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MIGRATION, INVASION AND METASTASIS

Group Leader

Laura Machesky FRSE

Research Scientists
Amelie Juin
Nikki Paul[1]
Karthic Swaminathan

Scientific Officer
Heather Spence

Graduate Students
Loic Fort
Anh Hoang Le
Vassilis Papalazarou[2]

[1]Pancreatic Cancer Research Fund
[2]CRUK Glasgow Centre, joint with Manuel Salmeron-Sanchez, University of Glasgow

We urgently need to improve our understanding of the mechanisms of metastatic cancer dissemination in order to develop new therapeutic strategies to improve patient outcomes. Our research addresses mechanisms of metastatic spread by determining the roles of key actin cytoskeletal proteins, such as the actin filament nucleation machinery and the bundling protein fascin-1.

The actin cytoskeleton is important not only for cell strength and migratory capacity but also for adhesion-dependent survival, membrane trafficking and establishment of polarity. Additionally, the actin cytoskeleton plays a key role in how a cell interacts with and remodels the extracellular environment. The extracellular matrix contributes to the development and homeostasis of organs and tissues, and in tumours, matrix and associated stromal cells provide key support for growth, invasion and metastasis. We aim to understand how various actin regulators control interaction with matrix and how tumours subvert both the actin cytoskeleton and the surrounding tumour stroma to gain advantages.

Role of actin nucleating proteins in cell migration, invasion and membrane trafficking

The Arp2/3 complex is the major inducer of actin filaments in response to extracellular signals. The Wiskott-Aldrich Syndrome Protein family of proteins (including WASP/N-WASP, Scar/WAVE, WASH, WHAMM and JMY) transmit signals to the Arp2/3 complex to trigger actin assembly. We aim to understand the mechanisms of regulation and the involvement of these proteins in invasion and metastasis of cancer as well as their normal cellular function. WASP family proteins regulate actin assembly in multiple cellular processes,

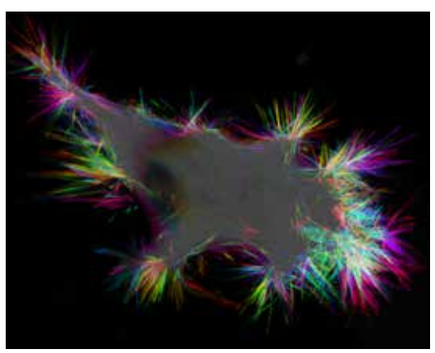

Figure 1
Fascin localises in highly dynamic filopodia in a MiaPaCa human pancreatic cancer cell.
Photo credit: Nikki Paul

such as endocytic trafficking, protrusion of lamellipodia and filopodia, cell division and assembly of invasive structures such as podosomes and invadopodia. Postdoc Ben Tyrrell studied the role of the WASH complex in assembling actin on endocytic vesicles to mediate trafficking of receptors and integrins. Deletion of the strumpellin subunit in melanocytes had no effect during development, despite defects in trafficking at the cellular level. Native gels revealed that, surprisingly, strumpellin null cells contained a partial WASH complex that appeared to recruit actin to endocytic vesicles, calling into question the previous view that each subunit of the WASH complex is essential for its function (Tyrrell *et al.*, 2016).

PhD students Loic Fort and Jose Batista (Robert Insall's group) have discovered a new highly conserved regulator of the Scar/WAVE complex that is responsible for controlling communication between the Rac1 GTPase and the Scar/WAVE complex. This protein allows cells spatial regulation of Rac1 signalling and subsequent actin assembly and protrusion. Future experiments will determine how this new regulator modulates cell migration, polarity and Rac1 signalling.

