To The Abercrombie Meeting and back again
A journey into the world of cell migration

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The 7th Abercrombie Meeting took place in Oxford this past summer. It was organized by The Royal Microscopical Society with the support of The British Society for Cell Biology. Michael Abercrombie was a pioneer in the field of investigating cell behavior using time-lapse microscopy. The meeting was focused on “multi-dimensional cell migration in development and disease” and it brought together many of the world’s leading researchers in the area, providing an opportunity to discuss the very latest advances and possible future developments in the field. The meeting sessions included Invasive Migration, Invasive Adhesions in Migrating Cells, Signaling in Migration, Immune Cell Migration, Migrations during Morphogenesis and Migration and Disease. As with all Abercrombie meetings, the conference delegates were treated to a staggering array of live cell imaging, in vivo imaging and images generated by the latest developments in microscopy.

Over 120 scientists met at The 7th Abercrombie Meeting in Oxford this summer to discuss the role of cell migration in development and disease. The Abercrombie Meetings have been held every five years since the death of Michael Abercrombie in 1979, to honor Michael’s pioneering use of time-lapse microscopy in the field of investigating cell behavior (see Dunn and Jones, Trends in Cell Biol 1998). The meeting was organized by The Royal Microscopical Society with the support of The British Society for Cell Biology, and the scientific organizers were Laura Machesky (University of Glasgow), Charles Streuli (University of Manchester) and Claire Wells (King’s College London). The meeting brought together many of the world’s leading researchers in the area and covered a variety of topics from cell migration and cell invasion to cell-substratum adhesion. Several talks highlighted the importance of identifying the most appropriate 3-dimensional matrix to the tissue types or investigated biological processes. The conference delegates were treated to a staggering array of live cell imaging, in vivo imaging and images generated by the latest developments in microscopy.

Roberto Mayor (University College London), opened the meeting with a keynote lecture on contact inhibition of locomotion, a phenomenon first characterized by Michael Abercrombie. He began by showing how contact inhibition of locomotion plays a key role in guiding the migration of neural crest cells during embryonic development. He went on to demonstrate that these cells display “collective chemotaxis,” where the cells respond much more efficiently to a chemoattractant (Sdf1) as clusters rather than as individual cells. He ended by showing his intriguing finding that neural crest cells are co-attracted via the complement fragment C3a and its receptor C3aR, revealing an unexpected role for complement proteins in early vertebrate development.

Invasive Migration

Peter Friedl (Radboud University Nijmegen Medical Centre) followed up on the theme of collective cell migration and using multiphoton microscopy to visualize the process in vivo. He showed that migration depends on the cell type and substrate, and that cells migrate along the tracks that provide the least resistance. His laboratory has discovered that collective cancer cell invasion is independent of β1 or β3 integrins-mediated mechanotransduction. Moreover, imaging of a tumor mass following radiation therapy showed that the invasive cells survive, representing an invasion niche. Interestingly, the combination of targeting integrins together with radiation therapy is effective in blocking invasion. The combination of these therapies has the potential to overcome the resistance of cancer cells to radiation therapy.

The role of integrins in cancer cell migration was discussed further by Maddy Parsons (King’s College London). She has found that silencing of β1 integrin results in fewer focal adhesions and leads to a reduced migration on 2D surfaces but enhanced invasion in 3D extracellular matrices (ECM). Cells with decreased levels of β3 integrin exhibit enhanced invasion in 3D cultures in the presence of fibroblasts, which secrete epidermal growth factor (EGF). Furthermore, she has discovered that activity of RhoA GTPase or focal adhesion kinase (FAK) can suppress the enhanced invasion of cells with lower β1 integrin levels, which demonstrates a specific role for different integrins in the modulation of FAK-RhoA-actomyosin signaling to regulate cell invasion.

Expanding of the theme of how matrix geometry affects migration, Erik Sahai (Cancer Research UK) talked about how cancer-associated fibroblasts (CAFs) enable cancer cell migration by remodeling the ECM to generate a permissive environment. CAFs promote cancer cell invasion in breast organotypic assays and the change from fibroblasts to CAFs persists even when cancer cells are no longer present. A global screen revealed that
transcriptional co-activator YAP/TAZ is upregulated in CAFs, and required for their tumor promoting activities. Regulated by the MST1/2 tumor suppressor, YAP/TAZ can be activated by a stiff matrix. Erik also presented his latest work modeling a theoretical framework for understanding cell migration in different matrix geometries. Lively discussion involved the possibility of putting CAFs into less stiff matrices or breaking the positive feedback of CAF phenotype, when after only 4–5 d the cells would revert to normal fibroblasts.

