Biophysical mechanisms behind cancer initiation in 3D mammary breast acini

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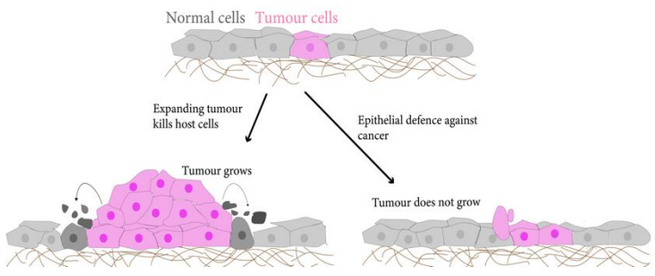
Introduction

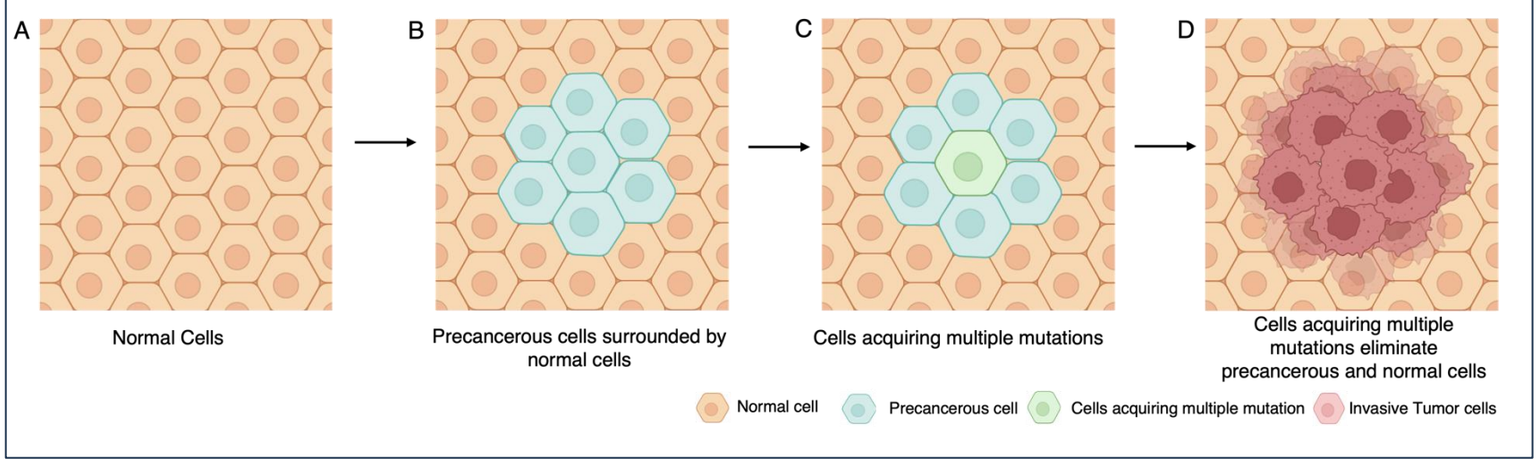
- Tumorigenesis is a multi-step process.
- Cells in an incipient tumor compete with those of the host tissue for their own growth and survival, and the outcome of this competition dictates the fate of the cancer[1].
- Such competition allows epithelial cells to recognize and remove misfit transformed cells- a phenomenon called **Epithelial Defense Against Cancer (EDAC)**[1].



Vishwakarma et al; Nat. Rev. Cancer. 2020

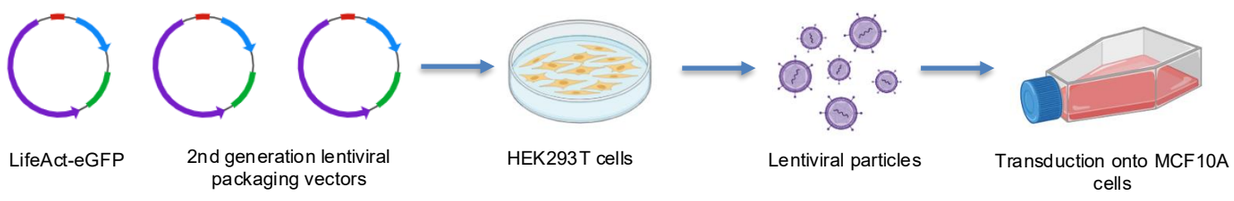
Key Question

- How does **mechanics of the tissue** change when normal cells undergo **accumulation of mutations** during breast cancer initiation? How does **tissue architecture, ECM mechanics, cell-generated forces, and mechano-transduction pathways** influence EDAC outcomes in 3D epithelial systems?



