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Cell matrix adhesion in cell migration

James RW Conway [1] and Guillaume Jacquemet [1,2]*

1: Turku Bioscience Centre, University of Turku and Åbo Akademi University, FI-20520 Turku, Finland.
2: Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, 20520 Turku, Finland

*: Correspondence to : Guillaume Jacquemet (guillaume.jacquemet@utu.fi)

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Abstract (200 words):

The ability of cells to migrate is a fundamental physiological process involved in embryonic development, tissue homeostasis, immune surveillance and wound healing. In order for cells to migrate, they must interact with their environment using adhesion receptors, such as integrins, and form specialised adhesion complexes that mediate responses to different extracellular cues. In this review we discuss the role of integrin adhesion complexes (IACs) in cell migration, highlighting the layers of regulation that are involved, including intracellular signalling cascades, mechanosensing and reciprocal feedback to the extracellular environment. We also discuss the role of IACs in extracellular matrix remodelling and how they impact upon cell migration.

Main text:

Introduction

Translocation of cells is a fundamental physiological process that is essential to both normal tissue homeostasis and the development of multiple diseases. In tissues, cells migrate within a complex three-dimensional (3D) environment composed of neighboring cells and extracellular matrix (ECM), where efficient directional migration requires tight control over both cell-cell and cell-ECM adhesion machinery. One of the hallmarks of the migration process is flexibility and plasticity, where cells can migrate using various modes that differentially rely on adhesion machinery (Figure 1). During single cell migration on two-dimensional (2D) surfaces, cells migrate principally using lamellipodia-based protrusions (Figure 2). In this context, migrating cells can display surprising heterogeneity by adopting and switching between modes when strong or weak cell-ECM adhesions are required (1). In 3D, cells often migrate collectively as either a cluster or a stream, which relies upon cell-cell junction dynamics. In this context, leading cells can use lamella- and/or filopodia-like protrusions to engage the ECM and guide the movement of the group (2). Single cells can also adopt various modes of migration in 3D, including amoeboid protrusive or blebby, lobopodial, lamellipodial and/or pseudopodial (3,4); many of which have also been observed in vivo (5–7) (Figure 1). Importantly, specific cell types display preferences towards a particular migration mode, but many also demonstrate a highly plastic nature, often shifting between migration modes to adapt to particular situations (Figure 1) (8–10). Mechanistically, these migration modes are driven by different pathways and cells adopting these can differ in their shape, their use of membrane protrusions and their reliance on cell-ECM adhesion machinery. For instance, during amoeboid migration, cells remain rounded and extend pseudopods or membrane blebs to move forward. Amoeboid migration can be relatively fast and involve a weaker, more “passive” adherence to the substrate (17 μm/min, speed of normal human neutrophil (11)). Amoeboid cells can also migrate without using their cell-ECM adhesions, through actin cortical flow and membrane protrusions (12–14). In contrast, during lamellipodial, lobopodial and pseudopodial migration cells are elongated and in order to move they protrude their membrane forward at the leading edge, and retract it at the trailing edge. These types of migration require constant disassembly and recycling of old cell-ECM adhesion sites, along with the formation of new adhesions at the leading edge, and this spatiotemporal balance is crucial for effective cell migration. Cells adopting elongated migration modes rely strongly upon their cell-ECM adhesion machinery to move forward, which results in slower migration speeds (0.234 μm/min, speed of normal human dermal fibroblast (15)) (16).

The ECM is an intricate proteinaceous mesh where cells are able to recognise characteristic signatures, which can vary dramatically depending on the tissue or disease assessed (17–19). In order to bind to the ECM, cells primarily utilise the integrin family of transmembrane receptors, where integrin-ECM engagement results in the formation of integrin adhesion complexes (IACs) that bridge the ECM and the cell cytoskeleton (Figure 3). Through this cell-ECM bridge, IACs orchestrate cellular behaviours including migration, proliferation and cell fate. This bridge also provides a platform for ECM deposition and remodelling. In this review we will focus on the role of integrin-mediated cell adhesion to the extracellular matrix and highlight this contribution to the migration process. Much of this work builds upon studies looking at lamellipodial migration in 2D, but with advances in intravital microscopy and 3D systems the complexities of different migration modes will also be discussed in these higher fidelity scenarios.

