Assessing the differential impact of RAS mutations on tissue mechanics and tumour formation

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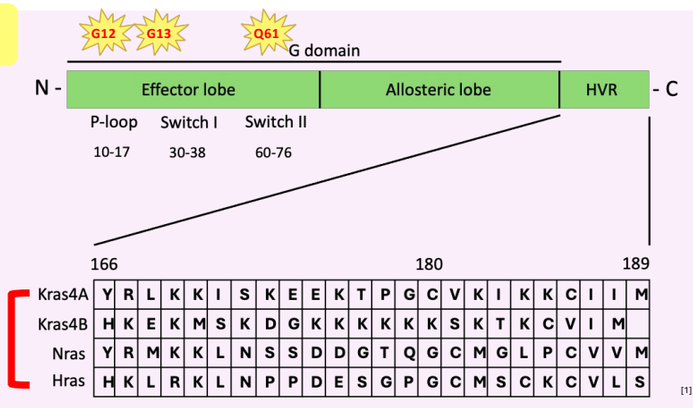
Background



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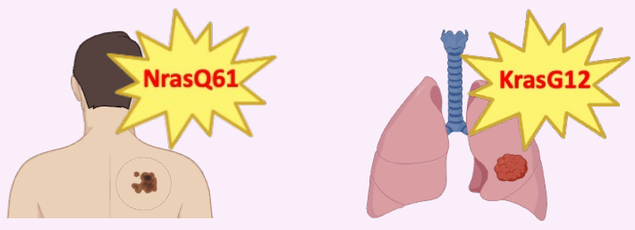
RAS mutational frequencies in cancer

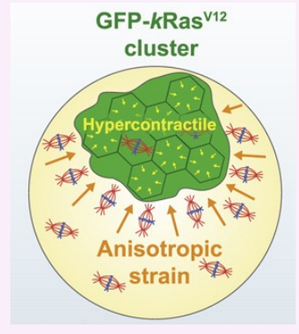


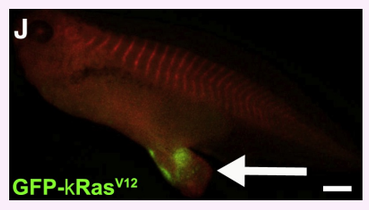
Melanoma
94% of RAS mutations are Nras

Lung cancer
96% of RAS mutations are Kras

[2]

RAS in mechano-regulation of cancer





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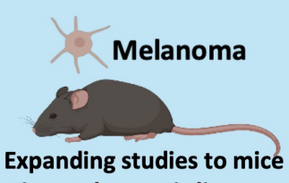
How do different RAS variants impact tissue mechanics and does this lead to different tumorigenic outcomes?

Aims

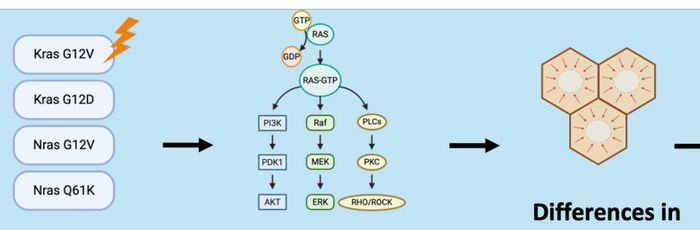
Examining Ras mutations in developing *Xenopus* embryos.



Expanding studies to mice using melanocytic lineages.

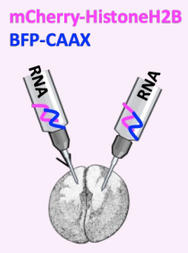
Exploring translational relevance of environment-specific mechanical cues.

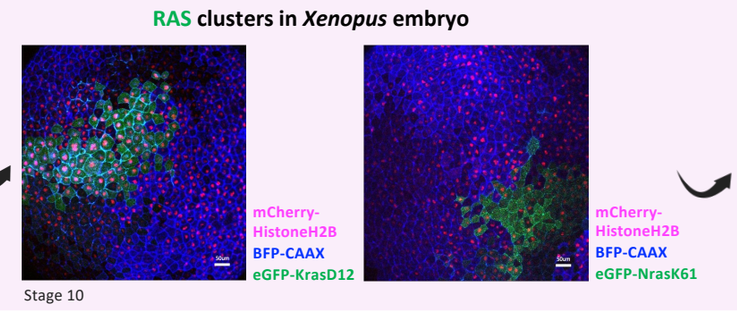
Hypothesis



Ras mutations → Distinct signalling networks → Differences in contractile force generation → Tumourigenesis in different tissue environments

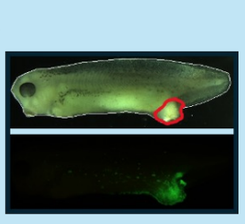
Workflow in *Xenopus laevis*



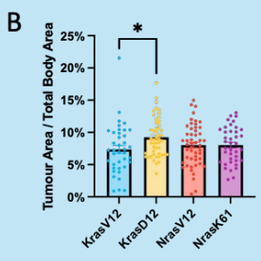


Key results

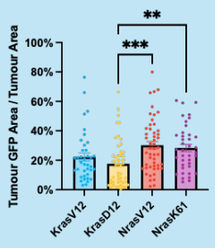
1. Different RAS variants produce subtly different tumours in terms of shape and cell content



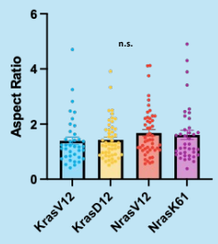
Demonstration image of how we calculated the ITLS (induced tumour-like structure) area.



