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Adhesion and Growth Factor Receptor Crosstalk Mechanisms Controlling Cell Migration

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Integrin, Growth Factor Receptor, Syndecan, Migration, Adhesion, Trafficking, Endocytosis, Signalling,

Abbreviations:

AKT - AKT Serine/Threonine Kinase
c-MET - Hepatocyte growth factor receptor
ECM - Extracellular matrix
EGF - Epidermal growth factor
FAK - Focal adhesion kinase
EGFR - Epidermal growth factor receptor
FAK - Focal Adhesion Kinase
FGFR - Fibroblast growth factor receptor
GFR - Growth factor receptor
HSPG - heparan sulfate proteoglycans
IAC - Integrin-associated complex
MAPK - Mitogen activated protein kinase
PI3K - Phosphoinositide 3-kinase
PKC - Protein kinase C
RCP - Rab-coupling protein
RTK - Receptor tyrosine kinase
TCPTP - T-cell protein tyrosine phosphatase / PTPN2
TGFβ - Transforming growth factor β
TGFβR2 - Transforming growth factor β receptor 2
VEGFR2 - Vascular endothelial growth factor receptor

Abstract

Cell migration requires cells to sense and interpret an array of extracellular signals to precisely co-ordinate adhesion dynamics, local application of mechanical force, polarity signalling and cytoskeletal dynamics. Adhesion receptors and growth factor receptors exhibit functional and signalling characteristics that individually contribute to cell migration. Integrins transmit bidirectional mechanical forces and transduce long-range intracellular signals. Growth factor receptors are fast acting and highly sensitive signalling machines that initiate signalling cascades to co-ordinate global cellular processes. Syndecans are microenvironment sensors that regulate GTPases to control receptor trafficking, cytoskeletal remodelling and adhesion dynamics. However, an array of crosstalk mechanisms exists, which co-ordinate and integrate the functions of the different receptor families. Here we discuss the nature of adhesion receptor and growth factor receptor crosstalk mechanisms. The unifying theme is that efficient cell migration requires precise spatial and temporal co-ordination of receptor crosstalk. However, a higher order of complexity emerges; whereby multiple crosstalk mechanisms are integrated and subject to both positive and negative feedback. Exquisite and sensitive control of these mechanisms will ensure that mechanical forces and pro-migratory signals are triggered in the right place and at the right time during cell migration. Finally, we discuss the challenges, and potential therapeutic benefits, associated with deciphering this complexity.

Summary Points

Cell migration requires precise co-ordination of adhesion dynamics, mechanical force application, polarity signalling and cytoskeletal dynamics.

Crosstalk mechanisms exist that integrate integrin, growth factor receptor and syndecan signalling, trafficking and function.

Spatial and temporal regulation of crosstalk mechanisms fine-tunes cell migration by co-ordinating adhesion dynamics, GTPase signalling, cytoskeletal dynamics and application of actomyosin-dependent traction forces onto the matrix.

Adhesion receptor and growth factor receptor crosstalk is subject to feedback mechanisms and a higher order of complexity; enabling functional integration of multiple crosstalk mechanisms.

Mathematical modelling and systems biology approaches will be essential to fully dissect the complex regulatory mechanisms co-ordinating cell migration. Understanding this complexity will provide insight into the fundamental mechanisms that drive cell migration, but also how these mechanisms are dysregulated in disease. Ultimately, these insights will inform patient stratification, efforts to prevent acquired drug resistance and development of novel therapeutic approaches in cancer.

Introduction

Cell migration plays a critical role in many physiological and repair processes, including developmental morphogenesis, maintenance of tissue homeostasis, wound healing and immune surveillance, and drives the pathogenesis of numerous neoplastic and inflammatory diseases. The topological, biophysical and biochemical characteristics of the local microenvironment directly control cell migration[1]. Consequently, to enable efficient migration, cells sense and interpret an array of extracellular signals to precisely co-ordinate adhesion dynamics, local application of locomotive mechanical forces, polarity signalling and cytoskeletal dynamics[1].

The extracellular matrix (ECM) is a 3-dimensional scaffold providing positional, structural and chemical information to co-ordinate cellular functions[2]. Haptotactic migration is characterised by directed cell motility towards or along an immobilised ECM substrate. However, *in vivo*, cells encounter a complex microenvironment incorporating fibrillar and laminar ECM substrates of varying rigidities and porosity. Moreover, cells are exposed to soluble growth factors and guidance cues, which may be diffusible or immobilised on ECM at discrete sites or as gradients.

As growth factors can bind a range of ECM proteins, the matrix can essentially act as a reservoir with the capacity to retain and release growth factors, following specific environmental or cell-mediated stimuli. The ECM is metastable; an apparently stable scaffold, that is subject to dynamic cell-mediated mechanical deformation, remodelling and turnover[3]. Thus, mechanical- and matrix metalloproteinase-mediated ECM remodelling and degradation can regulate the bioavailability of growth factors and chemokines[4]. Emerging evidence suggests that detection of disparate matrix and growth factor stimuli within a complex microenvironment, provides exquisite spatial and temporal control of the cellular processes required to drive cell migration[5].

Signals initiated downstream of individual adhesion receptors and receptor tyrosine kinases are relatively well understood[1,6,7], but the complex regulatory mechanisms that co-ordinate crosstalk between these different classes of receptor remain largely obscure. Evidence of adhesion and growth factor receptor crosstalk has existed for some time, but only recently has it been possible to gain insight into the mechanisms that orchestrate adhesion and growth factor receptor crosstalk spatially and temporally. Here we discuss emergent concepts in the field and consider how these regulatory mechanisms might co-ordinate different functions associated with cell migration (Figure 1).

