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REVIEW ARTICLE

Signal co-operation between integrins and other receptor systems

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The multicellular nature of metazoans means that all cellular processes need to be tuned by adhesive interactions between cells and their local microenvironment. The spatial organization of cells within tissues requires sophisticated networks of extracellular signals to control their survival and proliferation, movements and positioning, and differentiated function. These cellular characteristics are mediated by multiple inputs from adhesion systems in combination with soluble and developmental signals. In the present review we explore how one class of adhesion receptor, the integrins, co-operate with other types of receptor to control diverse aspects of cell fate. In particular we discuss: (i) how β3 and β1 integrins work together with growth factors to control angiogenesis; (ii) how α6β4 integrin co-operates with receptor tyrosine kinases in normal epithelial function and cancer; (iii) the interplay between β1 integrins and EGF (epidermal growth factor) receptor; (iv) signal integration connecting integrins and cytokine receptors for interleukins, prolactin and interferons; and (v) how integrins and syndecans co-operate in cell migration.

Key words: adhesion complex, adhesome, cytokine receptor, growth factor receptor, integrin, integrin signalling, syndecan.

INTRODUCTION

The formation of complex organs, the organization of cells within them, and the expression of tissue-specific genes require a combination of spatial instructions from the ECM (extracellular matrix) and intercellular adhesions, together with temporal signals from soluble factors such as GFs (growth factors), hormones and cytokines. The precise integration of the resulting intracellular signalling networks drives the principal cellular processes of suppression of apoptosis, cell cycle, migration and differentiation, which are all key to the morphogenesis and function of tissues (Figure 1). In the present review we provide an overview of some of the pathways that are activated co-ordinately by the integrin class of cell-matrix receptor and other receptor systems, and discuss the current knowledge of the mechanisms by which signal co-operation regulates cell fate and development in both normal tissue homoeostasis and disease.

Cell adhesions occur through a wide variety of cell-surface receptor systems, and one of the major classes are termed integrins. They are composed of α- and β-dimers, which combine to form 24 $\alpha\beta$ heterodimeric receptors, each with their own ligand specificities [1]. Integrins contain the binding sites for physical attachment of cells to the ECM, and they connect to various components of the actin cytoskeleton and signalling enzymes via their intracellular domains [2–4] (Figure 2). This provides the architectural framework for cells to organize their shape, respond appropriately to their microenvironment, and to establish stem-cell niches [5–7]. Integrins themselves can direct the type of ECM deposited and how it is organized on the extracellular face of cells. In turn, the spatial orientation of the ECM provides the context for cells to migrate directionally and to proliferate in specific orientations, contributing to the highly ordered patterning of multicellular tissues [8,9]. Some integrins are also cell–cell adhesion receptors, although this aspect of their function will not be discussed in the present review.

Central to the process of cell adhesion is integrin clustering. This occurs through integrin aggregation co-ordinated by: (i) close proximity or periodicity of ligand-binding sites within the cross-linked matrix of ECM molecules; (ii) lateral associations of integrins, possibly as repeating trimers, or via tetraspanins or lipid islands within the plane of the plasma membrane [10–12]; and (iii) interactions between integrin cytodomains via structural dimers (e.g. talin) [13]. Integrin clusters form a stable membrane platform with a high avidity for the ECM on the outside of the cell and, on the inside, they have multiple binding sites for adaptors and enzymes that become compartmentalized into plasma membrane-associated complexes. An adhesion complex is a multi-component complex that is frequently big enough to be seen by fluorescence microscopy and can be considered to be an organelle within the plasma membrane. There are a variety of types of integrin adhesion complexes, including focal complexes, focal adhesions, fibrillar adhesions, podosomes etc., and in the present review we refer to them collectively as adhesomes, as described in [2]. Adhesome is a useful word to call a broad collection of adhesion structures, based on '*adhes*ion complex' and the Greek '*soma*', meaning body. Intracellularly, adhesomes assemble the cytoskeleton, signalling enzymes, and possibly other membrane microdomains such as lipid rafts [14].

