiogenesis and support a model of guided self-organization, in which patterned ECM, together with chemical guidance cues, constrains an intrinsic endothelial tendency toward network formation to generate reproducible vessel architecture in vivo.

428

Compartmentalization and synergy of osteoblasts drive bone formation in the regenerating fin

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Topic

5 Tissue Formation

Abstract Content

Background: Zebrafish fin regeneration depends on coordinated communication and functional specialization of distinct cell populations. Despite its remarkable capacity to restore complex and patterned structures, including bone, the cellular organization and lineage relationships that drive regeneration remain not fully defined.

Methods: We combined single-cell RNA sequencing (scRNA-seq) with fluorescence-activated cell sorting (FACS), lineage tracing, targeted osteoblast (bone forming cell) ablation, and electron microscopy to analyze regenerative epidermal, osteoblast, and non-osteoblast blastemal cells to decipher their transcriptional state and lineage relationships during bone formation and tissue patterning.

Results: Single-cell transcriptomics revealed previously uncharacterized subpopulations and signalling pathways involved in bone regeneration. Combined with RNA in situ hybridization our analysis uncovered defined mesenchymal and epidermal tissue compartments in the regenerate. In particular, distal blastema-associated and bone forming domains were distinguished by region-specific marker gene expression. Lineage analyses showed that the majority of osteoblasts in the regenerating fin derive from *osterix*$^{+}$ osteoblasts, while *mmp9*$^{+}$ cells contribute to a limited number of joint cells. In addition, a distal blastema population appears to give rise to osteoblast progenitors, indicating spatial compartmentalization during appendage regeneration. Functional perturbation demonstrated that ablation of *osterix*$^{+}$ osteoblasts impairs bone matrix formation and disrupts joint patterning in the proximal regenerate, and that distal regions partially compensate for the proximal loss through increased Wnt signalling activity. In contrast, ablation of joint cells did not prevent joint formation. Regenerative growth and patterning further showed differential dependence on Fgf signalling across cell populations.

Conclusion: These findings (https://doi.org/10.1016/j.isci.2024.108841) demonstrate that fin regeneration is driven by distinct, interacting cell populations with defined lineage relationships and spatial organization. The observed plasticity and compensatory mechanisms provide insight into the regulatory principles underlying successful bone regeneration.

429

Analysis of Regulative Elements Associated with Mutations of SLC26A4 Causing CEVA Inner Ear Malformations

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Hearing loss (HL) is one of the most common sensory disabilities, affecting approximately 1–6 out of every 1,000 newborns. Congenital hearing loss is caused by genetic abnormalities leading to inner ear malformations (IEM). SLC26A4 (pendrin) is an anion exchanger expressed in the inner ear, thyroid, mucus, and β-intercalated cells in the kidney. Mutations in *SLC26A4* cause a common form of IEMS: enlarged vestibular aqueduct (EVA), often accompanied by incomplete partition type 2 (EVA/IP2). However, only 25% of patients have confirmed mutations in both copies of *SLC26A4*. It has been reported that a group of polymorphisms upstream of the *SLC26A4* gene, the CEVA (Caucasian EVA) haplotype, is frequently found in patients with monoallelic *SLC26A4* mutations. These may affect the regulatory elements of SLC26A4, the enhancers and insulators.

Zebrafish offer an ideal model due to their transparent larvae, accessible inner ear, and rapid vestibular behavior onset. We started an evaluation of the *SLC26A4* upstream regulatory elements using the Tol2-mediated transgenesis. So far, we identified the carriers of the two SLC26A4-related polymorphisms expressing EGFP in the facial epidermis, branchial arches and kidneys. These and other results will be presented at the meeting.

432

A Novel Zebrafish Luminescent Biosensor for Kidney Tubulopathy,Metal Toxicity, and Drug Screening

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Topic

1 Disease Models

Abstract Content

Background

An efficient endolysosomal pathway is crucial to mediate the reabsorption and processing of ultrafiltered solutes including low-molecular-weight (LMW) proteins by epithelial cells lining the proximal tubule (PT) of the kidney. The zebrafish pronephros is used as a model system for congenital or acquired disorders that impair endolysosomal processing in PT cells, causing an inappropriate loss of solutes and LMW proteins in urine. We previously developed a transgenic *½vdbp-mCherry* reporter zebrafish line for monitoring LMW proteinuria based on quantifying the vitamin D-binding protein (VDBP)-mCherry in urine by ELISA. Although this line was validated to assess PT dysfunction in endolysosomal disorders, its scope and applicability is limited due to narrow dynamic range, cost, and time-intensive nature of the assay.

Methods

Transgenic zebrafish line is generated after injection of expression vector cloned by using multisite Gateway cloning technology into one-cell stage eggs. NanoLuc luciferase activity was assessed in presence of both luciferin furimazine and hikarazine Z108.

Results

We generated a new reporter *½vdbp-NanoLuc* zebrafish line, in which vitamin D-binding protein is coupled to NanoLuc luciferase for detection of PT dysfunction and LMW proteinuria. We demonstrate the reliability and value of the *½vdbp-NanoLuc* biosensor in fish models of monogenic endolysosomal diseases, gentamicin and cisplatin-induced nephrotoxicity, and metal contamination. This novel reporter system yields mechanistic insights into cadmium- and copper-induced PT dysfunction and provides a platform for drug screening.

Conclusion

Our studies demonstrate the value of the *½vdbp-NanoLuc* zebrafish system for congenital and acquired dysfunction of the kidney proximal tubule.