A higher mean ITLS area as a percentage of body area was observed in GFP-KrasD12-injected embryos compared to that of GFP-KrasV12-injected ones.

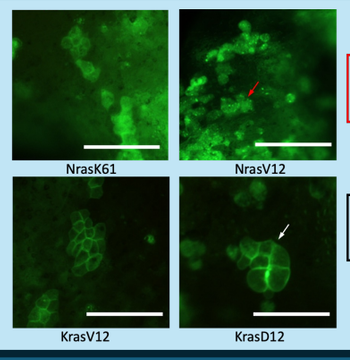


Nras-mutant ITLS had a significantly higher percentage of within-tumour GFP signals relative to tumour area, when compared to KrasD12.



Dot plot showing the mean length/width ratios of ITLS between all RAS mutant groups were not significantly different from each other. *p < 0.05, **p < 0.01, ***p < 0.001

2. Different RAS proteins have different cellular localisation

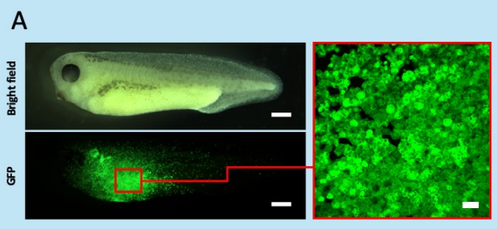


GFP+ intracellular particles

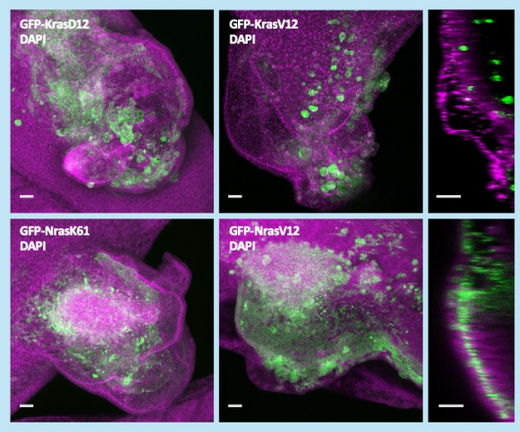
GFP at peripheral membranes

We observed an increase of GFP+ particle number in GFP-NrasV12-injected tumour cells at stage 38 (red arrow) compared to other RAS mutants (white arrow). Scale bars are 100µm.

3. Different RAS tumour cells might have different fates

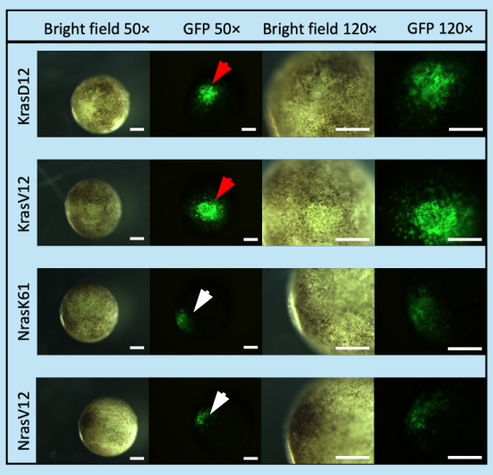


Injection of control GFP RNA into a single A4 blastomere results in a large proportion of fluorescent cells differentiating into epidermal cells with characteristic epidermal morphology. Scale bars are 500 µm (left panel) and 50 µm (right panel).



Confocal images of whole-mount stained ITLS showing that NrasV12 tumours are characterized by sheets of tumour cells exhibiting epidermal morphology, while other mutants are not. Scale bars are 50µm.