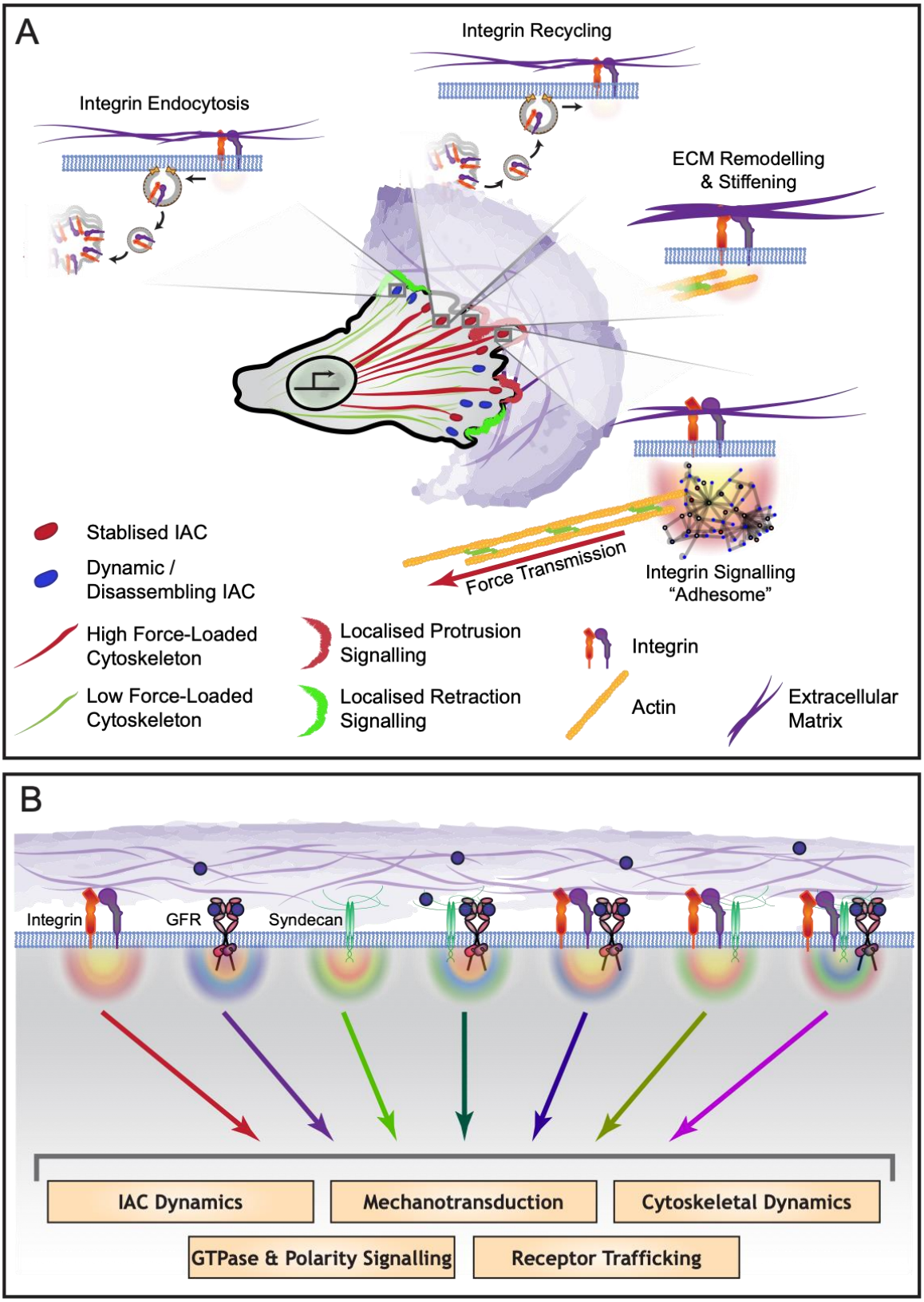

Fig 1: Adhesion receptor-growth factor receptor crosstalk regulates cell migration
A) Cell migration requires precise co-ordination of integrin-associated complex (IAC) dynamics, mechanical force application, mechanotransduction, polarity signalling and cytoskeletal dynamics. IAC dynamics are co-ordinated by receptor trafficking and mechanotransduction, membrane protrusion is spatially regulated by GTPase activity and actin dynamics. B) Crosstalk between integrins, GFRs and syndecans fine-tunes signalling outputs to spatially and temporally control cell migration.

Integrins: Mechanochemical Signalling Hubs

Integrins are the major class of ECM-binding adhesion receptor and functionally integrate the extracellular microenvironment with the inside of the cell. Integrins are transmembrane receptors that relay mechanical signals bidirectionally across the membrane, between the ECM and the contractile cytoskeleton. Thus, integrins enable cells to sense the mechanical properties of matrix and also to exert forces on ECM to control cell migration, invasion and tissue rigidity[1].

Integrin cytoplasmic domains lack inherent enzymatic activity, however, clusters of ligand-bound integrins recruit networks of hundreds of cytoskeletal and signalling molecules (collectively termed the "adhesome"). By establishing links to both the actin cytoskeleton and signalling moieties, and converting mechanical stimuli into biochemical outputs, integrin-associated adhesion complexes (IACs) regulate cell migration by co-ordinating mechanical force transmission and by propagating signals that drive membrane-distal intracellular events[1,8-10]. Thus, integrins can be considered bidirectional mechanochemical signalling machines that dynamically recruit signalling networks to control global cellular functions. Indeed, the signalling machinery at IACs can influence nearly all biological processes in metazoa[1].

Recruitment of protein networks to clustered integrins, at the cell-matrix interface, leads to spatial compartmentalisation of force transmission and mechanochemical signalling. Therefore, to co-ordinate local application of mechanical forces, pro-migratory signalling and cytoskeletal dynamics, cell migration requires precise spatial and temporal regulation of IAC dynamics[1,11]. So, dysregulation of any of the processes that co-ordinate adhesion dynamics by, for example, unconstrained activation or inhibition, has a major impact on cell motility[12-17].