433

Resident macrophage dynamics and function during zebrafish cardiac development

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Topic

4 Cardiovascular

Abstract Content

Resident macrophages arise from the yolk sac and play crucial roles during tissue development and function. In the mammalian heart, resident macrophages have been reported to interact with various cell types including cardiomyocytes and endothelial cells. However, the earliest timepoint at which macrophages are present in the vertebrate heart and their role(s) during cardiac development remain unknown. Previous reports have described a variety of macrophage populations in the developing zebrafish heart; however, their localization and role(s) during cardiac development remain to be determined. Here, by taking advantage of the optical clarity of zebrafish embryos and larvae, we observed the presence of resident macrophages in the developing heart as early as 24 hours post-fertilization (hpf). These macrophages lie both inside and outside the heart and are in close contact with cardiomyocytes. They can be observed from 24 hpf until 60 days post-fertilization, and based on single cell transcriptomic analysis, they can be classified into three populations, i.e., a *mfap4*$^{+}$ population, a *mpeg1.1*$^{+}$ population, and a *mfap4*$^{+}$; *mpeg1.1*$^{+}$ population. Ongoing studies using the *irf8* mutants, which display a reduction in the number of cardiac resident macrophages at embryonic and early larval stages, will provide insights into the functions of resident macrophages during early cardiac development and help identify their interacting cell types. Preliminary RNA sequencing analysis of *irf8* mutant hearts at 120 hpf reveals a down regulation in adrenergic signaling, which is critical for cardiomyocyte contractility. Together, these results show the presence of resident macrophages throughout the early stages of zebrafish cardiac development, and suggest the presence of distinct populations of macrophages. Further research will elucidate whether resident macrophages influence cardiac function and cardiomyocyte contractility.

434

Investigating the role of epigenetics in development and aging of the short-lived killifish

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

Aging is a complex phenomenon, characterized by various processes: the hallmarks of aging. One of these hallmarks is epigenetic alterations, including changes in chromatin accessibility, histone modifications and DNA methylation. These alterations play a crucial role in regenerative capacity as well as in aging-associated diseases including cancer and neurodegeneration.

The dynamics of DNA methylation are controlled by DNA methyltransferases and TET enzymes as the main players in active DNA demethylation. However, the physiological role of TET enzymes, especially in the context of aging, remains largely unknown.

To study the effects of DNA methylation levels on development, life span, health span and regeneration we are employing the short-lived killifish *Nothobranchius furzeri*, a freshwater fish inhabiting seasonal ponds in southeastern Africa. To overcome the dry season, the developing embryos can enter diapause, an arrest in development while remaining metabolically active. At the onset of the rainy season the embryos develop further until hatching state. After hatching the fish reach sexual maturity quickly and furthermore have a short lifespan showing typical signs of aging.

We successfully applied the CRISPR/Cas9 system and generated *tet1*, *tet2* and *tet3* mutant fish. Currently we are characterizing the respective lines, including life span experiments, analysis of development, regenerative capacity, and aging phenotypes. Interestingly, we observed that homozygous *tet1- knockout* animals are viable into adulthood but appear at sub-Mendelian ratios.

In contrast to the findings in *tet1* mutant *N. furzeri*, we observed homozygous *tet3 knockout* fish to appear in Mendelian ratios. This finding contrasts with published *tet3 knockout* mouse lines where embryonic lethality of homozygous *tet3* mutant mice was reported. *Tet3* homozygous *knockout N. furzeri* survive into adulthood and appear outwardly normal and healthy. However, *tet3* mutants have a reduced lifespan. We are currently performing *omics* analysis to identify the molecular basis for the apparent premature aging.

436

A multiplexed CRISPR/Cas9 screening pipeline to identify novel determinants of brain asymmetry

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Topic

3 Neurobiology

Abstract Content

The zebrafish epithalamus has proven to be an excellent tractable genetic model for understanding the decisions required to establish left- and right-sided character in the brain. Detailed embryological and forward genetics approaches have revealed that early axis determination is transmitted to the epithalamus via Nodal signalling, which in turn directs the asymmetry-breaking migration of the parapineal through FGF signalling. An as-yet-unknown signal from the parapineal inhibits a Cachd1-Wnt receptor complex in progenitors, promoting early neurogenesis on the left side. The subtype fate of the post-mitotic neurons is determined through a combination of Wnt signalling and the timing of neurogenesis: early born neurons tend to differentiate into lateral subtype neurons, predominant on the lefthand side.

Many of the genetic determinants underpinning this model are unknown. We have developed a reverse genetics screening pipeline to elucidate key genes and interactions that are necessary for lateralisation. We identify target genes through analysis of bespoke transcriptomes of epithalamic progenitors and habenula neurons at key stages in development and use multiplexed CRISPR/Cas9 to knock them out in sensitised transgenic backgrounds, validating the genetic lesions through next generation sequencing. Screening of a lateralised transgenic reporter indicates potential hits which are further examined by KASP genotyping and automated imaging of key marker gene expression (using a VAST Bioimager and spinning disk confocal) to generate high quality quantitative phenotype-genotype data. We have validated this method by knocking out genes with known roles in asymmetric development, for example lrp6 and axin1, and are identifying promising new targets.

Stable mutant lines will be generated for asymmetry genes identified in the screen in the future and we will use a combination of functional imaging and behavioural assays to better understand their function and the role of laterality in the central nervous system.

437

RNAquarium: Repository-Scale dual RNA-seq analysis identifies novel zebrafish viruses and illuminates host biology

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Topic

15 Infection and Immunity

Abstract Content

Most RNA-seq studies discard non-host reads, but what if overlooked insights are hidden in this biological “flotsam”? Previously, Balla et al. described a novel picornavirus and confirmed it in multiple public RNA-seq datasets, hinting at more undiscovered viruses. This hidden infection landscape may reshape host transcriptional states and confound functional genomics, yet no organismal-scale resource has linked pathogen discovery to host expression across the archive. We have created a dual-processed host–virome atlas of over 75,000 zebrafish SRA RNA-seq datasets, generating paired matrices of host gene expression and viral abundance.

While our pipeline recovers millions of non-host transcripts, we focus on the host+virome atlas containing ~75,000 zebrafish transcriptomes spanning diverse biological states along with ~180,000 viral transcripts spanning their full taxonomic breadth. We examined the transcriptomes by pretraining a deep model on this corpus. In its embedding space genes cluster by shared GO terms, and with few-shot learning on the Zebrahub dataset, the model predicts developmental stage and tissue identity, outperforming an XGBoost model. From the viral transcripts, we curated 27 likely fish-associated viruses, including 16 novel lineages, with evidence ranging from rare single-project transcripts to detection of a large poxvirus genome (>300 kb) across hundreds of datasets. Among viral discoveries are two influenza-like viruses, including a novel Influenza Z virus that may be a mosaic of internal and surface proteins from different ancestors. We also leveraged our paired dataset to characterize systematic zebrafish transcriptional changes associated with viral presence, confirming that presence of viruses we flag as likely fish-associated is linked with expression shifts in an innate immune program.