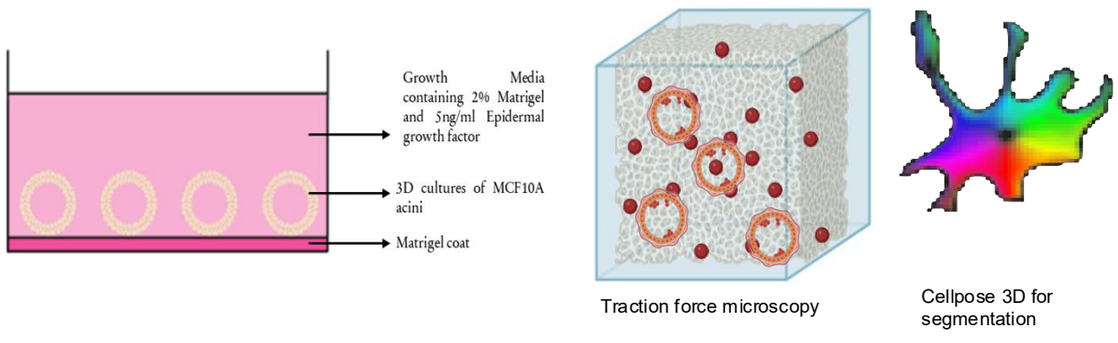
Methodology and Workflow

1. LifeAct-GFP MCF10A cell line to study actin dynamics during cancer initiation

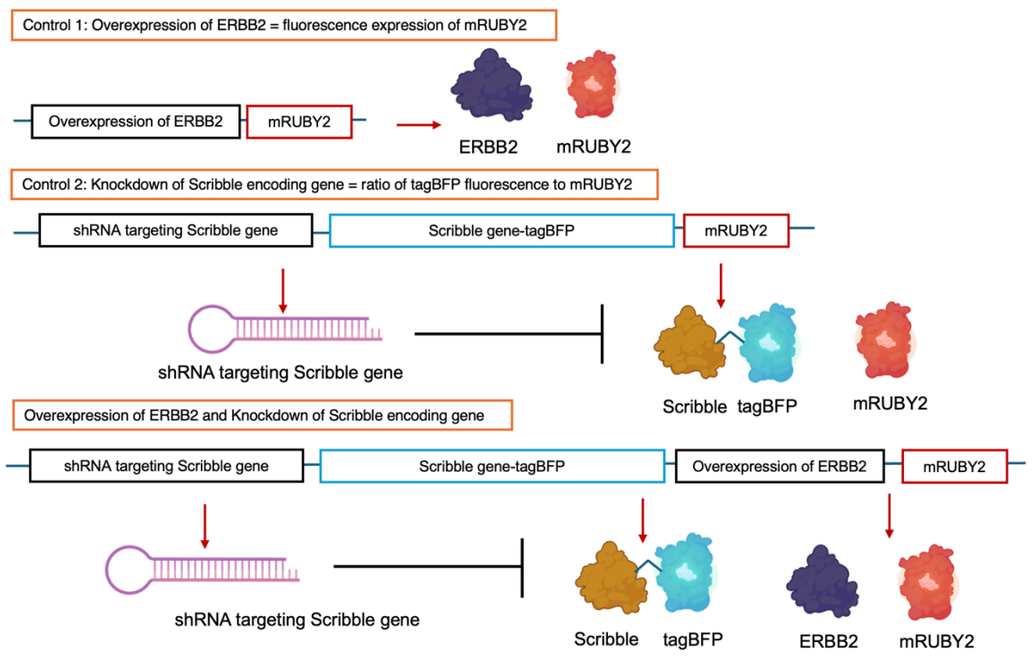


2. Establishing 3D cell cultures of acini, imaging for cytoskeletal markers and biophysical characterization

- 3D organotypic cultures of MCF10A Acini[4].
- Immunofluorescence staining for cytoskeletal biomarkers such as F-actin, E-cadherin.
- 3D cell segmentation using Cellpose and 3D force inference.
- 3D traction force microscopy to study forces during cancer initiation.