Cell migration in 3D environments is further promoted by the Rab-coupling protein, which coordinates recycling of α5β1 integrin and EGF receptor 1, as discussed by Patrick Caswell (Welcome Trust Centre for Cell-Matrix Research). He has also discovered that RacGAP I, a Rac- and Cdc42-specific GTPase activating protein, is a new substrate for PKB/Akt. His laboratory has found that phosphorylation of RacGAP I promotes its recruitment to IQGAP I within filopodia, triggering a localized suppression of Rac activity and an increase in the activity of RhoA at the leading edges of cells migrating in 3D. RacGAP I thus promotes Rac inactivation, extension of pseudopodia and invasive migration in carcinoma cells.

The SCAR/WAVE complex (a member of the Wiskott Aldrich syndrome protein family) is an important activator of the Arp2/3 complex, which stimulates actin assembly during lamellipodia formation. Hao Ran Tang (Beatson Institute) proposed that the loss of the SCAR/WAVE complex promotes cancer cell invasion. It consists of five subunits, all of which are essential for the stability of the whole complex. He presented work showing that, although blocking the Sra I subunit of the SCAR/WAVE complex inhibits cell migration on 2D surfaces, it enhances cell migration in 3D (in vivo). The work suggested that the focal adhesion kinase (FAK) phosphorylates and thus activates N-WASP, which then activates the Arp2/3 complex, promoting cell migration even when SCAR/WAVE is blocked. In turn, SCAR/WAVE blocks FAK, preventing the normal activation of N-WASP. Additionally, he showed that the smallest component of SCAR/WAVE, HSPC300, is required for cell invasion only in its free form, and not when it is incorporated by SCAR/WAVE complex.

In yet another example of collective cell migration, Harry Mellor (University of Bristol) showed that cell shape during angiogenesis is regulated by formin. The elongation of epithelial cells is accompanied by re-organization and stabilization of the microtubule skeleton. Using an siRNA based screen, he found that depletion of formin-like 3 (FMNL3, also known as FRL2) from epithelial cells affects the length and branch points of new blood vessels. He demonstrated that FMNL3 is required for microtubule re-organization during angiogenesis and for efficient elongation of epithelial cells, and it is therefore a crucial regulator of this process.

Invasive Adhesions in Migrating Cells

Philippe Chavrier (Institut Curie) presented his work studying dynamics of invadopodia, which play an important role in matrix degradation and cancer cell migration. He has found that MT1-MMP (membrane-type 1 metalloproteinase) is required for matrix degradation and for migration of breast carcinoma cells on 3D matrices. His laboratory has determined that the exocyst machinery, including the SNARE vesicle-fusion protein VAMP7, is required to deliver MT1-MMP to invadopodia. Recently, he has also found that WASH, a member of the WASP protein family, interacts with the exocyst complex. This ongoing work suggests that an interplay between VAMP7 and WASH controls endosomal actin and MT1-MMP dynamics by regulating the docking of the MT1-MMP endosomes at the plasma membrane.

Heptocyte growth factor (HGF) and its receptor tyrosine kinase Met (Met RTK) induce cell dispersal and epithelial morphogenesis. Richard Vaillancourt (McGill University) has found that the scaffold protein Gab I mediates the HGF-induced remodeling of the actin cytoskeleton. Met RTK induces cell dispersal and epithelial morphogenesis via recruiting and phosphorylating Gab I. Moreover, Gab I then recruits N-WASP to the Arp2/3 complex in response to HGF, suggesting that Gab I regulates actin polymerization through N-WASP recruitment. This work demonstrates a novel mechanism by which RTKs can regulate cell migration.