Cell migration relies fundamentally on co-ordination of integrin engagement and IAC turnover. Therefore, regulatory mechanisms exist that enable cells to fine-tune local integrin-mediated mechanical and chemical signalling. The integrin family is composed of 24 different heterodimers (pairs of α- and β-subunits). Each integrin heterodimer exhibits differential selectivity for specific ECM ligands. Moreover, the mechanical, biophysical and signalling properties of each heterodimer, within this large and diverse family, can be profoundly different. The signalling networks established, and the mechanical forces transmitted, following engagement of different integrin heterodimers, elicit highly divergent effects on cell migration. Consequently, even integrin heterodimers that have the capacity to engage the same ECM ligand can induce highly differential effects on cell migration; for example, the fibronectin-binding integrins, $\alpha5\beta1$ and $\alpha V\beta3$[18,19]. Engagement of $\alpha5\beta1$ leads to dynamic IAC turnover, random cell motility and signalling via the small GTPase RhoA. Whereas, $\alpha V\beta3$ suppresses IAC turnover and adhesion component dynamics, drives directionally-persistent migration and inhibits fibronectin-induced RhoA activity[13-15]. These differences are,

at least in part, due to differential mobility kinetics of individual receptors within IACs and their ability to sustain mechanical force transmission. At the single molecule level, ligand-engaged αVβ3 is immobile and stationary in IACs, whereas α5β1 exhibits actin-driven centripetal movement. Moreover, αVβ3 functions as a mechanosensor due to a rapid on/off binding rate, relative to α5β1, and requires clustering and recruitment of adaptor proteins to stabilise and reinforce adhesion. By contrast, α5β1 supports high ECM forces and regulates the magnitude of adhesion strength[20,21]. These heterodimer-specific characteristics are also likely to be mediated by recruitment of distinct adhesome signalling networks to the different integrins. So on-going studies are employing proteomic techniques to dissect heterodimer-specific signalling networks, in order to determine how they co-ordinate cell migration and global cellular processes.

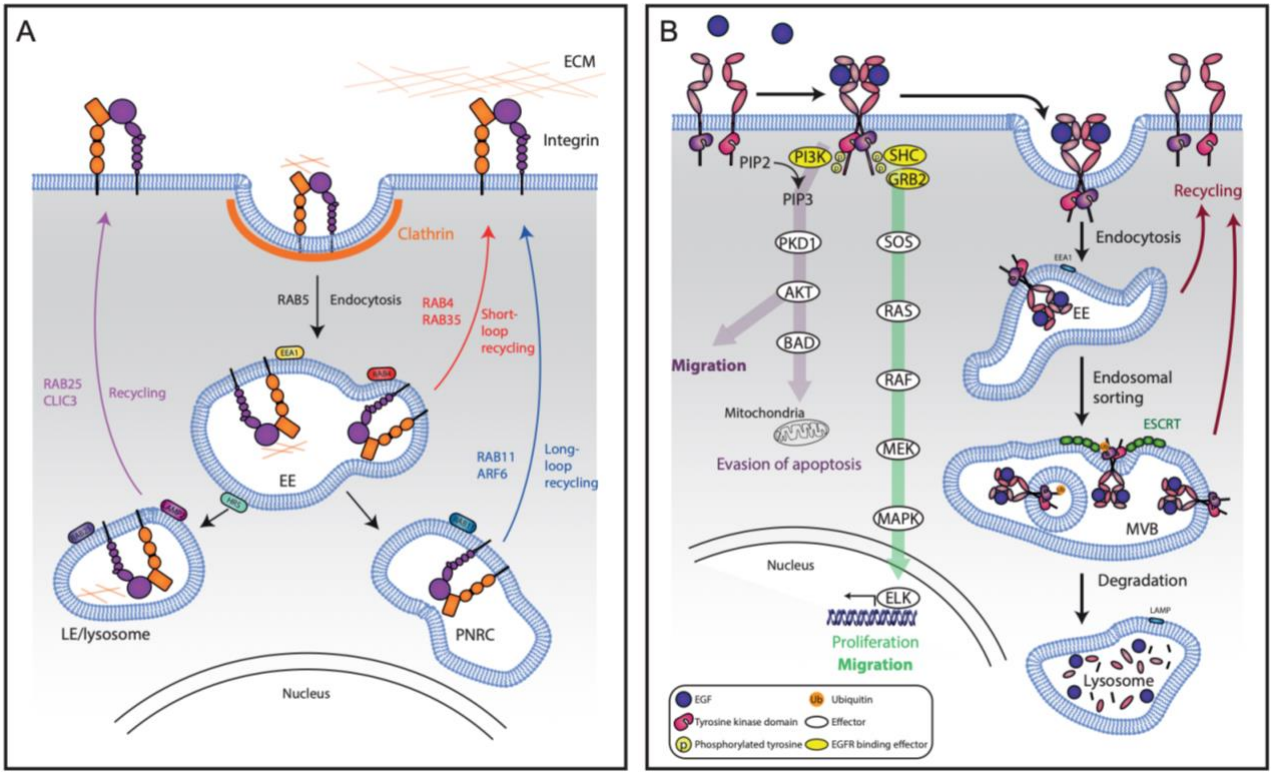

Fig 2: Receptor trafficking controls bioavailability and function of integrins and growth factor receptors
A) Integrin trafficking. Integrins are endocytosed via clathrin-dependent and -independent routes, and traffic to the early endosome (EE). Integrins are predominantly recycled rather than degraded[109]. Integrin recycling is generally associated with a fast short-loop pathway from the EE which is dependent on RAB4 and RAB35, or slower long-loop pathway from the perinuclear recycling compartment (PNRC) which is dependent on RAB11 and ARF6[66]. Integrins trafficked to the late endosome (LE) or lysosome can also be recycled, and the ECM ligand is degraded. This process has been shown to be mediated by CLIC3 for α5β1[110]. B) GFR signalling and trafficking - Exemplar RTK: EGFR. Ligand engagement triggers dimerization. Active ligand-bound EGFR forms a back-to-back homodimer, interacting primarily through trans- and juxta-membrane domains. EGFR activation induces an asymmetrical orientation of the tyrosine kinase domains (visualisation aided by monomers as different colours), which triggers autophosphorylation[111]. Signalling effectors are recruited to phosphorylated tyrosines, and mediate downstream signalling (PI3K-AKT and RAS-RAF-MAPK pathways shown in purple and green, respectively)[112]. EGFR activation stimulates receptor internalisation via clathrin-dependent and -independent routes[113]. Ubiquitin moieties on EGFR are detected by the ESCRT (Endosomal Sorting Complex Required for Transport) complex which sorts EGFR for lysosomal degradation, or recycling[114].

The impact that different integrins can have on cell migration demands that mechanisms exist to fine-tune the bioavailability and functions of different heterodimers. Consequently, during cell migration *in vivo*, heterodimer-specific integrin engagement and delivery to the cell-ECM interface is tightly regulated by receptor trafficking mechanisms (Figure 2A). Integrins are constitutively internalised and intracellular trafficking pathways regulate localised targeting of integrins back to the cell membrane[22-24]. Accordingly, co-ordination of receptor internalisation, sorting, recycling and degradation spatially and temporally segregates engagement of, and signalling from, specific integrin heterodimers[23,25-27]. As a result, integrin trafficking mechanisms operate to fine-tune integrin engagement and signalling during cell migration.