The kind of $\alpha\beta$ integrin heterodimer, and the composition, density and rigidity of ECM molecules, as well as the cell type,

Abbreviations used: Ang-1, angiopoietin-1; Cdk, cyclin-dependent kinase; ECM, extracellular matrix; GF, growth factor; EGF, epidermal GF; EGFR, EGF receptor; ERK, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; FGF, fibroblast GF; FN, fibronectin; GAG, glycosaminoglycan; GEF, guanine-nucleotide-exchange factor; HGF, hepatocyte GF; IFN, interferon; IGF-1, insulin-like GF-1; IL, interleukin; ILK, integrin-linked kinase; IRS, insulin receptor substrate; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LM, laminin; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; PAK, p21-activated kinase; PDGFR, platelet-derived GF receptor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PP2, protein phosphatase 2; PTP, protein tyrosine phosphatase; RTK, receptor tyrosine kinase; Shc, Src homology and collagen homology; SHP, Src homology 2 domain-containing protein tyrosine phosphatase; STAT, signal transducer and activator of transcription; TCPTP, T-cell PTP; TGF-β, transforming GF-β; VEGF, vascular endothelial GF; VEGFR, VEGF receptor; VN, vitronectin.

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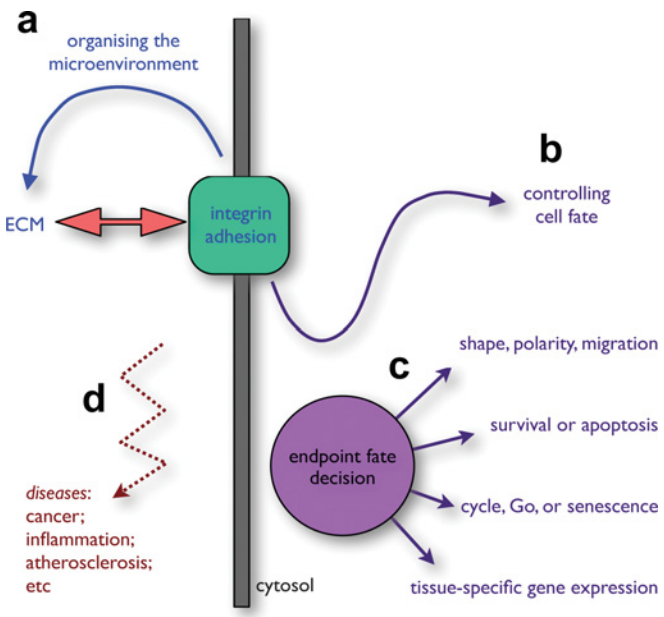

Figure 1 Concepts of integrin function

(**a** and **b**) Integrins are essential for matrix deposition and therefore they organize the microenvironment surrounding cells (**a**) and control cell-fate decisions (**b**). (**c**) Key aspects of cell fate controlled by integrins. (**d**) Disruption of the ECM–integrin–intracellular link leads to several of the important diseases of humankind.

specifies the profile of recruited proteins [15]. In turn, the type of adaptors and enzymes within adhesomes determines distal signalling events and thereby cell-fate decisions (Figure 3). Some of these are classical signalling enzymes, for example Src directly binds the β integrin tail and activated JNK (c-Jun N-terminal kinase) is detected in integrin adhesions [16,17]. Numerous signal-transduction pathways are triggered downstream of integrin engagement, including those involving Rho family GTPases, protein kinases, lipid kinases and phosphatases [18–20].

Some signalling enzymes are regulated by both integrins and GF/cytokine receptors, providing key nodal points at which the two systems can interplay [21]. Although the different receptor types can be activated separately by their own ligands, ECM adhesion can trigger GF receptors and vice versa. Moreover, in many cases engagement of both adhesion and GF receptors is required for an optimal output of sustained synergistic signalling. Integrins are relatively stable and the ECM is not diffusible, which means that integrins are microenvironmental sensors providing positional information over a sustained period of time. Depending on the location of cells, integrins determine whether a cell is able to respond to the more temporal signals provided by diffusible factors. Integrins are therefore positional checkpoints for cell-fate decisions induced by GFs or cytokines [22] (Figure 4).