Integrins

The integrin family is composed of 24 heterodimers generated from 18 α and 8 β subunits. These type I transmembrane glycoproteins combine a large extracellular domain with a short cytoplasmic tail (less than 50 amino acids; except for integrin β4 [>1000 amino-acids]) and can be broadly classified into RGD-, collagen-, laminin- and leukocyte-specific receptors. Inside the cell, the integrin tails function as platforms for the recruitment of regulatory elements. These IACs are then important for cytoskeletal reinforcement, along with downstream signalling cascades involved in survival, proliferation, polarisation and migration (Figure 3) (20–22). Most integrin heterodimers can interact with more than one ligand, with several heterodimers capable of binding the same ligand, but with different affinities or intracellular responses. For instance, both α5β1 and αVβ3 integrins can bind to fibronectin (FN), but only αVβ3 can bind to vitronectin (for a more exhaustive review of the various integrin heterodimeric combinations and their ligands see (23)). Interestingly, during FN adhesion initiation αVβ3 and α5β1 integrins cooperate, where αVβ3 initially outcompetes α5β1, but once engaged, αVβ3 promotes α5β1 activation and clustering, further strengthening cell adhesion (24,25).

At the plasma membrane, integrin functions are tightly regulated by intracellular trafficking and a conformational switch that modulates ECM binding, often referred to as activation (Figures 3 & 4). Integrin conformations can range from a bent to an extended open conformation, where the ligand affinity increases with stepwise opening (26–28). However, while this opening is important for the activation of several integrin heterodimers, not every integrins may follow this stepwise unfolding and some can instead assume a constitutively extended conformation (29,30). For specific integrin heterodimers, assessment of this unfolding can be performed using activation specific antibodies (31).

Integrin adhesion complexes (IACs)

Adhesion serves two major functions in migration. Firstly, it generates traction by linking the extracellular substratum to the cellular cytoskeleton, and secondly, it organizes the signaling networks that regulate migration. Integrin-ECM engagement leads to integrin clustering and the formation of macromolecular complexes that support adhesion with IACs. Given the broad range of cell types and extracellular environments within the body, it follows that IACs can take many forms in both migrating and non migrating cells (Figures 2, 5 & 6). By far the best characterised are FAs and FA-like structures, which are discussed further below (Figures 2 & 5). Additionally, some specialized cell types also utilise unique IACs, such as hemidesmosomes (32), podosomes (33), invadopodia (34) and the immunological synapse (35) not all of which are directly involved in the migration process (Figures 2 & 6).

Focal adhesions (FAs) and FA-like structures

The most well characterised adhesive structures involved in the migration process are focal FAs and FA-like structures. Microscopy-based studies of cells migrating on 2D substrates have classified FA-like structures in terms of maturation stage by assessing the components of the IAC, along with their subcellular distribution, shape and size (Figure 5). Many adhesions are thought to progress from early filopodial adhesions to nascent, focal and finally, fibrillar adhesions, but this process is heavily cell-type dependent and may begin at any of the maturation stages (Figure 5) (36–38). Early adhesions, such as filopodia and nascent adhesions, are very dynamic and support the migration process by enabling a constant probing of the cellular environment, while more mature adhesions, such as focal and fibrillar, allow the cell to exert traction on and remodel the ECM. Recent studies have further linked adhesion maturation with loss of talin and recruitment of tensin, which in fibroblasts can lead to metabolic reprogramming at these more stable complexes (39,40). At the nanoscale, FAs are vertically stratified into conserved layers: a membrane proximal integrin signalling