We have released our rich corpus of zebrafish transcriptomes and the pathogen-derived contigs detected within as an interactive online data portal, providing a community resource for interpreting infection as a hidden variable in zebrafish biology.

438

Drug repurposing in zebrafish identifies mitochondrial remodeling as a therapeutic target for COL6 myopathies

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Topic

2 Emerging Technologies

Abstract Content

Background
Collagen VI-related myopathies (COL6-RM) are a heterogeneous group of genetic disorders caused by mutations in the genes coding for collagen VI, a key component of the extracellular matrix. These conditions are associated with progressive muscle weakness that compromise life expectancy. Our previous studies in patients affected by COL6-RM, as well as in collagen VI knockout (COL6 KO) mouse and fish, have demonstrated mitochondrial defects in muscle fibers that ultimately lead to myofiber apoptosis and muscle wasting.

Methods
We tested over 300 FDA-approved compounds to evaluate rescue of motor function in COL6 KO fish embryos by measuring spontaneous coiling at 24 hours post-fertilization (hpf) and swimming performances at 5 days post-fertilization (dpf). Among the tested compounds, formoterol—previously described as a modulator of mitochondrial dynamics—showed beneficial effects. Mitochondrial content, assessed by crossing COL6 KO fish with the *Tg(Hsa.Cox8a:MLS-EGFP)* reporter line, showed no significant difference in mitochondrial DNA and in the expression of mitochondrial markers. We therefore characterized mitochondrial network organization of 48-hpf wild-type and COL6 KO embryos by confocal microscopy imaging with Leica Stellaris SP8 and AI-based Aivia software analysis. Mitochondria were segmented and classified according to their organization (isolated, associated, or complex network), and isolated mitochondria were further categorized by shape.

Results
COL6 KO zebrafish exhibited defective mitochondrial respiration, indicating impaired mitochondrial function, which was restored by formoterol treatment. This functional deficit was not associated with reduced mitochondrial content but rather with alterations in mitochondrial network organization, and these alterations were significantly improved by formoterol treatment.

Conclusion
Our results indicate that formoterol promotes remodeling of the mitochondrial network in COL6 KO fish, likely through transcriptional regulation of genes involved in mitochondrial dynamics and turnover, including *tfeb*, *drp1*, and *mffa*. Formoterol

treatment leads to a decreased abundance of isolated and swollen mitochondria COL6 KO fish, ameliorating mitochondrial function.

440

In vivo characterization of an orphan E3 ubiquitin ligase using zebrafish models

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Topic

1 Disease Models

Abstract Content

Protein homeostasis is essential for maintaining cellular function and relies on autophagy and the ubiquitin-proteasome system. Ubiquitylation is mediated by E3 ubiquitin ligases, which play key roles in regulating diverse cellular processes. The PA-TM-RING E3 family comprises 11 ubiquitin ligases characterized by three conserved domains: a protease-associated domain (PA), a transmembrane (TM) domain, and a RING domain, responsible for ubiquitin ligase activity. Here, we focused on RNF150 (RING Finger Protein 150), a poorly characterized member of this family. RNF150 expression is upregulated in neuroblastoma, where its expression correlates with poor prognosis. RNF150 is well conserved across vertebrates, with 68% sequence identity between the human and zebrafish proteins. This project aims to establish a zebrafish knockout model to investigate the in vivo biological function of RNF150. Due to the presence of two paralogous genes, *rnf150a* and *rnf150b*, in the fish genome, both genes need to be knocked out to achieve a complete loss-of-function model. Using CRISPR/Cas9, we first generated two single-knockout lines, one for each paralog. Deletions introducing premature stop codons were confirmed by sequencing, and reduced mRNA levels were verified by real-time PCR. Phenotypic characterization revealed distinct morphological alterations in the two lines, including differences in growth and craniofacial development, as assessed by morphological analysis and Alcian Blue staining, suggesting that each paralog may have distinct biological functions in different tissues. The double knockout model is currently under selection. Overall, this study highlights the value of zebrafish models for elucidating the biological roles of E3 ligases, many of which remain poorly characterized and lack identified substrates or defined functions.

441

FORGE: an evolutionary algorithm to create synthetic enhancers.

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Topic

4 Cardiovascular

Abstract Content

Targeted gene expression is constrained by the limited availability of compact regulatory elements with predictable tissue specificity. Here, we present FORGE, an interpretable computational pipeline for designing synthetic functional DNA elements from public regulatory datasets. Although FORGE is broadly applicable across sequence classes and biological contexts, we apply it here to the design of synthetic cardiomyocyte enhancers. FORGE identified sequence features associated with cardiomyocyte-specific activity and generated compact enhancer candidates. To experimentally test these designs, we used Sidewinder, a novel DNA assembly method for rapid *in vitro* synthesis of synthetic regulatory sequences. Using this combined approach, we generated a panel of synthetic cardiomyocyte enhancers and tested their activity in zebrafish embryos. Several candidates drove robust and restricted expression in cardiomyocytes, demonstrating that computationally designed regulatory elements can achieve tissue-specific activity *in vivo*. Together, these results establish an integrated platform for the design, synthesis, and validation of synthetic cardiac enhancers and provide a foundation for the broader development of functional DNA elements.

443

Impact of the Gut Microbiome on the Functional Development of the Enteric Nervous System

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Topic

3 Neurobiology

Abstract Content

Background

The gut brain axis is a dynamic, bidirectional interplay between the gut microbiome, the enteric nervous system (ENS), and the central nervous system. The ENS not only regulates critical gastrointestinal functions such as peristalsis, immune modulation, and nutrient absorption but also communicates with the brain via endocrine, immune and neuronal signaling, including the vagus nerve. While several correlative studies have implicated the gut-brain axis in mental health, gastrointestinal disorders often accompany neurological disorders. Despite the central role of the ENS in the gut-brain axis, its specific contribution to these disorders remains poorly understood.