Receptor crosstalk mechanisms introduce an additional level of complexity to the regulatory systems that orchestrate integrin function and signalling; most notably via crosstalk with growth factor receptor tyrosine kinases and/or syndecans. The common characteristic is that such co-operative mechanisms impact receptor engagement, signalling and trafficking. Accordingly, these mechanisms provide potential for positive and negative feedback mechanisms and afford further opportunity to fine-tune receptor function and downstream outputs.

Growth Factor Receptor: Tuneable Signalling Machines

Growth factor receptors (GFRs) are receptor tyrosine kinases (RTKs) that act as key regulators of cellular processes, such as proliferation, differentiation, survival and cell migration[28]. Accordingly, aberrations in GFR signalling have been linked to numerous diseases and disease-related processes. Stimulation of GFRs with extracellular ligands typically triggers an autophosphorylation cascade and activation of the RAS-RAF-MAPK and PI3K-AKT signalling cascades, but the specific signalling response is RTK-, ligand- and cell type-dependent (Figure 2Bi: Exemplar RTK - EGFR). Ligand engagement usually triggers rapid receptor endocytosis, which can either lead to receptor degradation or recycling[24] (the balance of which can be dictated by the specific stimulus[29]). An important facet of GFR function is that receptor internalisation is key for sustaining or modulating receptor signalling following stimulation; the so-called “signalling endosome” model (Figure 2Bii: Exemplar RTK – EGFR)[6,24]. Thus, GFR endocytosis is not simply a mechanism to switch off RTK signals, but a pre-requisite for a complete signalling response.

Crosstalk between cell-ECM adhesions and GFRs has long been evident in the phenomenon of anchorage-dependent cell survival. Whereby, GFRs signal inefficiently without integrin-mediated adhesion and initiate growth arrest or anoikis (detachment-induced apoptosis)[30-34]. However, accumulating evidence has led to the definition of numerous crosstalk mechanisms integrating integrin and GFR function.

Integrin-GFR crosstalk can be mediated by a diverse range of mechanisms affecting receptor expression,

activity, signalling and trafficking[35]. Thus, adhesion receptor and GFR crosstalk provides mechanisms by which IACs, representing localised foci of mechanical and biochemical signal transduction, are co-ordinated spatially and temporally by GFRs. Equally, integrins can directly influence the subcellular distribution, clustering and expression of GFRs[35].

Mechanisms of Integrin-GFR Co-operation and Signal Integration

Figure 3 shows the nine major mechanisms that enable integrin and growth factor receptor crosstalk; exemplar mechanisms are identified and discussed below, but this list of examples is not exhaustive. Some of the crosstalk mechanisms were initially characterised as co-ordinating global cellular functions, such as transcription, proliferation and cell survival, however, it is highly likely that all of these mechanisms will also directly impact cell migration.