Since the balance of normal tissue homoeostasis requires integration of spatial ECM signals with other receptor systems, dissecting the molecular mechanisms governing these processes is key to understanding many diseases. (In experimental terms, the interaction of cells with two-dimensional substrates have major differences in biological response to the more natural three-dimensional environments that cells are embedded within *in vivo*, which is important to bear in mind when considering how integrins work in tissues [23–25]. While two-dimensional cultures are unlikely to reveal how adhesion signals control biological endpoints in tissues, they are frequently used to

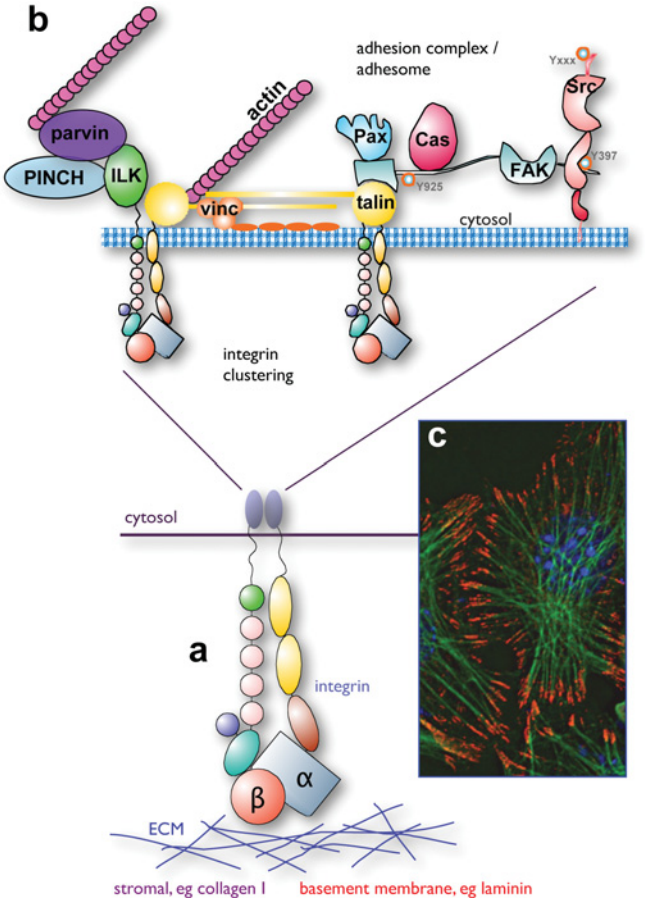

Figure 2 Integrin connections

(**a**) Integrins are α–β heterodimers that bind ECM proteins of stroma or basement membrane on the outside of the cells. (**b**) On the inside of cells, integrin clustering induces the formation of multi-protein aggregates called adhesion complexes, or sometimes known as adhesomes. Some of the many core adhesome proteins are shown. vinc, vinculin; Pax, paxillin, Cas, p130Cas. A fuller view of the proteins within the adhesome can be seen in [2], and some of the more recently identified components are discussed in [3]. (**c**) A fluorescence image showing integrin adhesions (red), the actin cytoskeleton (green) and nuclei (blue).

determine the initial signals that are engaged downstream of adhesomes.) Loss of connections between signalling networks contribute widely to diseases such as neoplasia. For example, one hallmark of cancers is 'self-sufficiency in growth signals', where certain GF receptors are no longer restrained by environmental cues so that their activation kinetics are extended, or they become otherwise deregulated [26]. Consequently, cancer cells can become independent of integrins, and thereby survive, proliferate or migrate within inappropriate ECM [27]. Conversely, some neoplastic cells take advantage of adhesion signals and up-regulate integrins at various stages of tumour progression, e.g. $\beta 6$ integrins are abnormally expressed in a wide range of carcinomas and activate TGF-β (transforming GF-β) [28], whereas $\alpha 6\beta 4$ integrin, in some metastatic carcinomas, switches from a strong adhesion receptor to a signalling receptor that combines with RTKs (receptor tyrosine kinases) to enhance downstream signals and directional migration [29].

SIGNAL INTEGRATION BETWEEN INTEGRIN AND GF RECEPTORS

Integrins are both sensors and machines. They sense the ECM content of the environment and communicate this information