Claire Wells (King’s College London) discussed the role of hypoxia in context of cancer metastasis. To resist the negative effects of hypoxia, cancer cells use pro-survival pathways that in many cases involve transcription factor called hypoxia-inducible factor-1 (HIF-1α). Her laboratory has found that HIF-1α can drive the formation of invadopodia and regulates the expression of several cytoskeletal-associated genes, including β-PIX (Pak interactive exchange factor). β-PIX is essential for efficient invadopodia formation driven by HIF-1α, demonstrating the connection between hypoxia and cancer metastasis. Questions raised in the discussion mentioned the possibility of β-PIX being connected to angiogenesis, which has not been identified, as well as an interesting perspective of examining the long-term effect of hypoxia on the described processes.

Dianne Cox (Albert Einstein College of Medicine) outlined how the stimulation-induced migration of macrophages into tumors can lead to increased invasiveness in cancer. She demonstrated that tumor-associated macrophages (TAMs) are recruited into tumors because of a paracrine interaction between cancerous cells producing macrophage colony-stimulating factor (CSF-1) and EGF-producing macrophages. She also showed that WASP, an activator of the Arp2/3 complex, is an important downstream effector of CSF-1. The inability of WASP-deficient TAMs to promote carcinoma cell invasion is due to defects in cell motility and in the presentation of EGF to carcinoma cells.

The migration of macrophages depends on the type of 3D environment, as shown by Veronique Le-Cabec (National Centre for Scientific Research). She demonstrated that macrophages exhibit ameboid migration in porous matrices (e.g., fibrillar collagen), but they show protease-dependent mesenchymal migration in dense matrices (e.g., Matrigel). Using mouse knockout models, she has discovered that macrophages require HcK, a phagocyte-specific kinase, to switch to a mesenchymal mode of migration, which requires podosomes. Moreover, a cell cycle inhibitor p27 KIP1 is upregulated during mesenchymal migration.
Stephen Weiss (Life Sciences Institute, University of Michigan) offered to the audience some “food for thought,” claiming that the only “simple” problems are those not studied carefully. He questioned the idea that cancer cells can choose either a protease-dependent or non-dependent strategy to migrate through the ECM depending on the ECM architecture. He showed that silencing of the secreted metalloenzymes MMP-1 and MMP-2 in combination has no effect on invasion of migrating spheroids of fibrosarcoma cells embedded within 3D gels of native type I collagen. However, silencing MT1-MMP blocks virtually all invasive activity. This finding was also confirmed using breast cancer cells embedded in human mammary gland explants. The requirement for membrane-anchored metalloproteinase MT1-MMP for invasion raises the question as to whether protease-independent migration really occurs in vivo. He suggested that it is only plausible in artificial ECMs or when the collagen network is devoid of the covalent cross-links that characterize normal tissues.

**Signaling in Migration**

Signaling pathways involving proteins such as Rac I are important for cell migration. Mark Bass (University of Bristol) showed that processive 3D migration can be driven by activation of Rac I at dominant protrusions. Rac I is regulated by RCC2, which binds and sequesters Rac I to prevent its activation. His laboratory has also discovered that coronin 1C, ECC2 binding partner, and a proteoglycan syndecan-4 are engaged in releasing Rac I from the plasma membrane for reactivation to promote protrusion. In contrast, caveolin is involved in degradation of Rac I.

Guillaume Charras (University College London) presented a new model for neutrophil migration, where neutrophils migrate through narrow microfluidic channels (5 μm × 5 μm in cross-section). His work shows that as the neutrophils migrate through the interstices, they form a two-layered actin gel: one at front of the cell, as found in cells migrating in 2D, and one at the cell-wall surface. The interaction between these two gels was hypothesized to create an outward pressure that facilitates cell movement.

SCAR/WAVE is important for activating the Arp2/3 complex. Sven Bogdan (University of Münster) showed that Ena, part of the Ena/VASP complex, has a role in regulating WAVE-dependent cell morphology and membrane protrusions in Drosophila blood cells (hemocytes). His laboratory has discovered that Ena activates WAVE by binding to the Abelson interactor (Abi) subunit of the WAVE complex. This interaction is required for proper lamellipodia formation and the normal functioning of WAVE at plasma membrane.