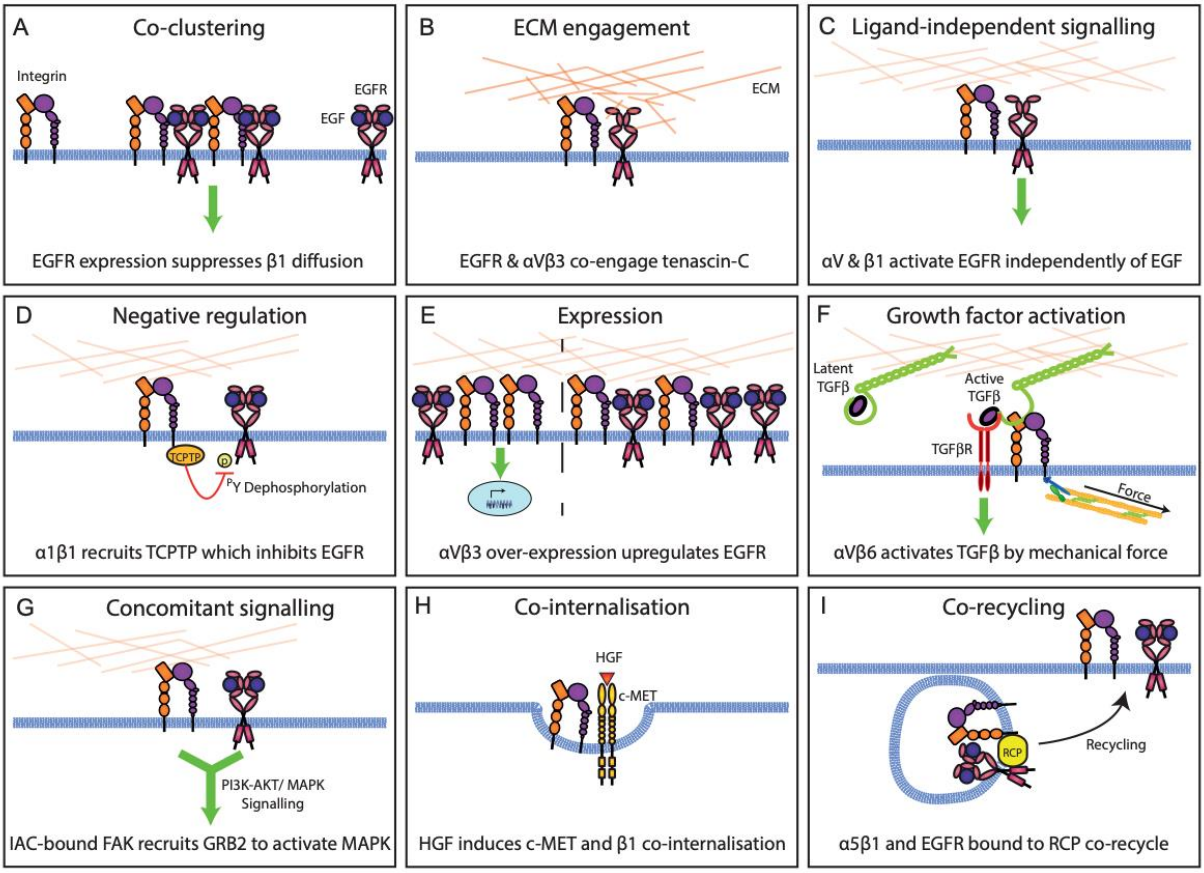

Fig 3: Integrin-growth factor receptor crosstalk mechanisms

Schematic depictions of integrin-GFR crosstalk mechanisms, with representative examples. Green arrows represent signalling. A) Integrin and GFR co-clustering positively regulates their signalling. B) Multivalent ECM molecules function as linkers co-ordinating integrin and GFR engagement and distribution. C) Integrins stimulate GFR signalling in a growth factor-independent manner. D) Integrins recruit negative regulators to suppress GFR signalling. E) Integrins upregulate expression of GFRs, and *vice versa*. F) Integrins activate growth factors (e.g. TGFβ) via mechanical force application or modulation of proteases, enabling juxtacrine or autocrine stimulation of GFRs. G) Integrins and GFRs initiate concomitant signalling by activation of common signalling pathways. H) Integrins and GFRs can undergo co-internalisation, and I) co-recycling; reciprocally controlling their availability at the plasma membrane.

Integrins and GFRs often co-localise at the cell surface, however few examples of direct binding exist[30]. Despite this, aggregation of integrins causes GFR co-clustering, creating a permissive environment by bringing GFR monomers into proximity with each other and common downstream signalling effectors (Figure 3A)[36]. GFRs may also influence integrin clustering, for example knockdown of EGFR results in increased membrane diffusion and decreased clustering of *Drosophila* orthologs of integrin β1[37]. Lipid raft plasma membrane microdomains have been implicated in these processes, as specialised regions that gather integrins and GFRs into signalling platforms[38]. Some ECM components can be engaged both by integrins and by GFRs (Figure 3B), for example tenascin-C can be bound by both EGFR and αVβ3, and therefore act as linkers co-ordinating the function of the receptors[39,40]. By controlling receptor clustering and actin dynamics, it is highly likely that these mechanisms co-ordinate cell migration, as well as survival.

Integrins can also regulate the activity of GFRs by mechanisms that are more direct than simply creating a permissive environment. Integrins are reported, in certain circumstances, to activate GFRs in a ligand-independent manner (Figure 3C). Integrin-dependent adhesion to fibronectin or collagen can stimulate EGFR phosphorylation in the absence of growth factor stimulation, via a mechanism that requires IAC signalling components (p130CAS and Src)[41,42]. Interestingly, ligand-independent activation of EGFR produces a different pattern of receptor tyrosine phosphorylation[42]. Integrin-mediated GFR activation may therefore induce unique signalling outputs compared to canonical ligand stimulation. Integrin-mediated ligand-independent activation of GFRs has been demonstrated for multiple other integrin subtypes and GFRs including platelet-derived growth factor receptor and hepatocyte growth factor receptor (c-MET), regulating cell migration and invasion, respectively[30,43,44]. Importantly, adhesion stimulated activation of GFRs does not appear to be a universal phenomenon and may be ECM ligand- and cell type-specific[45].

Integrins can negatively regulate GFR activity, via recruitment of RTK inhibitors to IACs, such as tyrosine phosphatases that dephosphorylate GFRs[46,47] (Figure 3D). One example of this is the recruitment of the phosphatase TCPTP (T-cell protein tyrosine phosphatase) by integrin α1β1[46]. Binding to collagen promotes an interaction between the integrin α1 cytoplasmic domain and TCPTP and promotes TCPTP-dependent dephosphorylation of EGFR, VEGFR2 and TGFβR2, and suppresses growth factor-dependent functions including survival, migration and differentiation[46-48].

Integrins and GFRs can affect the expression and surface levels of one another (Figure 3E). For example overexpression of αVβ3 upregulates EGFR expression[49], and sustained stimulation with HGF increases integrin α2β1 levels[50]. Integrins can also indirectly affect GFR function by interacting with, and/or modulating the activity of, growth factors (Figure 3F). Integrin-mediated activation of the growth factor TGFβ through mechanical force or proteases enables juxtacrine or autocrine stimulation of GFRs[51]. Integrin αVβ6 activates

TGFβ by binding and applying actomyosin- and RhoA-dependent force to latency-associated peptide (LAP) to induce a conformational change, releasing TGFβ from its inactive complex[51,52]. Whereas αVβ8 integrin activates TGFβ by promoting MT1-MMP-mediated cleavage of LAP[53]. Some integrins are also reported to bind select growth factors, such as αVβ3 which can bind insulin-like growth factor-1 and -2[54,55] and FGF1[56-58] to modulate cell migration. Integrin binding to growth factors promotes signalling of the corresponding GFR, possibly suggesting that integrins are involved in presenting growth factor to the GFR[30], although co-receptors such as syndecans may also be involved in this process[7].

Thus, integrin-GFR crosstalk can influence the activity of both receptor families, in a positive or negative manner. These mechanisms can be mediated indirectly, for example by a divalent ECM ligand, through facilitative mechanisms such as influencing receptor expression and clustering, and by direct mechanisms such as the recruitment of phosphatases to directly control receptor activity or local force-dependent activation of growth factor. However, in addition to promoting GFR signalling in a collaborative manner, integrins can independently activate common signalling pathways, enhancing signalling concomitantly (Figure 3G). Key shared pathways include RAS-MAPK, PI3K-AKT, and the downstream regulation of Rho-family GTPases. For example, the 'FAK-Src' complex, established at sites of integrin-ECM interaction, phosphorylates many RTK signalling effectors[59,60], including PI3K to stimulate RTK-dependent survival signalling[61]. The fully phosphorylated 'FAK-Src' complex also binds GRB2 and connects adhesion signalling to RAS activation and the MAPK cascade[59]. Additionally, GFRs can activate FAK directly or indirectly via Src, and both are central nodes downstream of both integrin and GFR signalling[30].

Concomitant signalling between integrins and GFRs can also regulate integrin-mediated force sensation[62], likely via EGFR- and PKC-dependent control of myosin-II contractility[63,64]. Moreover, Src-mediated phosphorylation of EGFR is required for rigidity dependent localisation of EGFR to early IACs[62]. As Src is not recruited to IACs in a force-dependent manner[64], a likely candidate for this role is the Src substrate p130CAS which is mechanically-sensitive and involved in EGFR localisation[64,42,65]. By controlling MAPK and AKT signalling, Rho-family GTPase activity, adhesion dynamics and contractility, concomitant signalling mechanisms must play a major role in co-ordinating and fine-tuning cell migration.

