Mechanomodulation of mutant clonal competition; relevance for early tumour formation.

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Principal supervisor's CRUK CC theme:

- Early Cancer Institute
- Thoracic Cancer Programme

Department for student registration: Department of Physiology, Development and Neuroscience

Department or institute where research will take place: Wellcome – MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre

MRes + PhD (1 + 3-year non-clinical)

MRes project outline:

Cancer is a deadly disease traditionally thought to initiate from the accumulation of genetic mutations in tissues. However, recent studies have shown that humans accumulate similar mutations to those found in cancer in their normal tissues over time. The question is then, how, despite the presence of these tumour mutations in our bodies, not all of us develop cancer. The answer resides in non-genetic environmental factors, which also contribute to tumour formation.

The aim of this project will be to investigate how non-genetic factors affect the mutant tissue. In particular, we want to explore the provocative idea that the physical interaction between different mutations present in the tissue creates an associated stress reaction decisive to establish whether a cancer initially forms. To do this, we will implement an interdisciplinary approach, combining genetic tracking of individual cells with long-term 3D cultures, to unveil the molecular mechanisms regulating cell behaviour in response to cancer mutations. These studies will the basis to establish prevention strategies based on manipulating mutant cells to limit the predisposition of high-risk patients of developing cancer in the first place.

MRes experimental plan:

In light of our previous work and preliminary observations, we propose that the physical collision between epithelial mutant clones stimulates a stress response, which ultimately shapes the dynamics of neighbouring clones promoting tumour formation. This represents a compelling hypothesis that may possibly reconciliate non-genetics aspects of early tumorigenesis, including the polyclonal nature of nascent tumours and the fact that tumour formation does not strictly depend on a set of given mutations.

In this rotation, we will aim at testing this hypothesis through two specific aims:
1- Define whether the collision between mutant clones leads to tumour formation.
To address this aim, we will make use of existing reporter mouse models, expressing fluorescent proteins that enable us to detect cells bearing mutations typically found in human cancers. Tissues from these mice will be grown using long-term 3D organ cultures to study mutant clonal competition.
2- How the mechanical stress resulting from epithelial mutant competition impact mutant clonal dynamics.
To this end, we will use of a similar approach to that used in Aim 1 but, in this instance, cells will

be treated with inhibitors that modulate the mechanical properties of the cells. The resulting data will determine the relevance of tissue mechanics in mutant clonal competition and early tumour formation.

In summary, this rotation will make use of traceable mouse lines, new long-term 3D cultures that recapitulate mutant clonal competition and tumour formation in vitro, confocal imaging, and an introduction to single-cell molecular characterization of cell states.

PhD project outline:

Mounting evidence shows that healthy human epithelial tissues accumulate cancer-associated mutations with age, revealing that mutations do not represent the sole cause of cancer. Instead, tumour formation is now believed to depend on a combination of genetic and environmental factors; with competition between adjacent mutant clones and changes in the mechanical properties of the mutated epithelium acting as critical modulators during early tumorigenesis. However, despite significant efforts in the cancer field, the exact events leading to the emergence of epithelial tumours remain largely unknown.

During this PhD project, we will aim at expanding on the MRes rotation to better understand how mutant clones synergise/compete to promote early tumour formation. In particular, we will expand our understanding how changes in the structural and mechanical properties of the mutant tissue impact on the stromal niche, promoting tumorigenesis. This represents multidisciplinary approach combining our expertise in tracing mutant clonal dynamics in vivo, a new long-term 3D culture approach, epithelial mechanobiology, single-cell molecular profiling, and mathematical network analysis in order to dissect the cellular and molecular mechanisms underlying mutant clonal competition.

This PhD project will have three specific aims:
1- Define how the mechanical stress resulting from epithelial mutant clonal competition impacts on the underlying stromal compartment and neighbouring clonal dynamics.
2- Investigate the molecular networks modulating epithelial clonal competition and their impact on early tumour formation.
3- Functional validation using 3D Organ cultures and CRISPR screenings

PhD experimental plan:

We hypothesise that the mechanical stress imposed by the clonal collision of epithelial mutant clones may be sensed by the underlying stroma, activating a wound healing-like response that would further favour the formation of tumours.

Here we will aim at testing this hypothesis through three specific aims:
1- Define how the mechanical stress resulting from epithelial mutant clonal competition impacts on the underlying stromal compartment and neighbouring clonal dynamics.
To this, we will investigate changes in tissue mechanics at stress areas localized at the boundary of mutant colliding clones, and explore the subsequent response of the underlying stroma. For this, we will use traceable mouse lines carrying mutations that mimic those typically found in the aging human oesophagus. Experiments will be performed using 3D cultures in vitro and in vivo validations.

2- Investigate the molecular networks modulating epithelial clonal competition and their impact on early tumour formation.
In order to gain mechanistic insight into the molecular networks modulating mutant clonal competition that may offer targets of potential clinical relevance to prevent tumour development,

growth and progression, we will perform spatiotemporal single-cell molecular profiling studies.

3- Functional validation using 3D Organ cultures and CRISPR screenings
The relevance of candidate targets (from Aim 2) will then be assessed in vivo and in vitro using 3D organ cultures (from mouse or human tissue, where relevant) placing particular interest on existing druggable targets with translational potential.
Main techniques:
-Long-term 3D organ cultures (Epithelioids)
-In vivo lineage tracing
-Live imaging of 3D cultures
-Single-cell molecular profiling (scRNA-seq, scATAC-seq)
-CRISPR screenings
-Confocal imaging

Key references:

-Colom, B. et al. Mutant clones in normal epithelium outcompete and eliminate emerging tumours. Nature 598, 510-514 (2021).

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-Alcolea, M.P. et al. Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change. Nat Cell Biol 16, 615-622 (2014).

-Bejar, M.T. et al. Defining the transcriptional signature of esophageal-to-skin lineage conversion. bioRxiv, 2021.2002.2019.431899 (2021).
1-Herms A#, Fernandez-Antoran D#, Alcolea MP#, et al. “Epithelioids: Self-sustaining 3D epithelial cultures to study long-term processes”. PREPRINT, bioRxiv, 2023, 522589, doi: https://doi.org/10.1101/2023.01.03.522589

-Martincorena, I. et al. Somatic mutant clones colonize the human esophagus with age. Science 362, 911-917 (2018).
Graphical abstract or figures:
Research passport requirementNo