layer (containing integrins, paxillin and focal adhesion kinase (FAK)), an intermediate force transduction layer (containing talin and vinculin) and an actin regulatory layer (containing both actin and actin regulatory elements) (41–44). Further unbiased characterisation of FA and FA-like structures by literature curation (45,46) or mass spectrometry (MS) has also highlighted the vast complexity of these structures. In particular the molecular composition of FA and FA-like structures is affected by the integrin heterodimers (25), the ECM ligands (47), mechanical forces (48–50), integrin activation state (51) and the maturation time (50). Despite these variations, compilation of multiple MS datasets from cells adhering to FN have revealed a core cell adhesion machinery of around 60 components, which is collectively termed the consensus adhesome (50,52). It is important to note that these unbiased MS studies have not all been correlated with microscopy-based studies and that the precise composition of the different types of FA and FA-like structures remains unknown. Furthermore, the assessment of cells in 3D and adhering to different substrates is necessary for the consensus adhesome to fully encompass the vast complexity of the cellular adhesion machinery.

The spatiotemporal regulation of the assembly and disassembly of FAs and FA-like structures is essential for efficient cell migration, where defects can lead to failures in tail retraction and loss of directionality (53,54). There are several mechanisms involved in the disassembly of IACs, including microtubule targeting (55–57), degradation by proteases (58–60), mitotic progression (61) and integrin endocytosis (62). Importantly, if these adhesion platforms are too stable, cells will be unable to protrude the leading edge or retract their tail, resulting in an inability to move. In contrast, reduced or unstable adhesions may compromise cell attachment to the substrate, traction force generation and the signal transduction pathways necessary for directed cell migration. Of note, individual integrins are surprisingly motile within FAs where their immobilisation can last less than 80 sec (63). In addition, the dynamics of free-diffusion and immobilization are different between integrin heterodimers and likely provide further functional specificity within different FA and FA-like structures (64).

While FA-like structures can be observed in cells migrating in 3D (65) (see (66) for review), a greater challenge is the visualisation of these small molecular complexes in a more complex in vivo setting. For example, lamella-like protrusions have been observed in leading cells during collective invasion in mouse models (7). However, higher resolution imaging is still required to fully elucidate the organisation and architecture of these structures, where one interesting in situ example was achieved through paxillin staining of human endothelial cells lining the vascular basement membrane of several tissues (67). FA-like structures have also been observed in migrating cardiac cells in the developing heart of zebrafish embryos, where their components regulate collective migration during development (68). Similarly, analysis of drosophila development has identified several defects resulting from dysregulation of IACs, emphasising the essential nature of these structures in normal tissue homeostasis (69–71). Hence, the conserved and essential role of FA-like structures is apparent at the organism level and many studies are now aiming to gain a more in-depth understanding of FA composition and dynamics at the nanoscale.

Clathrin plaques (CPs)

Recently, a class of atypical IACs, referred to as flat clathrin lattices (72,73), reticular adhesions (61), or clathrin plaques (CPs) (74) have emerged as prominent adhesive structures for cell migration in 2D and 3D environments (72,74) (See (75) for detailed review). It is important to note that it has not been established if these structures are identical, but they share many similar properties and will be referred to herein as CPs for clarity. In cells migrating in 2D, CPs are enriched in β5 integrin (61,73,76), which is required for their formation (73). Other integrins can also be recruited to these structures depending on the cell contractility

status (73). MS analyses of CPs has revealed an absence of classical IAC components and instead, an enrichment of components belonging to the integrin endocytic machinery, including clathrin, AP2, numb and dab2 (Figure 2) (61,73). In addition, unlike FAs, CP formation is not dependent on an intact actin cytoskeleton or on myosin contractility, but they maintain mechanosensitivity (61,76). Indeed, the exact relationship between CPs and the cell cytoskeleton remains to be fully elucidated, as CPs do not appear to be directly connected to actin, but rather surrounded by branched actin filaments and intermediate filaments (77). A functional and spatiotemporal interplay between FAs and CPs has also been described, where digestion of the ECM at FAs was shown to create topographical cues that dictated the future location of CPs, contributing to directional cell migration (74). Moreover, while most FAs dissociate during mitosis, CPs persist and maintain cell-ECM attachment (61). So, with emerging links to cell migration and adhesion in vitro, further work is required to not only elucidate their role in the migration process, but also to ascertain their requirement in vivo.

