CELL MIGRATION AND CHEMOTAXIS

Group Leader
Robert Insall FRSE

Research Scientists
Shashi Singh
Olivia Susanto
Luke Tweedy[1]

Scientific Officer
Peter Thomason

Graduate Students
Clelia Amato
Yvette Koh

Visitor
Vojtech Dostal

[1]CRUK Multidisciplinary Award

Cell migration in multicellular organisms must be suppressed, so that the architecture of tissues and organs remains consistent. In most early-stage cancer, it remains supressed. However, when tumours become metastatic, suppression of cell migration may be lost - cancer cells invade nearby tissues, and spread into the blood and lymph systems to form secondary tumours. Our group brings together multiple tools, from different disciplines, to improve understanding of how cell migration is controlled. We use *in vivo* models, cancer cells, model organisms and computational simulations, and apply a wide range of techniques, from genetics through biochemistry and microscopy to quantitative analysis of microscope movies and computational modelling.

We are interested in two related questions. The first is how cells are steered by external signals, a process known as chemotaxis, which is increasingly seen as a fundamental cause of cancer metastasis. Recently, our focus has shifted towards a related process, in which cells steer themselves by manipulating external signals. The second is the mechanics that drive cell migration, in particular actin.

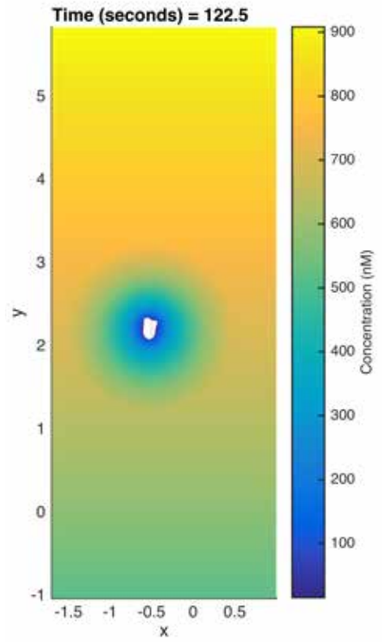
Mechanisms underlying chemotaxis: Pseudopods and self-generated gradients
Chemotaxis is emerging as a major driver of tumour metastasis. In the past, we have found that chemotaxis in *Dictyostelium* cells works by a different mechanism than that which is usually described. Pseudopods are constantly generated in random directions, then the ones that point in the best directions are selected and maintained. We are currently testing whether the same is true for cancer cells, using several melanoma lines. We have used chemotaxis chambers of our own design to show that melanoma cells are exquisitely chemotactically sensitive. They can navigate up a gradient of serum with unprecedented accuracy, irrespective of their stage – early melanomas are slower but still highly chemotactic. Our initial results suggest that the melanomas move like *Dictyostelium*, with many randomly generated pseudopods whose behaviour is biased to change the cell's direction.

The most interesting part of melanoma cells' response is that we find they make their own chemotactic gradients. LPA - which appears to be present at substantial levels in the tissue surrounding tumours - is a strong attractant for all the melanoma cells we have observed. But melanoma cells also break down LPA. This leads to a self-generated gradient, in which cells move out of tumours in response to gradients they are themselves creating. Thus tumours appear to need no external drivers to steer metastasis – they do it themselves.

We are now studying the details of self-generated gradients, using mathematical models to identify the range of possible behaviours, and experiments with a wide range of different cell types including melanoma, pancreatic ductal adenocarcinoma, immune cells and *Dictyostelium*.

We are collaborating with the Mathematics Departments of the Universities of Strathclyde and Glasgow to make different computational models representing moving cells. Our models already faithfully mimic some aspects of the movement of *Dictyostelium* cells. We are now using the model to test our predictions about the underlying mechanisms of chemotaxis, and the proteins that are involved. We are showing that chemotaxis is mostly likely mediated by several dissimilar mechanisms acting in parallel, including regulated pseudopod growth, pseudopod retraction and the control of adhesion. We can also determine which components can safely be ignored, which is increasingly important – hundreds of genes are

Figure 1
Computational modelling of the local gradient caused dynamically when a cell breaks down the chemoattractant in its vicinity.



newly associated with motility and invasion every year so we urgently need a mechanism to determine which are the most important.

Regulators of actin and the Arp2/3 complex

Most mammalian cells use pseudopods made of polymerised actin to power migration. Our current research focuses on the proteins and pathways that control these pseudopods. Actin drives nearly all cell movement, and the principal driver of actin is an assembly called the Arp2/3 complex. When turned on, the Arp2/3 complex causes new actin filaments to form and push against the membranes inside and at the leading edge of cells. We are particularly interested in the family of proteins that turns on the Arp2/3 complex. One such activator is SCAR/WAVE, which is a fundamentally important regulator of cell movement. Mutants in a variety of species show that it is required whenever cells need to make large actin-based structures such as lamellipods; without SCAR/WAVE such structures are either small and malformed, or completely absent. It is found as part of a five-membered complex with the Rac-binding protein PIR121, Nap1, Abi and HSPC300. Without the other members of the complex, SCAR is rapidly removed from the cell. The prevailing view in the field is that all these proteins act simultaneously as a huge, homogenous complex that couples Rac and lipid signalling to actin polymerisation. However, this view seems very simplistic in view of the size of the complex and its dynamic behaviour.

Our experiments are currently focused on identifying the activators and other proteins that regulate each component of the complex. We are using the Beatson's expertise in mass spectrometry to identify proteins that crosslink to SCAR in living cells at different migration rates. SCAR and the other complex members are phosphorylated at multiple sites but the biological significance of these phosphorylations is not understood. We have shown that control of SCAR phosphorylation is centrally important – nearly all the cellular SCAR is heavily phosphorylated but a rare dephosphorylated form seems to be particularly important. It is also very active in extending pseudopods, and very unstable, explaining its rarity. We are now seeking the phosphatases. We have also shown – very unexpectedly – that nearly all the same signals regulate the localisation of SCAR and its relative WASP. We are now seeking to understand what those signals are, and how they connect to upstream signalling molecules such as receptors and G-proteins. WASP's behaviour is slightly anomalous - there is a high degree of consensus among cell biologists about how it is controlled, but the standard view does a poor job of explaining the observed behaviour. We are working towards a more detailed and consistent narrative.

Publications listed on page 96