We hypothesize that the ENS, a key relay centre within the gut-brain axis, is also affected in neurological disorders, potentially explaining the frequent co-occurrence of gastrointestinal issues in such disorders.

Method

We are using zebrafish to study the functional development of the ENS *in vivo* and how the gut microbiome influences it. Using light-sheet microscopy, and *Tg(HuC:GCaMP6s)*TLN, we have imaged the entire ENS activity from 3 to 7 days post-fertilization (dpf) in the presence, and absence (germ-free), of the microbiome.

Results and Conclusion

In conventionalized larvae, the ENS activity intensifies from 3 to 6 dpf before declining at 7 dpf, highlighting a critical developmental period. Interestingly, germ-free larvae exhibit perturbed ENS activity, suggesting that the microbiome plays a role in shaping ENS development. Additionally, we are conducting single-cell transcriptomic analysis of the ENS and other gut cell populations to gain molecular insights into how the gut microbiome influences healthy ENS development.

444

Biofabricated Vascularized 3D Models for Translational Nanomedicine and Drug Delivery

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Topic

2 Emerging Technologies

Abstract Content

Advanced in vitro models that accurately recapitulate vascular architecture, transport phenomena, and tumor microenvironments remain a critical bottleneck in translating nanomedicine and drug delivery strategies to in vivo systems. To address this, we develop a modular biofabrication platform combining volumetric bioprinting, advanced hydrogel chemistries, and nanozyme-enabled therapeutics to engineer physiologically relevant, perfusable 3D tissue models.

Using volumetric printing of GelMA–PEG-based bioresins, we fabricate constructs with precisely controlled geometries and interconnected porosity, enabling enhanced transport beyond classical diffusion limits (~200 μm). Transport studies demonstrate strong dependence on molecular size, charge, and scaffold architecture, highlighting the importance of structure–function relationships in drug delivery systems. Complementarily, coaxially printed PEG-tyramine vascular conduits provide mechanically robust, elastic, and perfusable networks supporting endothelialization and angiogenic responses .

These platforms are further extended into vascularized tumor models, where spatially resolved nanoparticle–tumor interactions can be quantified. Using functionalized MnO_2 nanozymes, we demonstrate that nanoparticle surface chemistry critically governs penetration, catalytic activity, and therapeutic efficacy in 3D effects not captured in conventional 2D systems. Additionally, we develop cross-platform biomaterial inks (e.g., chitosan-based and nanozyme-initiated hydrogels) enabling tunable mechanical, biochemical, and responsive properties across multiple bioprinting modalities.

Beyond materials development, our work shows that geometry, curvature, and confinement directly regulate endothelial organization and tumor cell behavior, providing mechanistic insights into vascular biology and disease progression in engineered systems.

We envision these biofabricated platforms as a bridge between reductionist in vitro models and complex in vivo systems. In particular, their integration with zebrafish models offers a unique opportunity to validate vascular dynamics, nanoparticle transport, and therapeutic responses in a living organism with optical accessibility and genetic tractability.

We are actively seeking collaborations to couple our engineered vascularized models with zebrafish-based assays, enabling multi-scale validation of drug delivery, tumor progression, and vascular remodeling.

447

Investigating quorum sensing molecule c2-HDA and its role in Vibrio cholerae's interactions in the zebrafish gut microbiome.

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Topic

1 Disease Models

Abstract Content

Vibrio cholerae is an enteric pathogen which has been a persistent menace in human history. To date, developing nations face persistent cholera outbreaks. While major progress has been made to understand *V. cholerae* through the lens of its prime virulence factor, the cholera toxin, less progress has been made to understanding its interactions with the human gut microbiome. One of the ways by which bacteria communicate is via quorum sensing, where bacteria produce chemical messengers that regulate gene expression in a population dependent manner. One of such molecules is c2-HDA (cis-2-hexadecenoic acid), a diffusible signal factor that we have found to decrease the presence of certain T6SS (Type VI Secretion System) related proteins. The T6SS is a molecular nanomachine that is involved in inter- and intra-bacterial competition by penetrating an opposing cell's membrane and translocating toxic factors. We hypothesize that c2-HDA is involved the regulation of the T6SS and therefore the interactions with the gut microbiome. To investigate this, we use the zebrafish pathosymbiont, *V. cholerae* ZWU0020 and its known prey in competition, *Aeromonas veronii* ZOR0001 to study the c2-HDA and T6SS relationship in zebrafish. We conducted *in vitro* studies with *V. cholerae* to find that c2-HDA decreased T6SS activity, reducing its killing potential against *A. veronii*. We then use fluorescence microscopy to validate these findings *in vivo* with the zebrafish, imaging population changes in germ-free zebrafish larvae colonized with fluorescently marked *V. cholerae* and *A. veronii*. We established that c2-HDA can be toxic to zebrafish in combination with inflammation and obtained preliminary results regarding the impact of c2-HDA on V. cholerae mediated gut motility in zebrafish. Our research has highlighted c2-HDA as a promising member of the quorum sensing system for the gut microbiome dynamics, inhibiting T6SS activity, and that it may play a role in gut homeostasis.

450

When the gut turns blue: Investigating age-related intestinal changes in the short-lived killifish

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Topic

1 Disease Models

Abstract Content

Aging is a complex biological process involving multiple factors, including genomic instability, telomere shortening, stem cell exhaustion, and metabolic dysregulation. These factors can cause cellular malfunction. Subsequently, accumulation of malfunctioning or senescent cells can lead to chronic, low-level systemic inflammation, often termed “inflammaging”. In recent years, research has underscored the significant impact of intestinal health on overall health. Among other factors, intestinal barrier failure, also known as leaky gut syndrome (LGS), has been identified as a driver of inflammaging and a predictor of premature death. However, the age-dependent molecular mechanisms of changes in the intestinal permeability remain unclear despite LGS's impact on systemic health. We aim to address this knowledge gap by investigating the molecular mechanisms and genomic interplay of LGS in *Nothobranchius furzeri*.