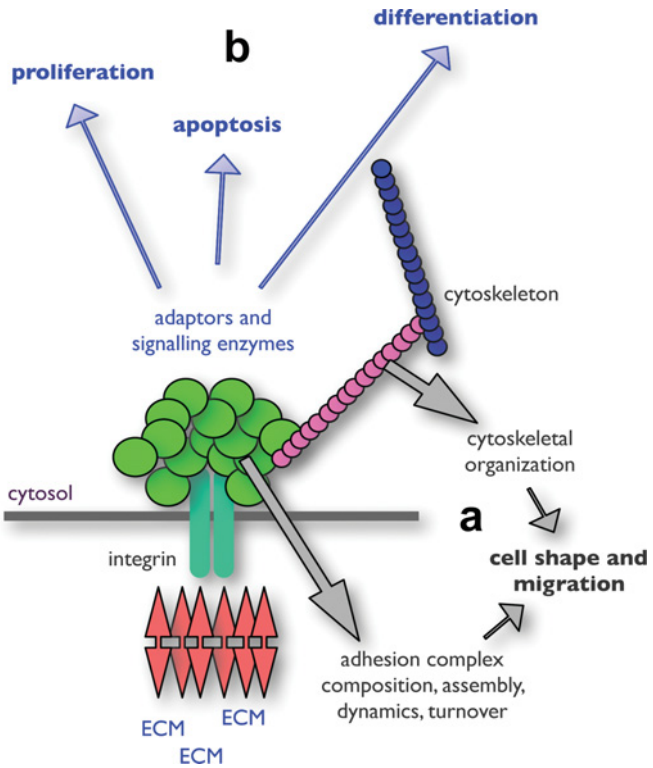

Figure 3 Integrin assemblies regulate cell phenotype

(**a**) Integrin assemblies between the ECM and adhesion complex organize the cytoskeleton, which controls cell shape, polarity and migration. (**b**) Recruitment of adaptors and signalling enzymes to integrin adhesions controls prolferation, apoptosis and differentiation.

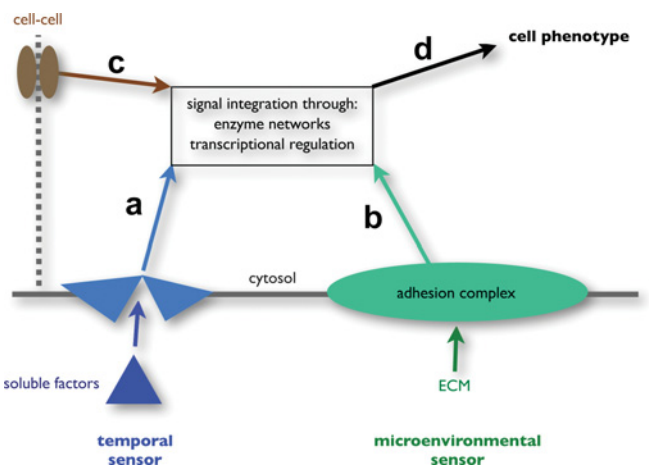

Figure 4 Integrin adhesion is a positional checkpoint for cell-fate decisions

(**a**) Soluble factors control development temporally, but their signals are only interpreted by the cell in the context of integrin adhesion complexes (**b**) and cell–cell interactions (**c**), which together provide microenvironmental checkpoints for cell fate (i.e. phenotype) decisions (**d**).

to the inside of the cell directly by receptor activation through 'outside–in' signalling. This leads ultimately to decisions on cell fate, which has been documented in some cases at the level of cellular gene expression profiles; in a recent example, $\alpha 6\beta 4$ integrin controlled the expression of genes involved in motility and invasion [30]. At the same time integrins are the core component of multi-protein machines that facilitate the attachment of cells to the