Recent work has demonstrated that GTPase signalling, converging downstream of αVβ6 integrin and EGFR, acts as a switch between integrin-mediated tumour cell migration and force-dependent activation of TGFβ[52]. This raises the intriguing notion that potential for a higher order complexity exists, whereby concomitant integrin-GFR signalling co-ordinates the activation of other GFR-dependent signalling networks.

Integrins and GFRs have shared trafficking routes, including those for internalisation and recycling[30, 66,67] and crosstalk between the receptor families affects the endosomal trafficking of both receptor types. Integrins and GFRs often exhibit a reciprocal relationship whereby the function of one receptor directly impacts the internalisation of the other (Figure 3H). For example, EGFR activity triggers internalisation of both EGFR and α2β1 integrin through different endocytic pathways[68]. By contrast, HGF stimulation promotes co-internalisation and co-trafficking of c-MET and β1 integrin, promoting sustained signalling from internalised c-MET[69]. Thus, co-internalisation of surface receptors functions as a key mechanism regulating receptor surface bioavailability in a co-ordinated and functionally integrated manner. While this mechanism was shown to regulate survival signalling, by co-ordinating the availability of integrins at the cell-matrix interface, it is likely to also influence mechanical force transduction and cell migration.

Integrin and GFR recycling is an important determinant of spatiotemporal receptor delivery and distribution at the cell-matrix interface, and controls IAC dynamics and cell migration (Figure 3I). Growth factor stimulation can directly control whether specific integrin heterodimers recycle through the rapid short-loop recycling pathway, or the slower long-loop pathway[25,27,70]. Integrins and GFRs can also recycle in a co-ordinated manner together. For example, α5β1 integrin and EGFR co-recycle[71]. Rab-coupling protein (RCP) binds directly to both β1 and EGFR, physically linking them, and enables co-ordinated delivery of both receptors to the membrane; impacting cell motility and EGFR signalling[71]. Co-ordinated regulation of receptor recycling has direct consequences for receptor availability at the cell surface, dynamics and levels.

RTK endocytosis is required to propagate a complete growth factor-induced signalling response ("signalling endosome" model)[6,24]. Recent work has demonstrated that internalised integrins continue to signal via FAK on endosomes and that endocytosis is required for complete ECM-induced, integrin-mediated MAPK, AKT and FAK signalling and resistance to anoikis[72,73]. The parallels between the "signalling endosome" model for RTKs[24], and the "endoadhesome" model for integrin signalling[72], raises the exciting possibility that integrin or GFR signalling on endosomes sustains or modulates signalling from cooperating receptors; introducing further complexity and potential for feedback mechanisms. Endosomal signalling, crosstalk and feedback would enable fine-tuning and spatial constraint of signalling kinetics and could potentially play an important regulatory role in the co-ordination of migration. This prospect, and the role such mechanisms play in cancer, will doubtless be the focus intensive study over coming years.

Due to the role of GFRs in regulating cell survival and proliferation, to date, many mechanisms of adhesion and GFR crosstalk have not been studied in the context of cell migration. However, the critical role that these mechanisms play in controlling receptor bioavailability and signalling outputs, means that they will play major regulatory roles in directing and co-ordinating cell migration.

The impact of specific ligands on GFR conformation and signalling kinetics is a new and rapidly developing field. Structure-function studies have revealed pronounced differences in strength and stability of GFR dimers formed, following engagement of different ligands[6,74]. Compared to EGF, the epiregulin and epigen ligands induce structurally unstable and weak EGFR dimers. However, surprisingly, these less-stable asymmetric dimers promote sustained and exaggerated EGFR-dependent signalling. By modulating the downstream signalling outputs, engagement of the different ligands induces EGFR-dependent differentiation, rather than proliferation[74]. Understanding the conformational dynamics of RTK stability and activation kinetics has led to novel approaches to design and screen for RTK-targeting drugs, which target specific active conformations, in order to overcome drug resistance mechanisms in cancer[75].

In the context of cell migration, an appealing model is that as cells navigate through ECM they encounter nanoscale quantities of locally-immobilised growth factors, enabling restricted activation of small clusters of RTKs in membrane subdomains. The local interpretation of these discrete signals would allow spatially constrained regulation of signalling kinetics to fine-tune migration. Moreover, by initiating differential signalling responses following engagement with different ligands, the same RTK would have the capacity to induce different migration modulating effects. It will be important in the future to determine the extent to which the IAC environment, and its impact of the architecture of lipid microdomains, modulates GFR dimer structure, symmetry and signalling kinetics.

Syndecans: Environmental Sensors and Signal Integrators

Syndecans play key regulatory roles in many physiological processes, including wound healing, angiogenesis, inflammation and neuronal patterning[7,76]. Syndecans are type-I transmembrane heparan sulfate proteoglycans (HSPGs) that act as adhesion receptors engaging ECM molecules and co-receptors for growth factors, cytokines and morphogens[7,76,77]. While not the primary receptors of ECM molecules or growth factors, syndecans cooperate with the prototypic receptors through simultaneous ligand engagement (Figure 4A).

Syndecan extracellular domains are substituted with long unbranched heparan sulfate chains that mediate adhesive interactions with heparin-binding domains (HBDs) in ECM macromolecules. The glycosaminoglycan chains also associate with HBDs in a range of different growth factors and GFRs, regulating immobilisation of growth factors, establishing gradients or essentially presenting growth factors to GFRs to promote dimerisation[78]. As nearly all classical ECM macromolecules contain at least one HBD, this means that syndecans have a potentially very large repertoire as adhesion receptors. By contrast, a more restricted group of growth factors and GFRs have HBDs and are potential syndecan substrates (including amphiregulin,