In a “pseudopod-centered” view of cell migration, Robert Insall (Beatson Institute) suggested that pseudopods can be generated and grow without direct control, and that anything that affects the evolution of pseudopods can contribute to cell steering. In Dictyostelium, SCAR (the Dictyostelium WAVE) is the principal regulator of pseudopod growth in crawling cells and it is mostly found phosphorylated and inactive in a cytoplasmic pool. Robert showed that an unphosphorylatable SCAR mutant is hyperactive, and is excessively recruited to the leading edge, resulting in large pseudopods that continually grow forward. In contrast, a phosphomimetic SCAR is weakly active, resulting in frequent small, disorganized pseudopods. He then demonstrated that SCAR in pseudopods is dephosphorylated, which increases activation by Rac and lipids, and promotes positive feedback of pseudopod growth. Thus, a regulated dephosphorylation of Dictyostelium SCAR controls normal pseudopod dynamics and is an important step for normal cell movement.

Alexis Gautreau (CNRS) showed that Arpin regulates the Arp2/3 complex, by localizing to the lamellipodium and competing with Arp2/3 activators. Arpin is activated by Rac, and its role seems to be as a “brake” in the “engine” of lamellipodial dynamics, because its depletion potentiates lamellipodial protrusion and increases cell migration. However, introducing purified Arpin into fish keratocytes decreases cell speed and lamellipodia persistence, resulting in frequent changes in the direction of migration. Arpin is thus analogous to the “steering wheel” of protrusion, regulating the directional persistence of lamellipodia. Moreover, he stated that Arpin is a highly conserved tumor suppressor gene, and is lost in invasive breast carcinoma. In the discussion that followed, we found out that Arpin is absent from plants, yeast, Drosophila and C. elegans.

Src, a non-receptor tyrosine kinase, and its substrate FAK are important in controlling cell adhesion, as discussed by Margaret Frame (University of Edinburgh). She indicated that Src and FAK affect both cadherin-dependent cell-cell contacts and integrin-mediated cell-matrix adhesions, and both these processes change in cancer during epithelial-mesenchymal transition (EMT). She presented work showing that inhibiting Src and FAK suppresses E-cadherin-dependent collective movement in 3D tumor environment, demonstrating the importance of these proteins in tumor formation and progression.

**Immune Cell Migration**

Intravital microscopy was used by Frederic Geissmann (King’s College London) to visualize human monocytes retained in the intra-microvasculature during inflammation. The crawling monocytes are dendritic and extend a number of filopodia-shaped structures. Monocytes crawling on the vascular endothelium express the chemokine receptor CX3CR1 but not the T-cell activation antigen Ly6C (Ly6C<sup>hi</sup> monocytes). Ly6C<sup>hi</sup> monocytes produce interleukin 1β (IL-1β), tumor necrosis factor (TNF) and KC in response to TLR7/MEK signaling. He demonstrated that crawling Ly6C<sup>hi</sup> monocytes adhere to the endothelium via LFA I and its ligand ICAM I, and that this adhesion is independent of chemokine receptor signaling. He discussed his work showing that CX3CR1<sup>hi</sup> monocytes are retained in kidney after kidney treatment with TLR7 antagonist, and that this retention requires CX3CR1 and chemokine receptor signaling. He also indicated that activated monocytes retained in kidney capillaries recruit polymorphonuclear leukocytes, produce IL-1β and damage endothelium in vivo. He proposed that chemokine receptor switch mediates this retention, which may cause kidney damage during TLR-mediated inflammation.

Milka Sarris (Institut Pasteur) addressed the question of leukocyte guidance by chemokines, by analyzing chemokine function.
directly within the interstitial tissues of live zebrafish larvae. She described a newly identified functional zebrafish homolog of the chemokine CXCL8, zCXCL8, which recruits zebrafish neutrophils to sites of bacterial infection. Her work suggests several roles for zCXCL8: it guides neutrophil interstitial migration by directionally biasing their step length (orthotaxis), it affects the random walk of neutrophils, causing faster accumulation (chemotaxis) and it restricts neutrophil motility as they approach the source of infection. She demonstrated that the distribution of secreted zCXCL8 shows a tissue-bound gradient, where the molecules are present on venous vasculature, and extracellular heparin sulfate proteoglycans (HSPGs) are required for optimal recruitment and guidance. She concluded by proposing that leukocytes perceive chemokines as surface-bound gradients (haptotaxis) and that this ensures robustness and precision of their guidance within tissues.