Signalling by IACs

IACs can integrate both biochemical (ECM composition) and mechanical (ECM stiffness) cues, and transduce this information through both biochemical signalling cascades and mechanical organisation of the cytoskeleton. In the context of directed cell migration, IAC signalling mediates durotaxis (migration towards stiffer substrates), chemotaxis (migration towards a higher chemokine concentration) and haptotaxis (migration towards higher ECM concentrations) (78). These signals also modulate the activation of transcription factors, such as YAP/TAZ or SRF, and can lead to changes in the gene expression profiles of cells (79). Some of these gene expression changes can be long lasting (days after the interaction) due to epigenetic changes (80), while others directly modulate the expression of adhesion molecules and support forward movement (81). Interestingly, removal of the nucleus had no discernible effect on short-term directional cell migration on 2D substrates, but was found to be paramount for efficient 3D migration (82,83). This suggests that a transcriptional response may not be required to initiate 2D migration and highlights the differential requirements for different migration modes in 2D and 3D environments.

IACs are phosphorylation platforms that are especially enriched for tyrosine phosphorylation, suggesting an important regulatory role of kinases and phosphatases at these signaling hubs (84,85). Indeed, many classical cell migration-linked signaling molecules and adaptors are regularly associated with IACs, including FAK, Src, and paxillin, as well as the ILK/PINCH/PARVIN and p130Cas/CRK complexes (86). Importantly, the activation of kinase signalling events upon integrin-ECM engagement can be rapid, exemplified by α5β1 integrin-FN binding, which activates FAK and Src in less than half a second (87). Similarly, small GTPase signalling downstream of IACs regulates cytoskeletal dynamics, membrane protrusions and cell contractility. In 2D, the small GTPases RhoA, Rac1 and CDC42 contribute to the precise spatiotemporal coordination of the migration process (88) and their activation is tightly regulated by integrin-mediated cell adhesion (53,89). In 3D, differential activation of specific small GTPases at the leading edge can define the mode of cell migration. For instance, mesenchymal migration is primarily driven by Rac1, while pseudopodial and amoeboid are regulated by RhoA (8). Importantly, dynamic regulation of these small GTPases allows cells to switch between migration modes, while also contributing to integrin activation and IAC formation (90).

Significantly, cell adhesion to different ECMs can lead to the formation of IACs with overlapping but distinct compositions, which in turn lead to different signalling outputs. For instance, MS analyses of FN or V-CAM-induced IACs identified RCC2 as a specific component of FN-induced IAC (47). In this context,

RCC2 was found to regulate the small GTPases Rac1 and Arf6, which resulted in modulation of directional migration on cell-derived matrices (47). The complexity of IAC signalling is further increased by the fact that different integrin heterodimers binding to the same ECM molecule can trigger different cellular responses that in turn lead to different types of cell migration. For example, in fibroblasts migrating on FN in 2D, αVβ3 favours lamellipodium-driven directional cell migration, while α5β1 engagement leads to RhoA-ROCK-mediated phosphorylation of cofilin and rapid, random migration (91,92). In this way, we can see that signalling by IACs is a finely-tuned process that provides an essential bridge, mediating external cues through complex and specific intracellular signalling cascades.