To assess the proportion of individuals who have developed LGS, we applied a non-invasive method called "Smurf Assay". This method utilizes the short-lived vertebrate model *N. furzeri*, a striking model for vertebrate aging due to its rapid lifecycle and demonstration of major characteristics of vertebrate aging. We gathered longitudinal data on the development of LGS upon aging to ensure a robust comparison of permeability changes. Our data revealed clear evidence of age-dependent LGS development in *N. furzeri*. Following the assay, analysis of harvested organs confirmed dye trapping exclusively in the organs of affected individuals. Beyond phenotypic screening, transcriptome and proteome analyses of the intestines and livers revealed significant differences in expression patterns and the upregulation of senescence marker genes in response to LGS. This underscores the significant impact of intestinal health on systemic health.

Moreover, this method provides a scientifically robust platform for studying LGS development in *N. furzeri*. This research will provide insights into the molecular drivers of aging and contribute to strategies for mitigating inflammaging and improving overall health in future translational research applications.

456

Defining the developmental role of FGF19 and modeling ligand-dependent tumor signaling using complementary zebrafish xenograft systems

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Topic

8 Metabolism

Abstract Content

Fibroblast growth factor 19 (FGF19) is an endocrine fibroblast growth factor that regulates bile acid, glucose, and lipid metabolism and contributes to systemic tissue homeostasis; aberrant activation of FGF19-FGFR4 signaling has been implicated in tumor progression in several cancers. Although dysregulated FGF19-FGFR4 signaling is well recognized in cancer, the physiological role of FGF19 across developmental stages in vivo and the contribution of ligand-dependent FGF19 signaling to tumor behavior remain incompletely understood.

To investigate the physiological function of FGF19, we generated fgf19 knockout zebrafish using CRISPR-Cas9 and characterized their phenotype across developmental stages. While *fgf19*-deficient embryos and larvae showed no obvious abnormalities, homozygous juveniles exhibited growth restriction and reduced survival, particularly when raised together with heterozygous and wild-type siblings. Adult mutants were analyzed histologically, and the contribution of maternal *fgf19* RNA during early development was also examined, suggesting a stage-specific requirement for FGF19 during development and juvenile growth.

The fgf19 knockout line establishes a powerful in vivo model for uncovering how ligand-dependent signaling shapes tumor behavior. Although zebrafish xenograft models offer major advantages for rapid in vivo tumor studies, their predictive value can be limited by incomplete compatibility between zebrafish ligands and human tumor receptors. This challenge is very relevant for the FGF19-FGFR4 axis, which has been implicated as a pro-tumorigenic pathway in colorectal cancer and other cancer types and has emerged as a therapeutic target.

To test how ligand identity shapes tumor behavior and response to FGFR4-targeted therapy in ZTX, we compare wild-type zebrafish, *fgf19* knockout zebrafish, and a humanized zebrafish with FGF19 in a KM12SM colorectal cancer xenograft model treated with FGFR4-targeting compounds. Together, these studies define the developmental role of FGF19 and establish complementary zebrafish models that enable more accurate and predictive preclinical evaluation in precision oncology.

459

Translating Zebrafish Tumor Models for Precision Medicine within Immune-Oncology

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Topic

1 Disease Models

Abstract Content

Background
Cancer remains the second leading cause of death worldwide, and treatment failure is often driven by circulating tumor cells and micrometastases that escape conventional therapies. Immuno-oncology approaches, including immune checkpoint inhibitors (ICIs) and autologous T-cell therapies, have improved outcomes in metastatic cancer, but only a subset of patients achieves durable benefit. This highlights the need for rapid and predictive preclinical models to support precision immunotherapy selection. The zebrafish patient-derived xenograft (zPDX) model offers a promising in vivo platform for real-time evaluation of tumor behavior and treatment response.

Methods
We developed a translational workflow combining an in vitro JEDI system with an in vivo zebrafish xenograft model. In the JEDI system, GFP-specific mouse T cells were co-cultured with GFP-expressing 4T1 tumor cells, and T-cell activation and tumor killing were quantified by Aurora spectral flow cytometry. In the zebrafish model, dissociated GFP-positive tumor cells were implanted into 2 days post-fertilization embryos, while DiI-labeled T cells were injected into the circulation. Longitudinal imaging was used to monitor T-cell–tumor interactions over time.

Results
This platform enabled dynamic visualization of T-cell trafficking, tumor cell distribution, and treatment-associated changes in vivo. The combined in vitro and in vivo approach provided a feasible framework to assess T-cell-mediated tumor killing and to examine the effects of immunotherapy in a rapid and scalable model system.

Conclusion
Our study establishes a zPDX-based strategy for evaluating immunotherapy response in real time. By integrating functional T-cell assays with longitudinal zebrafish imaging, this approach may improve preclinical testing and support more precise patient selection for future cancer immunotherapies.

460

Integrative Analysis of *dhx37* Reveals a Conserved Role in Germline Identity and a Potential Function in Zebrafish Sex Differentiation

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Topic

9 Germline and Early Development

Abstract Content

Background

The DEAD-box helicase *DHX37* plays a conserved role in germline maintenance and sex differentiation in vertebrates. However, its expression dynamics and cellular distribution in zebrafish (*Danio rerio*) remain unclear. We performed an integrative analysis to determine whether *dhx37* is also expressed in germline and gonadal somatic cell niches, thereby extending its known expression profile during early development and adult spermatogenesis.

Methods

We analyzed zebrafish single-cell RNA-seq datasets from early development (7–120 hours post-fertilization, hpf) and adult gonads (5–27 months) using Seurat and SCpubr. Additionally, we reanalyzed RNA-seq data from isolated primordial germ cells (PGCs; GEO: GSE122208) and incorporated novel experimental data from adult zebrafish testes undergoing spermatogenic regeneration after 17β-estradiol-induced arrest.

Results

dhx37 showed strong expression in germline stem cells (32.5% of cells, mean expression 0.53) and undifferentiated spermatogonia (15.1%, mean 0.14). Notably, it was highly expressed in PGCs at 7–12 hpf (47.1%, mean 0.54) and 14–120 hpf (54.7%, mean 0.38). After converting the data to transcripts per million (TPM), we observed a dynamic increase in *dhx37* expression from the shield stage (6 hpf: mean 1.657 TPM) to the 3-somite stage (11 hpf: 31.296 TPM), peaking at the prim-5 stage (24 hpf: 45.264 TPM). In our experimental data, *dhx37* was predominantly expressed in early spermatogonia A (57%, mean 0.24) and B (17.9%, mean 0.15), but minimally in spermatocytes and somatic cells (<2%), suggesting a role in germline maintenance during regeneration.

