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MOLECULAR CONTROL OF EPITHELIAL POLARITY

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Our group studies the gain and loss of collective cell polarity and invasion in prostate tumours. Our research is focused on two intersecting streams: 1) understanding the molecules that regulate collective cell polarity, and 2) developing the computational image analysis tools that allow us to dissect cell polarity.

Developing tools for collective 3-Dimensional (3D) invasion analysis

Traditionally, how cells move has been studied using single cells grown on glass or plastic. However, tumours are collections of many, not singular, cells. Dissecting how collective cell invasion is regulated requires developing methods to allow for 3D 'mini-tumours' (spheroids) to be grown, imaged and analysed *ex vivo*. Analysis methods for studying collective invasion have lagged far behind that of single cell analyses, primarily because of a lack of quantitative tools to do so. Our group aims to develop methods to overcome such limitations. We have a newly formed Industrial Partnership with Essen Bioscience to develop image analysis tools to automate this process and provide bioinformatics solutions to studying 3D cultures via live imaging.

In collaboration with the HICAR facility, our group is developing an integrated platform for high-throughput, high-content, live imaging–based analysis of spheroid invasion. We have developed lentiviral shRNA arrays to manipulate gene expression in spheroids in massive parallelism. We have coupled this to semi-automated multi-day imaging (both live and fixed) of spheroid invasion. Our analyses have been aided by the introduction of machine learning algorithms to classify different invasion phenotypes in a robust, quantitative method. This presents an exciting new possibility to examine, in a rapid fashion, the factors that regulate tissue formation and its disruption in cancer. We have used this to investigate mapping of signalling networks, and gene expression clusters stratifying particular cancer patient subgroups.

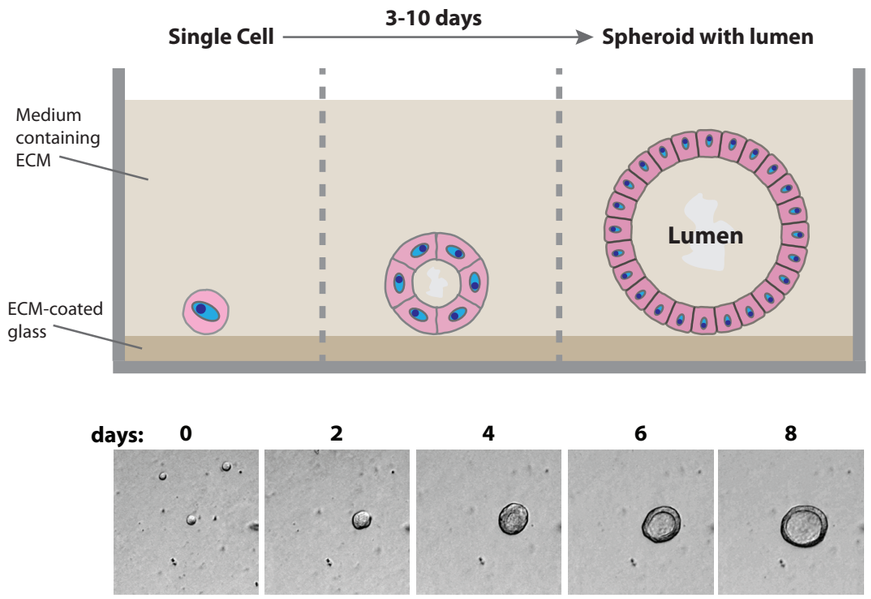

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

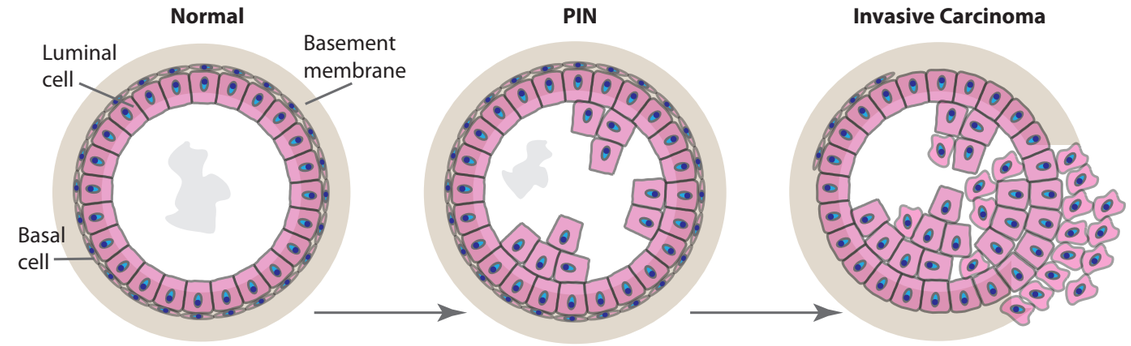

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies prostate cancer progression.

ARF GTPase circuits controlling cell invasion

The ARFome is a network of five GTPases, multiple regulatory proteins (GEFs, GAPs) and effectors that are involved in lipid signalling, cytoskeletal organisation and membrane trafficking. They form a highly overlapping network and are thought to share many of the same binding partners. This makes untangling specific functions for each GTPase difficult. We have performed a functional genomic screen to systematically interrogate each member of the ARFome family's influence on prostate cancer cell invasion.

In collaboration with the Ismail and Shanks groups, we are interrogating their function in prostate cancer cell invasion from spheroids. We find that many ARFome family members assumed as redundant have highly divergent and sometime opposing roles in invasion, and show that there may indeed be specificity of signalling between family members. In addition, we are focusing on how alternate splicing of ARFome proteins can contribute to divergent functions of such alternately spliced isoforms. This is an important preamble to identify which, and how selectively, ARF GTPases may be targets for future therapeutic inhibition studies.

Podocalyxin function in collective cancer cell invasion

Podocalyxin is mutated in some families with congenital prostate cancer. Additionally, amplification of Podocalyxin expression is a predictor of poor outcome in several cancer types. We are characterising the molecular mechanisms by which Podocalyxin promotes collective cell invasion.

In collaboration with the Zanivan group, we are using in-depth quantitative mass spectrometry to identify the interacting partners of Podocalyxin ('Podxl interactome') that control its pro-invasive function. Additionally, we are mapping the proteomic changes required during cancer progression to promote Podocalyxin function. Furthermore, we have collaborated with the Shanks lab / HICAR facility to develop a functional genomic approach to systematically evaluate each member of the Podxl interactome for its role in invasion from spheroids. Our current aim is for a rigorous dissection of the exact cooperating protein modules that promote Podocalyxin-driven invasion. Our future aim is to understand which of these *in vitro* modulators of invasion are consistently altered in prostate cancer patients, such that they may be potential therapeutic targets in the clinic in the future.

Understanding the effect of common genomic alterations on epithelial–stromal crosstalk in ovarian and endometrial cancer

In collaboration with the Zanivan group, we are developing a novel 3D co-culture of fibroblasts and epithelial cells to understand the role of epithelial–stromal crosstalk in ovarian cancer. Our approach combines quantitative mass spectrometry and high-throughput image analysis to understand how common genomic alterations in ovarian cancer affect both fibroblast and epithelial organisation. Our aim is to understand whether there are specific, druggable signalling events between fibroblasts and ovarian cancer cells that control cancer progression.

In collaboration with Samantha Stehbens (University of Queensland, Australia), we are investigating the effect of FGFR mutations in collective cell invasion in 3D cultures. This work is revealing that collective polarity defects underpin patient mutations in FGFR2.

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