Mechanosensing by IACs and the molecular clutch

At the leading edge of migrating cells, actin polymerises and flows backwards towards the cell body. This flow of actin connects to and pulls on integrin cytoplasmic tails via talin. This mechanical force is then transduced to the ECM through integrin heterodimers and drives cell protrusion. The efficiency of this cytoskeleton-Integrin-ECM bond to convert force into protrusion is variable and this modulates the migration response of the cell (93). The actin retrograde flow is modulated by both external and internal forces that are generated by myosin motors, membrane tension and substrate rigidity. This retrograde flow also contributes to the organisation and alignment of ECM-engaged integrins within FAs (94,95). Integrins demonstrate variable affinities for their ligands in response to these external stimuli and this provides a feedback mechanism for the cell to mediate intracellular responses (Molecular clutch dynamics reviewed in (96,97)). In addition, multiple IAC proteins, such as talin, vinculin and p130Cas, are mechanosensitive and their functions are strengthened by increased force (Figure 3) (98–100). The balance between these external forces, which are modulating adhesion strength, and the internal forces applied through actin cytoskeletal connections can then result in disassembly or reinforcement of IACs (96,97). Importantly, mechanical forces exerted on talin induce a conformational change that triggers a switch from talin-RIAM to talin-vinculin complexes and promote adhesion stabilisation and cell spreading (101–103). In this context, engagement of the ECM-integrin-actin molecular clutch can also contribute to local rearrangement of the plasma membrane, such as through the formation of glycosylphosphatidylinositol-anchored protein nanoclusters that also support cell spreading (104). Increasingly, our understanding of cellular mechanics is unveiling novel therapeutic opportunities (reviewed in (105)), and in vivo models are already showing promising preclinical efficacy when overalying their results with stiffness modulation of the cancer microenvironment (106,107).

Modulation of integrin functions and regulation of cell migration

Regulation of cell migration via integrin cytoplasmic tails

Integrin activity can be mediated by both ligand binding (outside-in activation) and by the recruitment of proteins to the integrin cytoplasmic tails (inside-out activation). Key integrin activators include talin, kindlin and tensin, while key integrin inactivators include ICAP1, SHARPIN and filamin-A (Figure 3 and (108) for review). Modulation of integrin activity has a strong impact on how cells interact with the ECM. Unsurprisingly, integrin activity regulators strongly contribute to cell migration, and their misregulation is often associated with diseases, such as cancer, fibrosis and cardiovascular disease (109). Recently, a mouse harboring an activating mutation in talin was used to demonstrate that increased talin-mediated integrin activity leads to more stable adhesions and impaired wound healing in vivo (110). Furthermore, in both fly and worm, defects in integrin activity lead to severe developmental deformities, suggesting a high level of evolutionary conservation that reinforces the importance of careful integrin regulation for multicellular organisms (111–113). Of note, only a small subset of known integrin tail binders have been implicated in the

regulation of integrin activity, instead they are likely to contribute to the migratory process by tuning the integrin response (114). For example, MENA, a member of the ENA/VASP family, binds to α5 integrin and modulates IAC signalling on FN or FN-rich matrices and can contribute to haplotaxis towards FN in vivo (115,116). However, the precise coordination of different integrin tail binders during cell migration remains poorly understood.

Regulation of cell migration by integrin trafficking

Integrin trafficking controls the membrane availability of integrin heterodimers through both clathrin-dependent or -independent pathways (Figure 4) (22,117). Importantly, both active and inactive integrins traffic through different compartments and are recycled at different rates (118). Once internalised, active integrin can also signal from recycling endosomes, a feature which in cancer cells contributes to anoikis resistance (119). Furthermore, migrating cells can maintain integrins in an active conformation, recycling them towards the leading edge, and this has been proposed to contribute to directional cell migration (120). Most integrin heterodimers are recycled back to the plasma membrane, with only a small proportion being degraded in the lysosomal compartment (121,122). Depending on the cell type, differential trafficking of heterodimers can also modulate specific responses to ECM cues, such as the formation of nascent adhesions upon cell spreading, or ruffling of the membrane at the cell front (123,124). This tight control of integrin recycling can also lead to migratory defects and eventually disease progression.