Conclusion

Our findings establish *dhx37* as a conserved marker of germline identity in zebrafish, with potential implications for sex differentiation and determination. This work provides a foundation for future studies on the regulatory mechanisms of *dhx37* in vertebrate reproduction.

462

A Multi-System Pathogenicity Assessment Pipeline: Validating Desmin VUS via Tissue Culture, Zebrafish and Xenopus laevis.

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Topic

1 Disease Models

Abstract Content

Background: Variants in the DES gene, which encodes the intermediate filament protein desmin, are associated with cardiomyopathy and myofibrillar myopathy. However, many remain classified as variants of uncertain significance (VUS), making clinical interpretation limited. To address this, we established a cross-species pathogenicity assessment pipeline designed to rapidly assess DES variants and prioritize the most disruptive candidates for downstream long-term mechanistic validation.

Methods: The first stage of our setup uses desmin-null cells alongside zebrafish and *Xenopus* models as rapid screening platforms. In cells, we examine the impact of desmin variants on filament assembly and aggregate formation, while transient in vivo expression in zebrafish and Xenopus enables rapid evaluation of skeletal muscle and cardiac phenotypes. This rapid cross-model initial screening allows efficient identification of high priority variants which will be advanced to the long-term phase of the pipeline, for functional and mechanistic validation by generating stable muscle cell lines and creating CRISPR/Cas9 zebrafish models.
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Results: Using this strategy, we assessed a DES VUS identified through the diagnostic services of biobank.cy CoE UCY and found clear evidence of pathogenicity in the initial screening phase. In cells, the mutated desmin failed to integrate into the filament network and instead formed cytoplasmic aggregates, a hallmark of desmin pathogenesis. In zebrafish and *Xenopus,* we observed severe and consistent cardiac and skeletal muscle defects, including impaired sarcomere disorganization, disrupted intersomitic boundaries, and defective cell attachment, as well as defective cardiac morphology and function. The preliminary findings validate our rapid assessment pipeline, demonstrating its use for identifying pathogenic DES variants, with our strongest hits now advancing into long term studies.

Conclusions: Together, this work establishes a scalable framework for the functional interpretation of DES variants and provides a powerful strategy to bridge genotype to phenotype in cardiomyopathy and myopathy, with potential applicability beyond desmin associated disease.

463

Phenotypic screening in zebrafish larvae for the identification of microalgae with lipid-reducing and appetite-suppressing effects, and comparative evaluation of high-fat diet induced obesity in adults

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Topic

17 Chemical Biology and Drug Discovery

Abstract Content

The zebrafish (*Danio rerio*) is a vertebrate model for studying metabolic regulation due to conserved endocrine pathways, genetic accessibility, and suitability for *in vivo* screening. It provides translational insight into human metabolic disorders, including obesity. Microalgae, rich in bioactive metabolites, may modulate energy balance, lipid metabolism, and appetite regulation. Combining zebrafish assays with microalgal bioprospecting and obesity models enables identification of conserved metabolic modulators relevant to human health.

Here, we demonstrate the potential of aqueous extracts derived from microalgae, as a source of bioactive compounds for advancing metabolic disease research. The phenotypic screening with the Nile red fat metabolism assay in zebrafish larvae demonstrated that aqueous extracts of *Nannochloropsis gaditana*, *Nannofrustulum shiloi* and *Porphyridium cruentum* lowered lipid accumulation > 40%. Screening for appetite reduction revealed that *Spirulina platensis* and *Tetraselmis striata* reduced the food intake of liposomes >30%. Bioactivity-guided molecular networking with liquid chromatography-tandem mass spectrometry identified compounds that could be responsible for bioactive properties.

In order to prepare proof of concept experiments in adult zebrafish, various protocols for diet-induced obesity were compared (diets with cholesterol, vegetable oil, animal fat, egg yolk or overfeeding). Our data indicated that overfeeding produced the most robust phenotype, corroborated by fat quantification through micro-computed tomography. However, the serum metabolome was able to discriminate between all feeding regimes. Currently, liver histology and qPCR analyses of lipid metabolism genes are ongoing, which will provide further insights into different obesity models.

Our findings highlight microalgae as sources of metabolic modulators and establish zebrafish diet-induced obesity models as a platform for *in vivo* validation of bioactive compounds relevant to obesity research.

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465

FUS Orchestrates Conserved Metabolic Rewiring Of The PPAR–carnitine Axis In ALS, Revealing Clinically Relevant Therapeutic Targets

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Topic

1 Disease Models

Abstract Content

Background

Mutations in the Fused in Sarcoma (FUS) gene are associated with a juvenile-onset form of Amyotrophic Lateral Sclerosis (FUS-ALS), characterized by rapid and severe motor neuron degeneration. In this study, we characterized a fus knockout zebrafish ***fus$^{-/-}$*** model that recapitulates key pathological features of ALS, including progressive paralysis, increased larval mortality, disrupted muscle fiber architecture, and compromised mitochondrial network integrity. To investigate the metabolic consequences of FUS loss, we adopted a multi-model approach encompassing ***fus$^{-/-}$*** zebrafish, ***Fus$^{\Delta NLS/+}$*** murine skeletal muscle, ***Fus***-depleted myoblasts, and edited mutant ***FUS*** human motor neurons.

Methods

Survival assay on zebrafish,Touch-evoked escape response (TEER) and locomotion assay,Drug treatments, Metabolomics, Immunohistochemistry zebrafish sections, Western Blot ,Mitochondrial activity measurements, qPCR.

Results

- Muscle dysfunction and mitochondrial defects in the fus-/- zebrafish at early stages
- Transcriptomic and metabolomic analysis of fus mutants point to defective fatty acid oxidation
- Integrated transcriptomic analysis of FUS-deficient in vivo and in vitro models recapitulates metabolic impairment
- PPAR-α rescue and Fenofibrate treatment rescues motor phenotype and extends lifespan in fus mutant larvae
- Metabolic profiling of juvenile FUS-ALS fibroblasts reveals mitochondrial dysfunction and impaired energy metabolism

Conclusion

These findings indicate that FUS plays a pivotal role in maintaining muscle and metabolic homeostasis,and that its dysfunction triggers energy imbalance and mitochondrial stress, processes that may contribute to the heterogeneity of FUS-ALS and point to metabolism-targeted therapeutic strategies.