Following on a similar theme, Michael Sixt (Institute of Science and Technology) outlined the directional guidance of leukocytes in vivo, by looking at the intravasation of mature dendritic cells into adherent lymphatic vessels of the skin. In a defined perimeter around the lymph vessels, dendritic cells switch from random motility to highly directional migration toward the vessel. Michael pointed out that this migration of dendritic cells is entirely dependent on the CC-chemokine receptor 7 (CCR7). This receptor is upregulated after contact with pathogens and it recognizes two ligands, CCL19 and CCL21, which are expressed by lymphatic endothelial cells. His findings show that deposits of CCL21 in the lymphatic endothelium decay steeply, creating an interstitial gradient of CCL21 around the vessels, which corresponds to the cell migration pattern. As CCL21 binds to glycosaminoglycans, and is thus immobilized on heparin sulfate residues within the interstitium, which results in haptotaxis. However, CCL19 is not immobilized on surfaces, it does not induce cell adhesion but it does polarize the cytoskeleton and is responsible for the chemotaxis of dendritic cells. He concluded that these functionally active interstitial gradients are therefore responsible for directionally guiding cells through the interstitium.

**Migration during Morphogenesis**

Andrew Ewald (Johns Hopkins Medicine) discussed the regulation of collective cell migration by cell-cell and cell-matrix adhesion, examined by using 3D organotypic culture assays and fluorescently labeled gene activation and inactivation strategies. The model he established is similar to in vivo terminal end buds (TEB) of the developing mammary gland. Cells in the elongating mammary ducts re-organize into a multilayered, stratified epithelium as a result of a shift in the orientation of luminal cell divisions, which give rise to a polarized luminal daughter cell that retains tight junctions and an unpolarized interior daughter cell. Intriguingly, the cells migrate collectively, and rearrange without forming leading cellular extensions. The study presented demonstrated that even after depleting E-cadherin, the cells remained adherent to one another, and myoepithelial cells appear to restrain elongating ducts. Additional genetic events may be required for sustained epithelial dissemination. He suggested that reductions in cell polarity and adhesion observed during breast cancer progression may reflect partial recapitulation of a normal developmental program.

The use of Drosophila embryos to investigate how Drosophila macrophages (hemocytes) are able to prioritize key guidance signals and ignore others was demonstrated by Will Wood (University of Bath). A hierarchy of responsiveness to damage, apoptosis and development growth factors exists in hemocytes and it varies over time. Will discussed his ongoing work on this hierarchy, showing that it involves various cues including the levels of calcium in a wound or the PVR signaling pathway (Drosophila homolog of human PDGF and VEGF receptors). He pointed out that these cues are also used as guidance signals to direct hemocytes along their highly stereotyped migratory routes through the developing Drosophila embryo.

Cell-cell and cell-ECM adhesive forces are involved in directing collective cell migration during Xenopus gastrulation. Douglas DeSimone (University of Virginia) showed that mechanical force applied locally to C-cadherins on single Xenopus mesendoderm cells is sufficient to induce polarized cell protrusion and the persistent migration typical of individual cells within a collectively migrating tissue. Local tugging forces on cadherins are likely to occur in vivo through interactions with neighboring cells, and these forces coordinate changes in cell protrusive behavior. He showed that plakoglobin, a member of the catenin family, is localized to cadherin adhesions under tension and is required for both mechanoreponsive cell behavior and assembly of the keratin cytoskeleton at the rear of these cells. He finished the talk by concluding that cadherin-dependent, force-inducible regulation of cell polarity in single mesendoderm cells represents an emergent property of intact tissue.

**Migration and Disease**

Claudia Wellbrock (University of Manchester) discussed cellular signaling pathways involved in melanoma cell invasion. Normally, keratinocytes in the epidermis of the skin suppress melanocyte motility and E-cadherin plays a major role in the cell-cell communication between these cells. She outlined her work showing that a reduction in E-cadherin expression increased melanocyte migration. P120-catenin, which normally stabilizes E-cadherin, is highly expressed in invasive melanoma, in contrast to many other cancers. She demonstrated that in melanoma cells, E-cadherin is retained in vesicles and its expression is deregulated, and that inhibition of the kinase MEK increases invasiveness of melanoma cells.