As integrin internalization contributes to IAC disassembly, misregulation of integrin endocytosis often leads to impaired cell migration with cells displaying tail retraction defects (125). Hence, changes in integrin recycling can lead to profoundly different phenotypes depending on the context. In ovarian or pancreatic carcinoma, preferential recycling of α5β1 over αVβ3 integrins promotes a switch from mesenchymal to pseudopodial cell migration on cell-derived matrices and cell invasion into FN-rich ECM (126,127). Mechanistically, α5β1 integrins are co-recycled with growth factor receptors, such as EGFR (Figure 4) (128). This can lead to increased EGFR signalling and the local activation of the PI3K/Akt pathway, in turn promoting filopodia formation in a RhoA- and FHOD3 formin-dependent manner (8,129). In agreement with these studies, the activity of RhoA in the invasive tip of metastatic tumour cells has been tracked *in vivo*, where pancreatic tumour cells show a clear polarization (130). Furthermore, in ovarian carcinoma the small GTPase Rab25 promotes invasive migration through 3D matrices (131,132) and is associated with increased aggressiveness of epithelial cancer cells in vivo (133). Rab25 is known to directly associate with β1 integrin and promote the recycling of α5β1 integrins towards the cell surface (131). Interestingly, in head and neck squamous cell carcinoma tumours with mixed populations of cells expressing the GTPase Rab25, or with Rab25 knocked out, only cells lacking Rab25 invaded towards lymphatic vessels, away from the primary tumour (134). This example further highlights the context-dependence of integrin recycling pathways, which can result in profoundly different phenotypes. This complex, regulation of integrins functions via intracellular trafficking could help to explain the limited clinical success of anti-integrin therapies, such as cilengitide (targeting RGD receptors such as αVβ3 integrin), which in some context, displays preclinical efficacy but in others drives cancer cell invasion (8, 109,135).

Regulation of cell migration by integrin co-receptors and the glycocalyx

In migrating cells, integrin functions are further modulated via cross-talk with co-receptors, such as receptor tyrosine kinases (RTKs), CD98hc, neuropilin and syndecan-4 (Figures 3 & 4) (136–139). In particular, stimulation of cells with growth factors often leads to cytoskeletal rearrangement, chemotaxis, increased cell

migration and invasion. Indeed, multiple growth factor receptors have been described to synergise with integrin signalling (see (140,141) for reviews). For instance, EGFR stimulation can trigger a rapid change in the composition of IACs (142), while integrins can also influence the subcellular distribution, clustering and expression of growth factor receptors, along with their signalling (140,141). IACs are also associated with additional surface molecules, including selectins, chemokines and the glycocalyx (Figures 3 & 4) (143–145). The glycocalyx is a glyocoprotein- and carbohydrate-rich coating that surrounds many eukaryotic cells and is often associated with cell fate decisions and cancer progression (144). Importantly, the glycocalyx can facilitate integrin clustering upon ligand binding (146) that can contribute, for instance, to glioblastoma progression and dissemination (147).

Furthermore, syndecans (a small family of transmembrane proteoglycans) can also strongly modulate integrin functions and cell migration. Most ECM molecules possess both integrin- and syndecan-binding sites, and IAC formation on several matrix ligands requires engagement of both syndecan and integrin (136). In fibroblasts, Syndecan-4 is required for integrin-mediated adhesion and signalling on FN and contributes to cell migration in vitro and to wound healing in vivo (89,148,149). Moreover, Syndecan-4 signalling regulates the differential recycling of αVβ3 and α5β1 integrins, which guides cell adhesion dynamics and migration mode (54). Cumulatively, co-receptors and the glycocalyx provide an important layer of regulation for IACs, fine-tuning their responses to extracellular cues.