466

Shared mechanisms in SCA36 and C9ORF72-ALS/FTD: Insights from novel zebrafish models

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Topic

1 Disease Models

Abstract Content

Background

Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), and Spinocerebellar Ataxia type 36 (SCA36) are progressive neurodegenerative diseases. While ALS and FTD primarily affect motor neurons and the frontal and temporal cortex leading to motor impairment and cognitive decline respectively, SCA36 mainly targets the cerebellum, with additional motor neuron involvement resulting in impaired motor coordination.

Despite these differences, C9ORF72-linked ALS/FTD and SCA36 are caused by similar intronic hexanucleotide repeat expansions located in the first intron of the *C9ORF72* and *NOP56* genes. These expansions lead to the formation of RNA foci and the production of toxic dipeptide repeat proteins (DPRs), including poly(Glycine-Proline) (polyGP). While a combination of gain- and loss-of-function mechanisms has been proposed in C9ORF72-linked ALS/FTD, their potential contribution to SCA36 remains largely unexplored.

Methods

We developed zebrafish models of C9ORF72-ALS/FTD and SCA36 to investigate the contribution of polyGP toxicity and gene loss-of-function to neurodegeneration, alone or in combination. Phenotypic, neuronal, locomotor, proteomic and metabolomic analyses were performed to identify disease-specific and shared pathways.

Results

Our results reveal distinct roles of polyGP across disease models. In SCA36, polyGP displays mild intrinsic toxicity that is strongly exacerbated by modulation of *nop56* expression. In contrast, in C9ORF72-ALS/FTD, polyGP toxicity appears to be primarily mediated through modulation of *c9orf72* expression.

Both models exhibit clear neurodegenerative phenotypes, and proteomic and metabolomic analyses identified overlapping pathways, suggesting convergent mechanisms of neurodegeneration.

Conclusion

This study reveals previously uncharacterized divergent and convergent mechanisms driving neurodegeneration in C9 ALS/FTD and SCA36. Our findings highlight the context-dependent role of polyGP and loss-of-function pathways in both diseases. We also establish zebrafish as a powerful in vivo model for uncovering complex pathogenic mechanisms. Comparative studies between C9 ALS/FTD and SCA36 provide a framework for identifying shared therapeutic targets, advancing therapy development for these untreatable conditions.

467

Phenotypic characterization of KCNT1 variants in a zebrafish model of developmental and epileptic encephalopathies.

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Topic

1 Disease Models

Abstract Content

Background
Developmental and epileptic encephalopathies (DEEs) are severe early-onset disorders characterized by drug-resistant seizures and neurodevelopmental impairment. Among the genes of interest implicated in this pathology, is KCNT1, which encodes a sodium-activated potassium channel ($K_{Na1.1}$) involved in the regulation of neuronal excitability. To date, 64 missense gain of function (GOF) variants were discovered and associated with altered channel function mostly in heterologous cell lines. However, the *in-vivo* consequences of distinct KCNT1 variants remain incompletely understood, hence the use of a zebrafish model providing valuable insights for rapid functional assessment of neurological phenotypes.

Methods
To investigate the functional impact of KCNT1 variants, zebrafish embryos (AB wild-type strain) were microinjected at the one-cell stage with plasmids encoding wild-type KCNT1 (WT) or two disease-associated variants (R428Q and R474C). Larvae were assessed for spontaneous locomotor activity at 1 and 2 days post-fertilization (dpf) respectively through tail coiling activity and touch-evoked escape response assays. Seizure susceptibility at 6dpf was evaluated following exposure to pentylenetetrazole (PTZ), a proconvulsant commonly used in zebrafish models.

Results

At 1dpf, both variant-expressing larvae displayed reduced activity compared to both controls. At 2dpf, R428Q larvae showed a decrease whilst R474C larvae exhibited an increase in spontaneous activity compared to both controls. At 6dpf, baseline activity showed an increase with R474C compared to all other genotypes. Following PTZ exposure, R428Q larvae demonstrated severe seizure susceptibility compared to a mild susceptibility in R474C. These findings indicate that distinct KCNT1 variants are associated with divergent behavioral phenotypes *in-vivo*.

Conclusion
This study establishes a zebrafish model for the comparative functional characterization of KCNT1 variants and reveals variant-specific behavioral alterations. These results highlight the importance of this approach for investigating the *in-vivo* impact of KCNT1 dysfunction and provide a foundation for future mechanistic studies and therapeutic screening in KCNT1-related DEEs.

469

Evaluation of Lichen-Derived SB5 as a Glioblastoma Therapeutic Agent Targeting Prohibitin in a Zebrafish Xenograft Model

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Topic

1 Disease Models

Abstract Content

Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by high invasiveness and resistance to standard therapies including surgery, radiotherapy, and temozolomide. Due to the high recurrence rate and poor prognosis of GBM, discovering novel bioactive compounds is essential. This study explores the therapeutic potential of SB5, a lichen-derived depside compound, as a candidate for GBM treatment. The anticancer effects of SB5 were first evaluated in vitro using U87 glioblastoma cells through MTT assays and qualitative toxicity assessments. To evaluate in vivo efficacy, a zebrafish xenograft model was employed. Fluorescently labeled U87 cells were microinjected into zebrafish larvae, and tumor progression was monitored following SB5 treatment. Furthermore, molecular docking analysis (PDB ID: 4E3G) was conducted to investigate the interaction between SB5 and its potential target, prohibitin (PHB). In vitro assays demonstrated that SB5 significantly inhibited U87 cell proliferation in a dose-dependent manner. Notably, the zebrafish xenograft model confirmed the in vivo potency of the compound, as SB5-treated larvae showed a substantial reduction in tumor volume and fluorescence intensity compared to the control group. Molecular docking revealed favorable binding affinity between SB5 and PHB, suggesting that the its mechanism of action is linked to the modulation of mitochondrial-associated pathways. Our findings demonstrate that SB5 exerts potent anti-tumor activity in both in vitro and in vivo zebrafish xenograft systems. By identifying PHB as a key molecular target, this study suggests that SB5 is a promising mitochondrial-targeting lead compound for the development of next-generation glioblastoma therapies.