Role of actin regulatory proteins in colorectal and pancreatic cancer

N-WASP is established as a key driver of formation of invadopodia and of cancer cell invasion *in vitro*, but much less is known about its potential role *in vivo*. MRC-funded clinical research fellow Hayley Morris found that loss of N-WASP accelerated tumour progression of APC-driven colorectal cancer in a mouse model. Her findings suggest that N-WASP could have a tumour suppressive role in colorectal cancer, even though it is a promoter of invasion and metastasis in other models. Postdoc Amelie Juin is studying the role of N-WASP in pancreatic

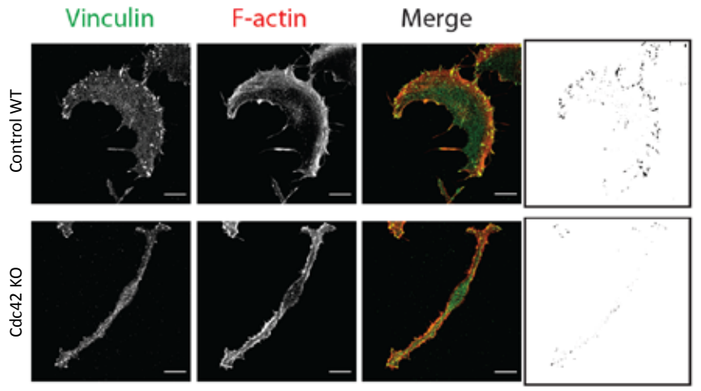

Figure 2
Vinculin and F-actin (actin filament) staining in normal and Cdc42 knockout melanocytes. Loss of Cdc42 causes a disruption of focal adhesion organisation in melanocytes.
Photo Credit: Nikki Paul

ductal adenocarcinoma (PDAC). Amelie has discovered a role for N-WASP in tumour invasion and metastatic spread to secondary sites. Using cells cultured from the tumours, she uncovered how N-WASP drives matrix remodelling by tumour cells and potentiates chemotactic signalling loops that mediate cell egress from the primary tumour. In collaboration with Ines Anton (Barcelona), we have uncovered a role for the N-WASP interacting protein (WIP) in cancer cell invasion and regulation of the formation of matrix degrading invadopodia protrusions (Garcia *et al.*, 2016).

Fascin is an actin bundling protein that is not expressed in normal epithelial cells, but is often upregulated in the most invasive and aggressive cancers (Fig. 1). PhD student Loic Fort and Pancreatic Cancer Research Fund postdoc Nikki Paul are studying how fascin upregulation affects the initiation of pancreatic cancer from precursor lesions (pancreatic intraepithelial neoplasia). Nikki leads our ongoing efforts to develop and test fascin-1 inhibitor compounds together with Drug Discovery. The team has developed some exciting new inhibitor compounds that can potently inhibit actin bundling *in vitro* and which are being tested in cellular assays.

Role of actin regulatory proteins in melanoblast migration and melanoma

We previously showed that loss of Rac1 causes major defects in melanoblast migration and proliferation during development. We continue to investigate the roles of RhoA and Cdc42 in melanoblasts with PhD student Emma Woodham, postdoc Nikki Paul and scientific officer Heather Spence, together with Cord Brakebusch (BRIC, University of Copenhagen). Cdc42 is crucial for melanoblast migration and cells lacking Cdc42 show defects in polarity, migration and integrin-based adhesion (Fig. 2). Postdoc Karthic Swaminathan has discovered a role for the Scar/WAVE complex in melanoblast migration and melanoma tumour development and progression, suggesting that the Scar/WAVE complex may be an important melanoma target downstream of Rac1. New postdoc Jamie Whitelaw will study the role of the Scar/WAVE complex in cancer cell invasion and explore possible links between this complex and protein translational control. New PhD student Anh Le will study how Rac1 P29S, a driver mutation found in around 5% of sun-exposed melanomas, affects cell migration pathways.

Role of extracellular matrix in migration and invasion of tumours

Pancreatic ductal adenocarcinomas contain a dense fibrous stroma rich in collagen, fibronectin and other components. This is thought to serve both as a barrier to chemotherapeutic treatment and an inducer of more aggressive behaviour of the tumour cells. PhD student Vassilis Papalazorou has been engineering surfaces of various stiffness to test how this affects pancreatic cancer cell migration (Fig. 3). Vassilis is co-supervised with Manuel Salmeron-Sanchez (Engineering, University of Glasgow). Our goal is to better understand the crucial properties of desmoplastic stroma so that PDAC treatments could be improved in the future.

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Figure 3
ASPC1 human pancreatic cancer cells on rigid matrix (40kPa) or soft matrix (1kPa) showing phospho-FAK (red), Actin filaments (green) and the nucleus (DAPI)
Photo Credit: Vassilis Papalazorou