4. Differences in early oncogenic clusters

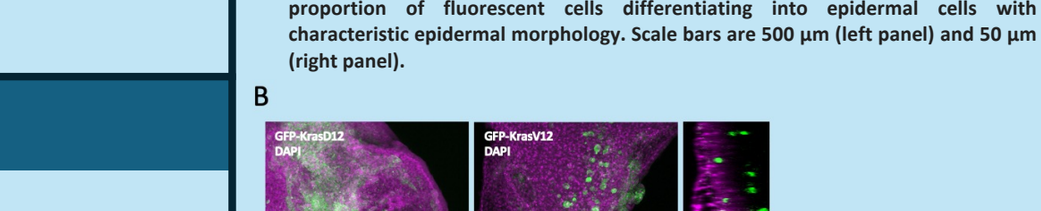


Bigger and centrally localised Kras mutant clusters

Smaller and peripherally localised Nras mutant clusters

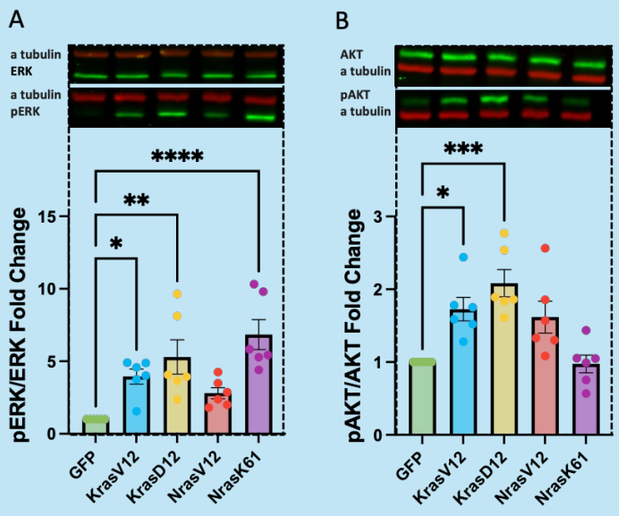
At stage 10, in GFP-KrasD12- and GFP-KrasV12-injected embryos, the clusters were generally bigger in size and located in the centre of the animal cap (red arrow). On the contrary, in GFP-NrasK61- and GFP-NrasV12-injected embryos, the clusters were generally smaller in size and located on the edge of the animal cap (black arrow). Scale bars are 400µm.

6. Mechanical differences between different RAS variants



(A) We adopted laser ablation as a direct measurement of junctional mechanical tension. We used a 355nm high-power laser to physically cut the cell edge at the sites of cell-cell adhesion labelled with mCherry-CAAX. The tension could be thus visualized as the outwards recoil of the vertices following ablation. XY coordinates of the two vertices were tracked in ImageJ using the MTrackJ plugin in ImageJ and fitted to a Kelvin-Voigt model [4].
(B) Cropped region of confocal time-lapse stills showing laser ablation at a cell edge in a GFP-KrasV12 cluster.
(C) Recoil measurements.
(D) The initial recoil rates within KrasV12 clusters ($p < 0.001$), KrasD12 clusters ($p < 0.01$) and NrasV12 clusters ($p < 0.05$) were significantly higher than in GFP clusters (Kruskal-Wallis with Dunn's tests).
(E) K values showed no significant differences between groups (Kruskal-Wallis with Dunn's tests), suggesting that initial recoil velocity can act as an indicator of local junctional tension.

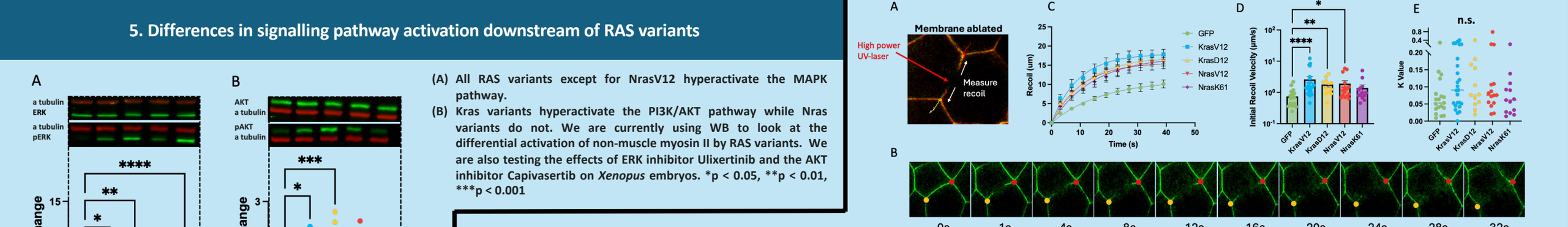
5. Differences in signalling pathway activation downstream of RAS variants



(A) All RAS variants except for NrasV12 hyperactivate the MAPK pathway.
(B) Kras variants hyperactivate the PI3K/AKT pathway while Nras variants do not. We are currently using WB to look at the differential activation of non-muscle myosin II by RAS variants. We are also testing the effects of ERK inhibitor Ulixertinib and the AKT inhibitor Capivasertib on *Xenopus* embryos. *p < 0.05, **p < 0.01, ***p < 0.001

Do melanoma cells with different RAS mutations respond differently in collagen matrices with varying stiffness?

Experiments in melanoma cell lines



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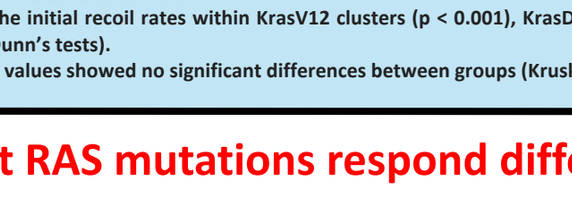
Conclusions

RNA injection of different RAS variants results in:

1. Subtle differences in tumour size.
2. Difference in RAS intracellular localisation.
3. Possible difference in cell fate during *Xenopus* embryos development.
4. Differential activation levels of MAPK and PI3K/AKT signalling pathways.
5. Differences in the level of contractile forces generated by cells.



Acknowledgements

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References

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