471

Exposure route determines biodistribution and cardiovascular toxicity of polystyrene micro- and nanoplastics in zebrafish embryos

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Topic

10 Toxicology

Abstract Content

Micro- and nanoplastics (MNPs) can reach developing organisms through multiple exposure routes, yet most experimental studies rely on a single paradigm. Here, we investigated how particle size and exposure route jointly determine polystyrene particle biodistribution and developmental toxicity in zebrafish embryos, focusing on cardiovascular function.

Zebrafish embryos were exposed to 50 nm and 1 µm polystyrene particles (PS-MNPs) via three complementary routes: bath exposure (waterborne uptake), yolk microinjection (maternal nutrient-associated exposure), and blastomere microinjection (direct embryonic exposure). Fluorescently labelled particles were used to quantify biodistribution and whole embryo burden up to 96 hpf. Vascular development was assessed in Tg(fli1:EGFP) embryos by quantifying angiogenic area and skeletonized vascular network, while cardiac function was evaluated in Tg(myl7:EGFP) embryos through ventricular fractional area change, chamber-specific heart rate, and rhythm variability.

Particle biodistribution was dependent on exposure route and size. Bath exposure preferentially increased the internal burden of 50 nm particles, whereas yolk injection promoted retention and subsequent redistribution of 1 µm particles toward the yolk–intestinal region. Blastomere injection resulted in a broader embryonic distribution of both particle sizes. These route-dependent toxicokinetic patterns translated into distinct organ-specific outcomes. Vascular alterations were mainly detected following yolk exposure, with embryos exhibiting a simplified vascular network, together with reduced angiogenic area, particularly after exposure to 1 µm particles. Cardiac function was similarly affected, with yolk exposure emerging as the most disruptive route, reducing ventricular contractility for both particle sizes. In contrast, atrioventricular coupling showed route-specific and bidirectional changes, indicating that particle effects on cardiac rhythm depend on their internal distribution.

Our findings demonstrate that exposure route is a key determinant of PS-MNPs biodistribution and developmental toxicity. Route-resolved approaches are therefore essential to understand MNPs' hazard during early vertebrate development.

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472

High-content zebrafish imaging platform identifies a novel autophagy activator

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Topic

8 Metabolism

Abstract Content

Activation of adaptive autophagy is a promising strategy to promote cellular function and prevent the onset of age-related diseases. Transcription factor EB (Tfeb) is the master regulator of a network of genes, collectively known as CLEAR elements, involved in the process of autophagy and lysosomal functions, and thus an important therapeutic target for clearance of unwanted materials.

Many Tfeb studies to date have been performed in vitro, with important differences in Tfeb signaling depending on the cell-type. In particular, signaling cues that activate Tfeb in response to energy deprivation and other physiological challenges at the organismal level are less well-understood and require in vivo investigations. Transparency of zebrafish larvae facilitates the image-based measurement of Tfeb activation in vivo, a strategy that can be used to identify novel inducers of Tfeb. To this end, we developed and characterized a muscle-specific transgenic zebrafish reporter line by generating a reporter construct with the fluorescent protein ZsGreen tagged to Tfeb (*Tg(actc1b:Tfeb-ZsGreen)*).

A high-content image acquisition and analysis setup was used to screen natural bioactives on this line. By classifying the images of treated zebrafish larvae into responders and non-responders using an A.I.-based image analysis script depending on the presence and absence of Tfeb nuclei, respectively, we successfully identified a potent Tfeb activator, molecule A. Its beneficial effect was further explored in *Caenorhabditis elegans* models, where treatment with molecule A increased mobility in models of muscle aggregation, and decreased aging-related protein aggregates in naturally aged worms.

This new platform represents a rapid and efficient technology for the identification of new Tfeb inducers for potential development of human interventions. The findings from this study contribute to the understanding of *in vivo* Tfeb activation and its implications for muscle health and aging.

474

Investigating the mechanisms underlying the coordinated function of pluripotency transcription factors during embryonic development

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Topic

13 Epigenetics and Gene Regulation

Abstract Content

During early embryogenesis, the zygotic genome is silent, and growth is sustained by maternally provided resources. For gastrulation to occur, the embryo must begin to transcribe its own genome. Zygotic genome activation (ZGA) is triggered with a predictable timing by a restricted cohort of pluripotency transcription factors (TFs), which in zebrafish are Nanog, Pou5f3(Oct4) and Sox19b(Sox2) (NPS). NPS regulate ZGA in a coordinated manner, mutually promoting or restricting each other's activity. Here we take a multiscale approach to investigate NPS combinatorial binding and mutual dependencies in orchestratating developmental transcriptional programs.

At the genomic scale, we are generating single molecule footprinting maps of NPS binding during ZGA, to visualize their concomitant binding at adjacent sites on chromatin fibers. Exploiting the genetic tractability of zebrafish embryos, we will evaluate changes in TF co-binding patterns upon truncation of intrinsically disordered regions, known facilitators of TFs' subnuclear localization and target recognition. This will provide quantitative information on whether NPS interchangeably, exclusively, or concomitantly occupy co-regulated loci, and inform on which protein features guide their recruitment at shared targets.

At the developmental scale, we are investigating the requirements of the three factors for critical developmental hallmarks. Phenotypic analysis of the progeny of fish carrying different combinations of wt, heterozygous or homozygous NPS deletions revealed a complex genetic interaction network for the regulation of several key processes, including animal pole adhesion to the yolk cell, epiboly initiation and gastrula morphology. While there seems to be a clear separation of roles between the three factors, their ability to correctly operate appears to be calibrated by the presence and level of the other two.

Our work aims to mechanistically understand how NPS coordinate their mutual positioning at cis-regulatory elements during ZGA, ensuring that only the appropriate transcriptional programmes are activated with the correct timing and magnitude.