Integrin adhesion complexes serve as platforms for ECM remodeling

Stromal cells constantly secrete, deposit and remodel ECM molecules. The properties of the resulting ECM (molecular composition, topology and bulk mechanical properties) then guide the migration behaviour of other cells. For instance, early work found that cancer-associated fibroblasts (CAFs) assemble tracks composed of thick collagen fibres and FN, which facilitates cancer cell invasion into a 3D ECM (150). Furthermore, these tracks have established significance for collective migration, where integrins play an essential role in this invasive progression (151). ECM generated and remodelled by CAFs is also generally stiffer, which drives both cancer cell invasion (152) and proliferation (80). Furthermore, this can also provide a source of energy for cancer cells during starvation or stressful conditions (153).

Mechanistically, all the pathways which regulate IACs are likely to be implicated in ECM deposition and remodelling. One of the best understood examples is the assembly of FN fibrils, which is a multistep α5β1-dependent process and requires cells to apply mechanical force. Soluble FN molecules are first captured by α5β1 integrins in talin-rich FAs at the cell periphery. α5β1 integrins then move inward to leave the FA and populate fibrillar adhesions. During this translocation, the mechanical forces exerted by α5β1 integrin cause a conformational change in FN that exposes self-association sites and allows fibril elongation and maturation (154). It is therefore not surprising that pathways regulating integrin trafficking and activation also contribute to FN remodelling. For instance, modulation of integrin activity via tensins has been implicated in FN fibre formation (39). As another example, FN fibrillogenesis is also strongly modulated by the composition of the underlying ECM (155). In endothelial cells, active α5β1 integrin is recycled together with FN and this process regulates FN secretion and fibrillogenesis (156). The tightly regulated delivery of ECM molecules is not limited to FN as, for instance, collagen type X was recently shown to be secreted near FA (157). Collectively, integrins play an essential role in the assembly of the ECM by stromal cells, laying the groundwork for multicellular tissue formation.

Future perspectives

Given the important contribution of IAC to cell adhesion and migration, it is not surprising that integrins and integrin-associated molecules are considered to be attractive drug targets, where anti-integrin therapies are already used in the clinic to treat clotting disorders, multiple sclerosis and inflammatory bowel disease (109,135,158). However, current therapies aimed at targeting cancer or fibrosis have been met with disappointment. This may be partially explained by our limited in situ understanding of IAC structure and function. To address this, we are seeing advances in intravital microscopy and targeted mass spectrometry, using endogenously tagged fluorescent proteins or biotin ligases, that are providing high resolution characterisation of both the spatiotemporal organization and molecular composition of IACs in a more physiologically relevant scenario (159–161).

There remains an important place for 2D studies however, where reductionist scenarios are necessary to initially deconvolve the sheer complexity of these structures, which contain hundreds of proteins (50). Importantly, these signaling events (mechanical and biochemical) are transduced and coordinated within an IAC that can contain several integrin heterodimers, all binding to a complex mixture of ECM. In order to tease out the individual functions of each component, as well as the complex feedback loops and compensatory mechanisms involved, it is likely that mathematical modeling (162) or deep learning approaches will be required. As we increase our understanding of IACs, their complexity continues to baffle even the most sensitive experimental set ups. Moving forward, we expect to see multidisciplinary approaches tackle this complexity from many angles, to the eventual goal of understanding the complete structure and function of IACs, and to be able to apply this knowledge for therapeutic benefit.