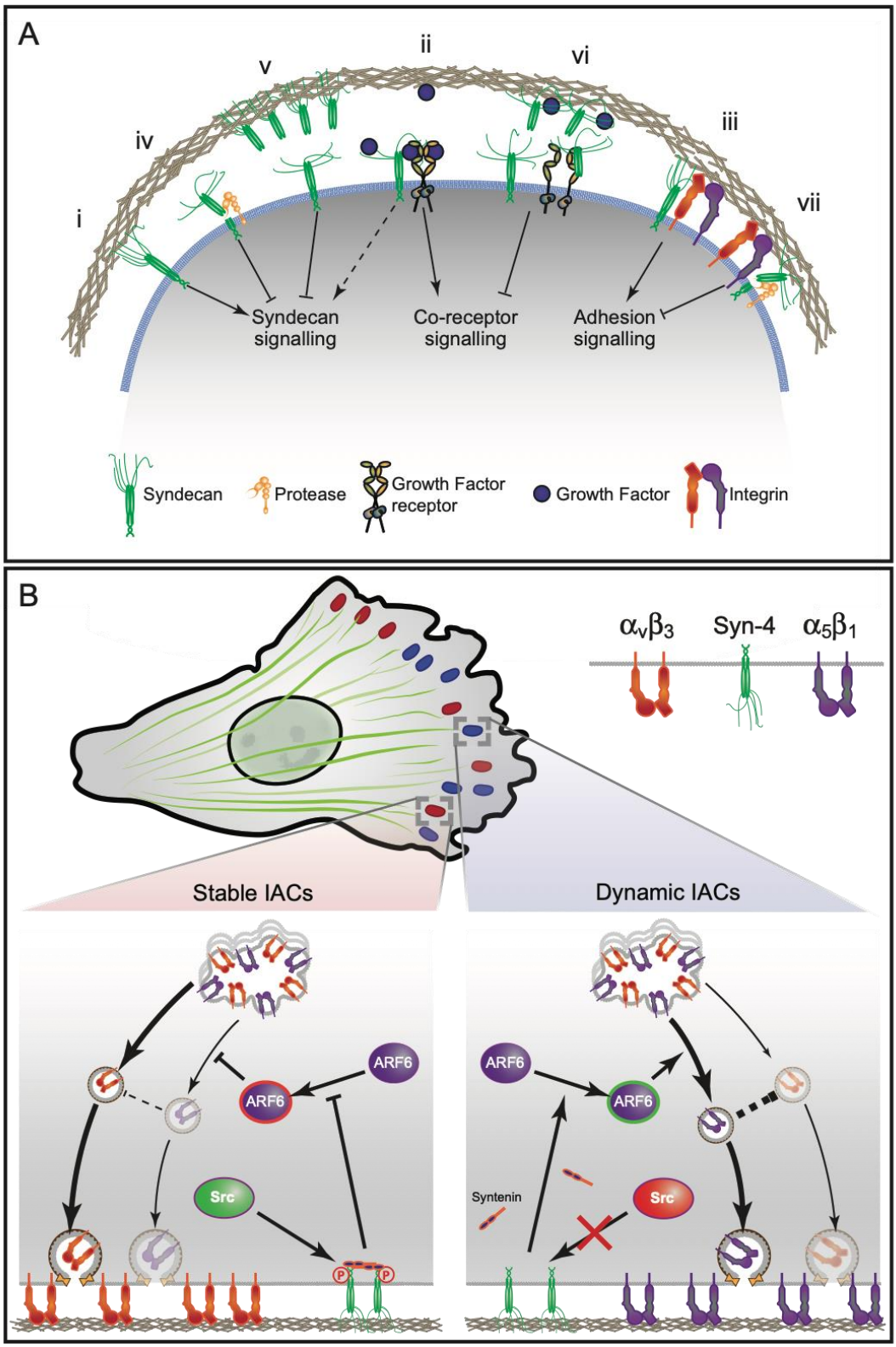

Fig 4: Syndecans: Integrators of integrin and GFR trafficking and signalling
A) The role of syndecans and the impact of syndecan shedding on cellular functions. Syndecans act as ECM receptors (i) and co-receptors for growth factors and GFRs (ii) and co-operate with integrins to initiate a full adhesion response (iii). Syndecan extracellular domain shedding terminates syndecan signalling (iv), competes with intact syndecans for ECM ligands (v), and growth factors or GFRs (vi), and inhibits crosstalk with integrins. B) Syndecans regulate adhesion complex dynamics by controlling ARF6-dependent integrin, and potentially GFR, recycling.

heparin-binding-EGF, FGF2, IGFBP, VEGF, PDGF-B, PIGF, TGFβ, HGF, FGFR and VEGFR2)[79,80]. FGFs bind to FGFRs with high affinity, but this interaction is stabilized and subsequent signalling events are amplified in the presence of HS[81,82].

The extracellular core proteins of syndecans are also reported to directly bind to integrins, both in *cis* and in *trans*, and to GFRs[26,83-87]. Indeed, in the case of syndecan-1, binding of integrin αVβ3 to the syndecan extracellular domain provides a 'docking face' for IGF1R[85-87]. Syndecan-4 can simultaneously bind EGFR and α6β4 integrin to form a trimolecular complex that is required for EGF-mediated motility[85]. Due to these properties, efforts are underway to develop anti-angiogenic and invasion inhibitory drugs based on different syndecan extracellular core proteins[85,86,88,89].

Syndecans can function as ECM adhesion receptors and physically link integrins, growth factors and GFRs, via both heparan sulfate chains and direct protein-protein interactions. Consequently, syndecans are uniquely placed to co-ordinate adhesion receptor GFR crosstalk mechanisms[7]. However, a further level of mechanistic complexity is introduced by the fact that syndecan extracellular domains can be cleaved, at a membrane-proximal site, by a range of secreted and membrane-associated proteases[90]. Shed ectodomains compete with intact syndecans for ECM ligands, growth factors or GFRs; impacting the signalling capacity of both the syndecan and the prototypic receptor[90-92] (Figure 4A).

It is becoming increasingly clear that syndecan signalling plays an equally important role in co-ordinating and integrating adhesion and GFR function during cell migration. Accumulating evidence is leading to a model of syndecans as molecular antennae that sense and interpret the biochemical and biophysical properties of the local microenvironment and initiate a wave of signalling outputs to spatially and temporally co-ordinate the migratory response (Figure 4B).

Surprisingly little is known about the cytoplasmic signals initiated directly downstream of syndecans following association with growth factors. However, it is clear that binding of syndecans to ECM ligands initiates a co-ordinated cascade of cytoplasmic signalling events, so it is likely that associations with growth factors and/or GFRs will also activate specific signal transduction mechanisms.