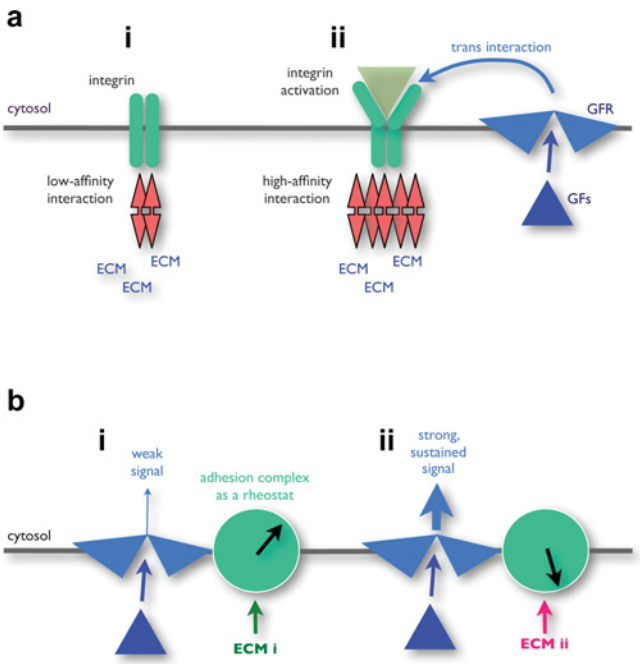

Figure 5 Integrins are sensors and machines

(**a**) The mechanical links between integrins and the ECM can be enhanced by *trans*-signalling from GFs or other ligands. (**b**) Integrins sense the ECM, which affects ligand activation of GF receptors. In the ECM (i), the rheostat activity of adhesion complexes is low, only permitting ligand–receptor interactions to deliver weak or transient signals. (ii) The ECM provides stronger more sustained signals.

ECM and/or migration through (or across) it, and in this respect ligand-binding activity is controlled via 'inside–out' signalling.

GF regulation of integrins

GFs can influence integrin function. One mechanism is by altering the expression of integrin α/β pairs or adhesome components. For example FGF-2 (fibroblast GF-2) increases $\alpha 5\beta 1$ integrin expression in endothelial cells, whereas TGF-$\beta 1$ elevates $\beta 1$ and $\beta 5$ integrin levels and induces *de novo* synthesis of $\alpha v\beta 6$ in keratinocytes [31,32]. How integrins are regulated has been discussed recently in the context of development and integrin trafficking, and a newly discovered mechanism for controlling integrin levels is through microRNAs, where the let-7a microRNA directly affects $\beta 3$ integrin expression [33–35]. Moreover, tetraspanins can control integrin function in several ways, for example CD151 regulates $\alpha 3$ and $\alpha 6$ integrins through glycosylation and trafficking [36–38].

Soluble factors also indirectly activate the ability of integrins to bind to the ECM via conformational molecular changes in the integrin molecules [39,40] (Figure 5a). In this context, the most important role of integrin activation is in dynamic cellular responses, such as in immune cell function, tissue repair and embryonic development. For example, immune cells, changing from circulating to migrating cells, undergo chemokine-regulated integrin activation [41]; platelet integrins become activated in the clotting cascade [42]; and keratinocyte integrin usage and function switches during wound repair [43].

The topic of inside–out signalling has been discussed widely [44]; however, it is not known whether or not regulated inside–out signalling occurs in the normal homoeostasis of static cells within tissues. Most normal epithelial, mesenchymal or neuronal cells maintain their spatial organization *in vivo*, and it remains

to be established whether the ability of integrins to bind ECM is regulated or constitutive.

Integrin regulation of GF receptors

GFs direct cell-fate decisions during development. However, the signalling responses of GF receptors (i.e. RTKs) are intricately controlled by adhesion to the ECM, where synergy leads to an optimal response. Integrin interception of GF signalling can occur at the receptor level, where integrins may influence expression, localization or post-translational modification of RTKs, or levels of GFs themselves. Moreover, in some cases integrins directly regulate GF activation prior to receptor binding, for example the αv integrins control TGF-β signalling via latency-associated peptide [45]. Integrins and RTKs can also interconnect via shared downstream signalling molecules [46].

One mechanism for activating GF receptors is through integrin clustering, in which integrin–RTK complexes can trigger RTK activity in the absence of the GF [47,48]. Several RTKs can be stimulated simply by adhesion to the ECM, including PDGFR (platelet-derived GF receptor), EGFR [EGF (epidermal GF) receptor; also known as ErbB1], bFGFR (basic FGF receptor), VEGFR [VEGF (vascular endothelial GF) receptor] and c-Met [HGF (hepatocyte GF) receptor] [21]. Such interactions are generally specific to the integrin subunit. For example, the receptors for insulin, IGF-1 (insulin-like GF-1) and VEGF are found in immune complexes with αvβ3, whereas ErbB2 and c-Met receptors specifically associate with α6β4 integrin. EGFR is more promiscuous, interacting with β1, β3 and β4 integrins. Many of these receptor–receptor connections are likely to be indirect within an adhesome, and it will be interesting to determine whether they can be visualized either by direct imaging or by FRET (fluorescence resonance energy transfer;), which has been successful for viewing integrin α–β subunit interactions [49].

The clustering of specific integrins within lipid microdomains can also influence RTK activation by their enrichment into those areas, thereby facilitating RTK activation by *trans*-phosphorylation [50]. Examples of integrin–RTK interactions at lipid rafts include α6β4 with EGFR in keratinocytes and α6β1 with PDGFR in oligodendrocytes [51,52].

