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MIGRATION, INVASION AND METASTASIS

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The impact of the tumour microenvironment and the peri-tumour extracellular matrix on metastasis presents an opportunity to develop new therapies. Pancreatic tumours are especially fibrotic, causing starvation for nutrients and enhancing invasive behaviour. We are studying how tumour cells balance the usage of their cytoskeletal machinery to migrate and invade, with the assembly of macropinocytic structures to take up nutrients and thus survive in nutrient-depleted conditions. We aim to exploit vulnerabilities caused by the cancer microenvironment that could be targeted against metastasis and to model the metastatic niche using bioengineering.

One of the ways that tumour cells survive in the hostile tumour microenvironment is by repurposing their actin cytoskeletal migration machinery to take in large gulps of the surrounding liquid by macropinocytosis. Migration and macropinocytosis use the same basic actin machinery, and therefore can compete with each other - but the mechanisms controlling this competition are not well understood. PhD student Anh Le recently discovered an important role for the RAC1-interacting protein CYRI-A in regulating the balance between macropinocytosis and invasive cell migration (Le *et al.,* 2021, *Journal of Cell Biology*). Together with PhD student Savvas Nikolaou, they found that CYRI-A and CYRI-B were both important in resolving macropinocytic cups, by opposing actin assembly and allowing actin to disassemble for engulfment of macropinosomes. Interestingly, cells depleted of CYRI-A and CYRI-B were unable to perform macropinocytosis, but showed enhanced invasive migration, suggesting a competition between these processes (Le *et al.,* 2021, *Journal of Cell Biology;* Le & Machesky, 2022, *Bio. Protoc.*).

One of the major receptors trafficked by CYRI-B-dependent macropinocytosis was integrin alpha5 beta1 (Le *et. al,* 2021, *Journal of Cell Biology*). Postdoctoral researcher Jamie Whitelaw found that CYRI-B knockout cells displayed enlarged and less dynamic focal adhesions. He performed a Bio-ID screen and identified several differences in the composition of adhesions in the absence of CYRI-B (Whitelaw *et al.,* in preparation).

Savvas Nikolaou went on to study the effects of depletion of CYRI-B in KRas- and p53-driven pancreatic cancer and to determine how CYRI-B affected tumour progression and metastasis *in vivo*. His studies suggested that CYRI-B might function as a buffer for the effects of hyperactivation of RAC1 downstream of KRas in early tumour progression. In contrast, CYRI-B seemed to play an important role in metastatic progression, which might be due to its key function in trafficking of integrins and other surface receptors via macropinocytosis (Nikolaou *et al.,* under review).

Associate scientist Amelie Juin discovered that the MAP-kinase kinase MAP4K4, which is known to impact actin dynamics and cell migration, also had a role in the progression of pancreatic ductal adenocarcinoma. She is continuing to explore downstream signalling pathways that accelerated tumour progression upon MAP4K4 depletion (Juin *et al.,* manuscript in preparation).

Postdoctoral researchers James Drew and Vassilis Papalazarou discovered that collagen-6 acted as a mechanosensitive component of the metastatic tumour microenvironment of pancreatic ductal adenocarcinoma (Papalazarou, Drew *et al.,* J. Cell Sci., 2022). Collagen-6 is a heterotrimeric collagen, composed of up to six different alpha chains, which assembles into elastic fibres in collagen-1 rich connective tissues. Pancreatic tumours are known for their high levels of fibrous extracellular matrix and depositions of collagen-I. We found that when cancer cells experienced a mechanically soft environment

Collagen-6 was upregulated and its expression contributed to invasive migration and establishment of metastases. Collagen-6 could be produced both by cancer cells and stromal cells and our study suggested that it could be expressed early during metastasis as an important promoter of a new metastatic niche in pancreatic ductal adenocarcinoma.

PhD student Hakem Albilasi is studying the interactions between chronic myeloid leukaemia (CML) cells and mesenchymal stem cells. He found that CML cells can interact with mesenchymal stem cells and is studying the mechanisms and consequences of this interaction.

PhD students Sonia Rolo and Elaine Ma are studying the effects of mechanosensing on expression of various target genes involved in invasion and migration in pancreatic ductal adenocarcinoma. Elaine is also developing novel hydrogels for the culture of tumour cells and organoids in conditions where she can use bioengineering to control stiffness and composition of the matrix (Figure 1). These hydrogels will serve as an excellent platform to ask specific questions about the effects of physical parameters and matrix composition on the 3D growth of cancer cells. Juda Milvidaite is also exploring bioengineered materials, such as alginate hydrogels, for the growth and preservation of organoids and tumour samples in collaboration with the Biotech company Atelerix. Together, we are developing new models for the tumour and metastatic niche to build better model of the complex cancer microenvironment.

Publications listed on page 106

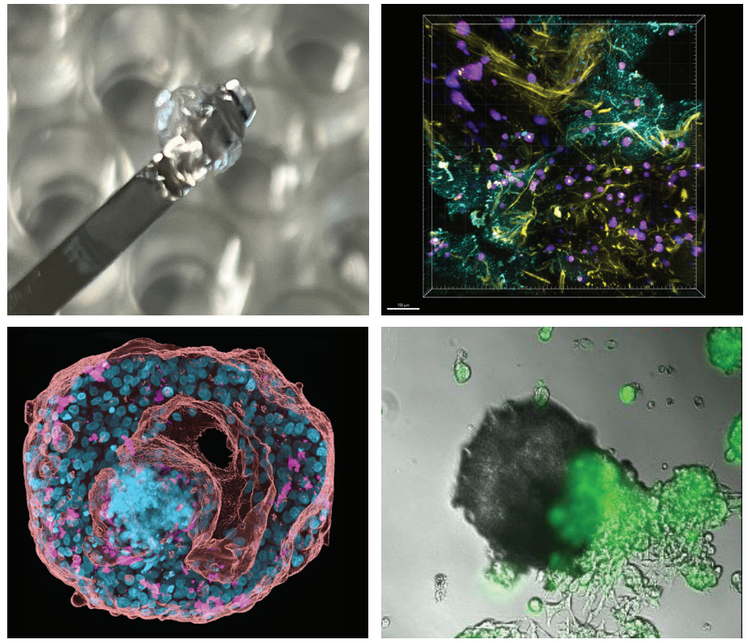

Figure 1
Polyethylene glycol (PEG) hydrogels functionalised with fibronectin and collagen 1 provides a scaffold for 3D cell culture *(top left: PEG hydrogel).* This synthetic hydrogel mimics the biochemical and physical properties of the extracellular matrix to support growth of pancreatic ductal adenocarcinoma (PDAC) spheroids *(top right: PDAC cell culture in PEG hydrogels; fibronectin (yellow), collagen 1 (cyan), phalloidin (purple), DAPI (blue).* Co-culture of PDAC cells and liver spheroids within the PEG hydrogel provides a 3D *in vitro* model of PDAC metastasis in the liver *(bottom left: co-culture of PDAC cells and liver spheroids; collagen 1 (magenta), DAPI (cyan), phalloidin (red) and bottom right: co-culture with PEG hydrogels; PDAC cells (green), liver spheroid (grey)).*