3. Cloning constructs for overexpression of ERBB2 and downregulating Scribble to study breast cancer initiation



Approach:

- To accumulate mutations in cells within 3D acini, we identified potent breast cancer genes .
- We aim to initiate cancer via the accumulation of mutations, which deregulate Akt signaling pathway and cell polarity by overexpressing ERBB2[2] (a receptor tyrosine kinase) and downregulating Scribble[3], respectively .
- We use this cloning strategy to transduce the expression of both the genes into MCF10A acinar cultures with different fluorophores and image them to study the fate of the mutants over time.

Results

1. MCF10A 3D Organotypic model of breast cancer initiation

Scale = 10 microns

2. Establishment of LifeAct-GFP MCF10A cell line to study cytoskeletal mechanics of breast cancer initiation

LifeAct–GFP

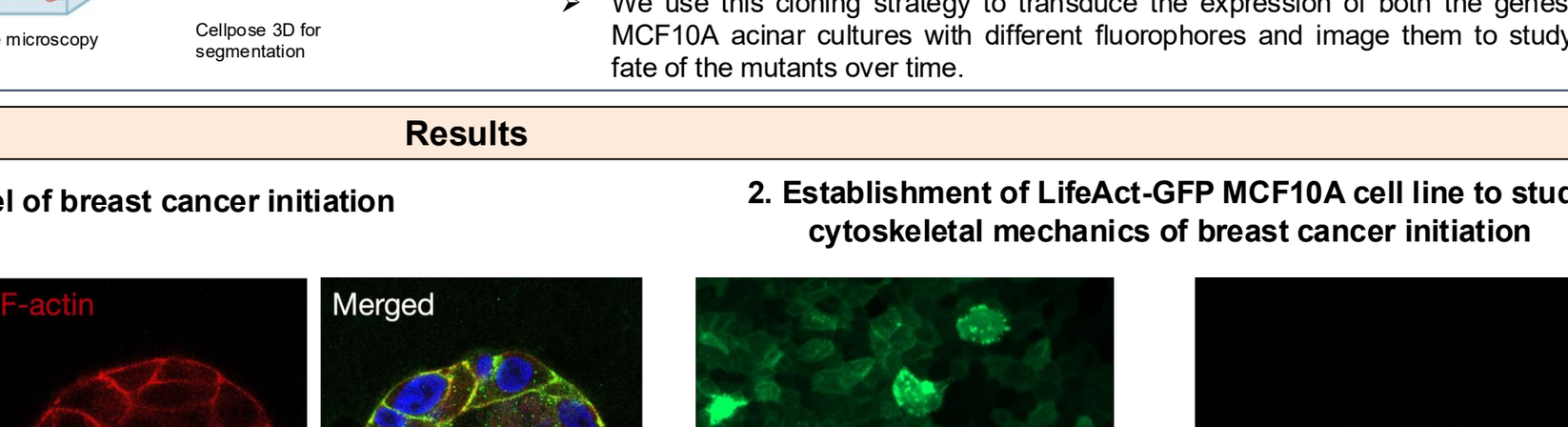
Negative control

Scale = 50 microns

3. LifeAct MCF10A cell line successfully forms acini

To validate, we have imaged **LifeAct-GFP** MCF10A organoids to visualize the acinar morphogenesis process.

1. Proliferation
2. Apicobasal polarization
3. Luminal cell death (Apoptosis)
4. Establishment of hollow lumen



References

1. Kajita, M., Sugimura, K., Ohoka, A. *et al.* Filamin acts as a key regulator in epithelial defence against transformed cells. *Nat Commun* **5**, 4428 (2014).
2. Leung, C., Brugge, J. Outgrowth of single oncogene-expressing cells from suppressive epithelial environments. Nature 482, 410–413 (2012).
3. Zhan, L. et al. Deregulation of Scribble Promotes Mammary Tumorigenesis and Reveals a Role for Cell Polarity in Carcinoma. Cell 135 (2008/11/28).
4. J, D., SK, M. & JS, B. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures - PubMed. Methods (San Diego, Calif.) 30 (2003 Jul).

Future directions

- To sporadically transfect invitro 3D acinar models with the developed construct along with its respective controls.
- To perform long term live imaging to study F- actin dynamics during breast cancer initiation.

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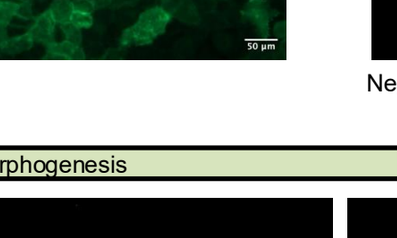
Meet the lab



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