Summary points

- Cell migration displays a high level of plasticity and a broad range of cell-ECM dependencies, with many cell types applying different modes depending on the situation
- IACs provide a bridge between the ECM and intracellular signalling cascades
- Integrin clustering leads to the formation of specialised adhesion complexes and guides directional cell migration
- Fine-tuning of IAC formation and stability takes multiple forms, including mechanical feedback, trafficking and co-receptor modulation of ligand affinities
- IACs facilitate stromal remodelling of the ECM to guide migratory behaviour for multicellular tissue development

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Writing – Original Draft, G.J. and J.R.W.C; Writing – Review and Editing, G.J., J.R.W.C.; Visualization, G.J. and J.R.W.C.;

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Figure Legends

Figure 1: Three-dimensional migration of single and collective cells

(**A**) Schematic illustrating the various modes of 3D cell migration described as well as their key characteristics. (**B**) Representative images of cancer cells migrating on cell-derived matrices using amoeboid (fibrosarcoma cell), mesenchymal (fibrosarcoma cell) or pseudopodial (ovarian carcinoma cell) modes of migration. Cells were transfected with life-act GFP to visualise the actin cytoskeleton and imaged using a spinning disk confocal microscope (for methods see (8)). Videos are provided as supplemental information.

Figure 2: Adhesion structures found on a typical lamellipodia-driven cancer cell migrating in 2D

Schematic of a typical cancer cell with (**A**) invadopodia, (**B**) filopodia, (**C**) CPs (purple) and other IACs (black), as well as (**D**) lamellipodia. (**E**) U2OS cell expressing lifeact-mturquoise was plated on fibronectin and imaged using an airyscan confocal microscope. The video is provided as supplemental information. (**F**) U2OS cells were plated on vitronectin for 24 hr, stained for F-actin, paxillin and integrin β5, and imaged using structured illumination microscopy.

Figure 3: Integrin outside-in and inside-out signalling and activation

(**A**) Schematic representation of integrins at the plasma membrane in both bent (inactive) and extended (active) conformations, where collagen fibres are promoting clustering and IAC formation. (**B**) Downstream signal transduction from the IAC complex, with reinforcement of the actin cytoskeleton. (**C**) Summaries of the key components of an inactive integrin heterodimer (left), an open integrin heterodimer undergoing mechanical activation (middle), and a fully active integrin heterodimer (right).

Figure 4: Recycling and cross-talk of IACs with receptor tyrosine kinases (RTKs)

(**A**) Clustering and co-signalling of RTKs at the plasma membrane. (**B**) Recycling of integrins either alone, or in concert with RTKs.

Figure 5: Maturation of FA and FA-like structures on a typical fibroblast
(A) Schematic of a typical fibroblast where adhesions are thought to mature along a progression model of filopodia adhesion (i), to nascent (ii) and focal adhesions (iii), then finally to fibrillar adhesions (iv). (B) U2OS cells expressing RFP-tagged Myosin-X were plated on fibronectin for 2 hr, stained for F-actin, phospho p130CAS and paxillin, and imaged using structured illumination microscopy. (C) Human fibroblasts were plated on fibronectin for 24 hr, stained for F-actin, fibronectin and paxillin, and imaged using structured illumination microscopy.

Figure 6: Adhesion structures found on specialised cell types
Schematic representations of (**A**) the immunological synapse, (**B**) a podosome and (**C**) a hemidesmosome.

Video 1: Amoeboid cancer cell migration.

Fibrosarcoma cell migrating on cell-derived matrices using amoeboid mode of motility. Cells were transfected with life-act GFP to visualise the actin cytoskeleton and imaged using a spinning disk confocal microscope.

Video 2: Mesenchymal cancer cell migration.

Fibrosarcoma cell migrating on cell-derived matrices using mesenchymal mode of motility. Cells were transfected with life-act GFP to visualise the actin cytoskeleton and imaged using a spinning disk confocal microscope.

Video 3: Mesenchymal cancer cell migration.

Ovarian carcinoma cell migrating on cell-derived matrices using mesenchymal mode of motility. Cells were transfected with life-act GFP to visualise the actin cytoskeleton and imaged using a spinning disk confocal microscope.

Video 4: 2D cell migration.

U2OS cell expressing lifeact-mturquoise were plated on 2D fibronectin and imaged using an airyscan confocal microscope.