EPITHELIAL POLARITY

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A feature of most tumours is that they become less organised as they progress. Tissue organisation is thus the strongest predictor of poor outcome. Our laboratory studies the molecular mechanisms of how cells organise to form tissues, and how this goes awry during tumour formation. We aim to understand this process such that we can identify new drugs for therapy in cancer.

Our group studies the gain and loss of collective cell polarity and invasion in 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, cell movement has been studied using single cells grown on glass or plastic. Tumours are collections of many, not singular, cells. Dissecting how collective cell invasion is regulated requires developing methods to allow for 3D 'mini-tumours' (organoids) 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 an 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.

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's influence on prostate cancer cell invasion.

In collaboration with the Blyth, Leung and Zanivan groups, we are interrogating their function in metastasis. We find that many ARFome family members assumed as redundant have highly divergent and sometimes opposing roles in invasion, and show that there is specificity of signalling between family members. In two publications this year we identified that the ARF6 GTPase is a vulnerability in PTEN-null ovarian cancers, by regulating the membrane transport of active integrin cargoes required for invasive behaviours into the extracellular matrix. In contrast in prostate cancer cells, we found that the ARF3 GTPase regulate cell-to-cell adhesion and metastasis by controlling the membrane transport of the cell adhesion regulator N-cadherin. These studies identify that the ARF GTPases may be targets for future therapeutic inhibition studies to control cell movement in cancer.

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 used our functional genomic approach to systematically evaluate each member of the Podxl interactome for its role in invasion from spheroids. In collaboration with the Blyth and Leung groups, we identified a molecular mechanism of how Podocalyxin controls

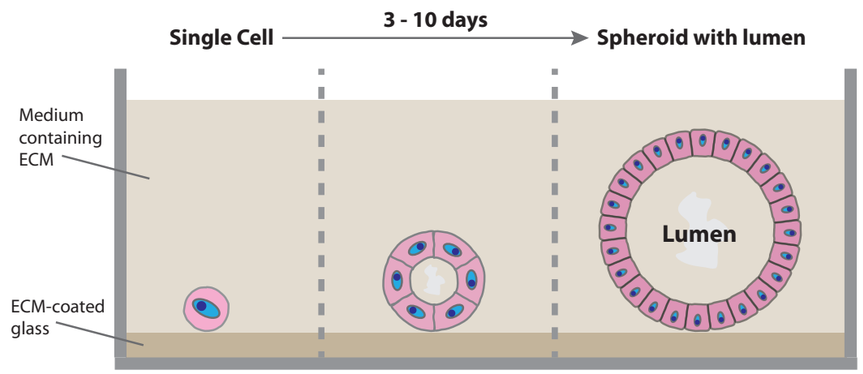

Figure 1
By culturing cells on glass-bottomed chambers coated with extracellular matrix (ECM), we direct the self-assembly of single cells into multicellular spheroid structures with a single, central lumen. This process occurs over 10 days, allowing us to study the dynamics of tissue formation.

prostate cancer metastasis and tumour growth *in vivo*. In collaboration with the Sansom laboratory, we are extending these studies to colorectal cancer, where elevated expression of Podocalyxin is associated with very poor outcome. Our current aim is for a rigorous dissection of the exact cooperating protein modules that promote Podxl-driven invasion. Our future aim is to understand which of these *in vitro* modulators of invasion are consistently altered in cancer patients, such that they may be potential therapeutic targets in the clinic in the future.

Phosphoinositide signalling in cell polarity and metastasis

A major new direction of the laboratory is to understand how a particular class of membrane-associated lipids, phosphatidyl-inositol phosphates (PIPs), contribute to tissue formation and its alteration during metastasis. We previously discovered pathways for how these lipids control the ability of cells to assemble into tissues. We identified in PTEN-null prostate and ovarian tumours that the ARF6 GTPase is required for invasive activities in cancer. In collaboration with Owen Sansom's lab, we are examining how these lipids control the disruption to tissue organisation and overgrowth that occurs during colorectal cancer progression.

Publications listed on page 102

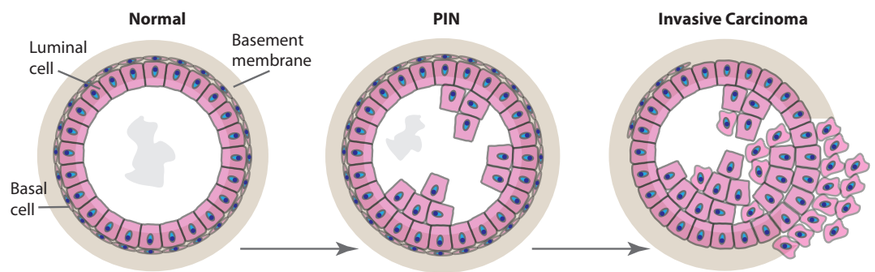

Figure 2
3D cultures of cells to form cysts (also called spheroids or organoids) also allows us to model the loss of normal tissue architecture that occurs in cancer. For example, the progressive disrupted organisation of Normal, to Prostatic Intraepithelial Neoplasia (PIN), to Invasive Carcinoma typifies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