Semaphorins inhibit cell migration by collapsing the actin cytoskeleton, which is mediated by the MICAL family of proteins. Jonathan Terman (The University of Texas Southwestern Medical Center) showed how a member of the MICAL family, Mical protein, directly associates with and disassembles actin filaments, which are its specific substrate. He has found that Mical oxidizes a methionine residue in actin crucial for filament assembly. He suggested a specific, oxidation-dependent mechanism
that selectively regulates actin dynamics and inhibitory/repulsive cellular responses induced by semaphorins.

Rho GTPases are intracellular signal transducers that coordinate cell migration. Anne Ridley (King’s College London) has found that RhoA plays a key role in T-cell trans-endothelial migration, where it is associated with protrusion and retraction events, presumably acting through downstream factors that include ROCK (1 and 2) or mDia. RhoB is known to regulate surface β1 integrin levels and focal contact dynamics. Anne showed a novel role for RhoB as a key regulator of the uPA receptor (uPAR)-dependent responses in prostate cancer cells. uPA (urokinase-type plasminogen activator) plays an important role in promoting cancer migration, invasion and adhesion to vitronectin. Her group has found that RhoB affects uPAR-induced integrin expression, association of uPAR with integrins and integrin activity. In addition, uPAR activates RhoB and stimulates RhoB expression. Interestingly, as pointed out in the ensuing discussion, depletion of uPAR has no effect unless it is depleted in cancerous cells.

Eph/ephrin signaling plays a key role during cancer cell migration, particularly during contact inhibition of locomotion, as shown by Kate Nobes (University of Bristol). By analyzing the dynamics of prostate cancer cells co-cultured with fibroblasts, she has demonstrated that contact inhibition of locomotion between cancer cells is mediated by EphA signaling. Ephrins and their receptors have a role in “social interactions” among cells and they regulate the cytoskeleton and cell adhesions. Her group has discovered that invasive PC-3 prostate cancer cells exhibit homotypic contact inhibition, but that contact inhibition is abnormal when they collide with fibroblasts or endothelial cells, due to elevated levels of EphB3 and EphB4 in PC-3 cells. She pointed out that an integrated response to both repulsive EphA and attractive EphB signaling pathways dictates whether a cancer displays contact inhibition of locomotion. Finally, she concluded that the migration of aggressively invasive cancer cells can switch from restrained to invasive, depending on the Eph-receptor profile of the cancer cell and the reciprocal ephrin ligands expressed by neighboring cells.

Kurt Anderson (Beatson Institute) presented the use of fluorescence life-time imaging of FRET-based biosensors to study regulation of cell migration. He discussed the latest findings in his work, where using a mouse model of pancreatic cancer he showed how he can investigate the spatial and temporal activation of proteins such as Rho or Src kinase. He demonstrated that metastatic cells show high Src activity in central and in peripheral tumor regions.

The meeting ended with a study of migration of leukocytes in zebrafish and how this can be correlated with human disease, from Anna Huttenlocher (University of Wisconsin-Madison). Both wounds and tumors generate hydrogen peroxide (H₂O₂), which is sensed by leukocytes and contributes to their rapid recruitment to damaged tissue. Wounding activates Src family kinases (SFK) in neutrophils and her group has found a member of this family, Lyn kinase, to be a redox sensor. She demonstrated that Lyn kinase mediates the initial neutrophil recruitment to wounds in zebrafish larvae and its activation in neutrophils depends on wound-derived H₂O₂ after tissue injury. Her group has also identified a Src family kinase Fyn as a redox sensor in the epithelium that mediates wound resolution. She summarized the talk by stating that redox and SFK signaling are important immediate wound signals that integrate early wound responses by leukocytes and epithelial cells and subsequent wound resolution.

Overall, the 7th Abercrombie Meeting was a great success, with many highly insightful talks covering a wide range of areas in cell migration. An impressive array of techniques used to study migration was presented, including many stunning images and movies. All the talks were followed by vibrant discussions, which continued on through dinner and late into the night, demonstrating the high level of interest generated by the talks and posters. As a relative newcomer to the field, I learnt a great deal about the incredible diversity of the field of cell migration, the range of tools available to study it, and the current state of the art in our understanding about what drives cells to migrate (and stop). It was a very enjoyable experience and I am already looking forward to the next meeting in 2017.