The short, well-conserved, cytoplasmic domains of syndecans interact with a number of signalling molecules and adaptors and are vital for their pro-migratory function. Syndecan-4 engagement by ECM ligand leads to a well characterised series of small GTPase modulatory events that regulate cytoskeletal reorganisation, integrin trafficking and IAC dynamics. Syndecan-4 stimulation initiates IAC disassembly in response to rapid induction of RhoG activity, through a mechanism consistent with caveolin-mediated integrin endocytosis[93].

This is followed by a wave of Rac1 activation and suppression of RhoA activity, which enable formation of protrusive lamellipodia and nascent IACs[94]. Subsequently, RhoA is activated to promote stress fibre formation and force application[95,96]. Finally, syndecan-4 activates ARF6 to drive $\alpha5\beta1$ integrin recycling and co-ordinate IAC dynamics[15]. Modulation of RhoG, Rac1 and RhoA are regulated by syndecan-4-mediated PKCα activity, whereas regulation of syndecan-4-dependent ARF6 activity is controlled by Src. Src-mediated syndecan-4 phosphorylation suppresses ARF6 activity to promote recycling of $\alpha V\beta3$. By acting as a switch to dictate whether $\alpha5\beta1$ or $\alpha V\beta3$ integrins are delivered to the cell-matrix interface, syndecan-4 exhibits precise control over IAC dynamics and the biomechanical response to the local microenvironment during cell migration[15].

During cell migration *in vivo*, the stimuli that initiate temporal activation of GTPase activity, must be spatially constrained to ensure that receptor trafficking and IAC and cytoskeletal dynamics are precisely co-ordinated. It is interesting to note that Src, the kinase that regulates syndecan-4-dependent $\alpha5\beta1$ or $\alpha V\beta3$ recycling can be activated both by integrin-mediated adhesion and by GFR signalling. Raising the possibility that syndecan-4 functions as a nexus integrating both ECM-associated and growth factor signals in order to co-ordinate integrin recycling. Indeed, ARF6 has also been implicated in trafficking of GFRs, and can be directly activated by GFR signalling[97-102], so it is conceivable that these mechanisms may also control GFR recycling. The emerging picture, therefore, is that syndecan-4 operates as a microenvironmental sensor that integrates multiple physical and biochemical extracellular signals in order to orchestrate cell migration; determining where and when specific receptors should be engaged.

Interestingly, the equivalent phosphorylation site in syndecan-2 has been shown to be phosphorylated by EphB2 RTK and regulates neuronal morphogenesis[103]. So, it is feasible that other syndecans regulate similar mechanisms, but the specific cargos and stimuli are likely to be dependent on cellular and microenvironment context.

Concluding Remarks

Complex regulatory networks and trafficking itineraries control receptor signalling to precisely co-ordinate efficient cell migration. Integrins, GFRs and syndecans each exhibit functional and signalling characteristics that directly and individually contribute to cell migration. Integrins are mechanosensitive and mechanoresponsive signalling hubs that enable bidirectional transmission of locomotive forces and distal propagation of signalling outputs. GFRs are highly sensitive signalling machines, with rapid kinetics, that initiate kinase cascades to co-ordinate transcription and GTPase activity. Syndecans are microenvironmental sensors that regulate GTPases to control receptor trafficking, cytoskeletal remodelling and adhesion

dynamics. However, an array of crosstalk mechanisms adds further strata of complexity to the system. Moreover, the individual crosstalk mechanisms are not mutually exclusive and growing evidence suggests a higher order of complexity; whereby multiple receptor crosstalk mechanisms are integrated. It is likely that, *in vivo*, regulation and integration of these mechanisms will be subject to both positive and negative feedback that will depend fundamentally on the spatiotemporal restriction of kinase, phosphatase and GTPase activity downstream of extracellular signals. Exquisite and sensitive control of these mechanisms will ensure that mechanical forces and pro-migratory signals are triggered in the right place and at the right time.

In order to dissect positive and negative feedback pathways, within mechanisms that integrate multiple extracellular stimuli with cascades of kinase, phosphatase and GTPase activity, it will be necessary to employ mathematical modelling and systems biology approaches. Ultimately, this will enable a greater understanding of the fundamental processes that co-ordinate physiological cell migration and will also help us to understand how these processes are dysregulated in disease.

To date, most studies analysing adhesion and GFR crosstalk in cell migration have relied on *in vitro* models, usually using 2D substrates. While these approaches permit dissection of complex signalling pathways and analysis of adhesion dynamics and traction forces, they are poor substitutes for 3D microenvironments encountered *in vivo*. Recent developments in microscopy and tissue engineering enable detailed analysis of cells in 3D ECM, organomimetic 3D co-culture systems, *ex vivo* organoids and *in vivo*. Consequently, it is now imperative to extend these studies to determine the impact of receptor crosstalk in complex microenvironments. Indeed, while many of the mechanisms described in this review orchestrate cell migration, it is probable that they will have differential effects on different modes of migration (e.g. mesenchymal, ameoboid, streaming and collective migration); suggesting that specific mechanisms will be dominant during particular developmental, morphogenesis, homeostatic and disease-associated events.

Integrin- and GFR-mediated processes such as proliferation, gene expression, cell survival and cell motility are exploited by tumour cells to promote cancer progression, invasion and metastasis[104] and are recognised as clinically relevant targets for cancer therapeutics[105-107]. However, integrin-targeting drugs have not met initial expectations in the clinic, and RTK-targeting agents, almost universally, result in acquired drug resistance[6,105]. Integrin-GFR crosstalk between has been implicated in the lack of efficacy of an integrin targeting drug, as low-doses of $\alpha V\beta 3/\beta 5$-targeting cRGD/Cilengitide promoted VEGFR recycling, angiogenesis and tumour growth[108]. Essentially, inhibition of one pro-angiogenic receptor, accelerated the pro-angiogenic functions of another receptor. Cilengitide has subsequently failed to reach the primary endpoint in a Phase-3, and three separate Phase-2, clinical trials. This evidence alone provides a compelling rationale to understand the complex regulatory mechanisms that integrate integrin, GFR and syndecan function.

By using systems-level approaches to dissect adhesion receptor and GFR crosstalk mechanisms, it should ultimately be possible to design novel therapeutic strategies to target metastasis-promoting mechanisms, reduce acquired drug resistance and stratify patients to predict when specific treatments are likely to be effective. However, such endeavours will have to reach beyond the field of cell biology and will demand integration with large-scale clinical datasets.

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