Although adhesion directly activates GF receptor signalling in some experimental models, our view is that under homoeostatic conditions *in vivo*, the interactions between adhesion and GF receptors have a regulatory role. Thus the activated integrin behaves as a rheostat to control RTK signalling output, by modulating its intensity and duration (Figure 5b). This means that the ECM environment of the cell determines how the cell responds to soluble factors. For example insulin and IGF receptors only activate downstream signals efficiently in mammary epithelial cells on a basement membrane, but not stromal, ECM, correlating with location-dependent survival within tissues [53–55]. The corollary is that altered expression or composition of integrin α and β subunits significantly changes GF signalling responses. This causes profound cell-behavioural changes in diseases where the ECM is altered, for example in fibrosis and cancer, or if there are mutations or epigenetic changes in integrin levels such as in blistering diseases and cancer.

Below are some examples of integrin subunit-specific partnerships with RTKs in modulating cellular responses and developmental processes.

β3 and β1 integrins collaborate with RTKs to control angiogenesis

The development of new capillaries from pre-existing blood vessels (i.e. angiogenesis) occurs in embryonic development, reproduction, tissue regeneration and in pathological conditions such as cancer, rheumatoid arthritis and diabetic microvascular disease. Vessel morphogenesis requires a co-ordinated regulation of cell proliferation, migration and differentiation into tubes, providing an elegant example of a developmental co-operation between GFs and integrin–ECM adhesion [56].

The role of αvβ3 integrin

Both GFs and integrins are central to angiogenesis. For example, VEGF-A regulates angiogenesis through the RTK, VEGFR-2 [KDR (kinase insert domain-containing receptor) or Flk-1], which is specifically expressed on endothelial cells. Mice lacking VEGFR-2 show an impaired vasculature and early embryonic lethality [57]. Tumour development and ischaemic diseases often display increased VEGF production, and inhibiting the ligand or its receptors reduces tumour growth in multiple models [58]. Similarly, integrins also regulate angiogenesis; for example, endothelial αvβ3 is highly up-regulated at active angiogenic sites associated with tissue regeneration, inflammation and tumours, and blocking-antibodies or small-molecule inhibitors against αvβ3 potently inhibit tumour angiogenesis *in vivo*, with the latest therapies being delivered via nanoparticles [59,60].

There are several lines of evidence indicating that a direct interplay between these two receptor types, i.e. αvβ3 integrin and VEGFR-2 occurs at the biochemical level. (i) Attachment of HUVECs (human umbilical-vein endothelial cells) to αvβ3-specific ECM ligands [VN (vitronectin), fibrinogen] increases VEGF-A-induced mitogenesis, and a β3 integrin-blocking antibody prevents tyrosine phosphorylation of VEGFR-2 by its cognate ligand [61]. (ii) The β3 (but not β1 or β5) integrin forms a physical complex with VEGFR-2 in co-immunoprecipitates, an interaction that requires tyrosine phosphorylation of the β3 integrin cytoplasmic domain [62]. (iii) αvβ3 and VEGFR-2 also co-regulate angiogenesis via activation of shared downstream molecules; for example, c-Src complexes with both VEGFR-2 and αvβ3, it directly phosphorylates the cytosolic tail of β3 integrin in response to VEGF, Src regulates the activation and ligand-binding of β3, and it is required for tube formation [63]. c-Src also modulates VEGF-induced vascular permeability and blood vessel development in animal models, and VEGF promotes c-Src-mediated FAK (focal-adhesion kinase) recruitment to a related integrin, αvβ5 [64,65]. (iv) Another adhesion-complex protein, ILK (integrin-linked kinase), which binds cytoplasmic domains of both β1 and β3 integrin, is also implicated in VEGF-induced capillary formation [66]. Since PKB (protein kinase B)/Akt activation by VEGF is important for angiogenesis, and ILK contributes to Akt activation via rictor [rapamycin-insensitive companion of mTOR (mammalian target of rapamycin)], there may also be downstream co-operation through this signal integrator [66,67].

Interestingly, subunit-specific deletion experiments question the role of αvβ3 for angiogenesis *in vivo* [68]. Mice lacking β3 and β5 integrins display normal developmental angiogenesis and are viable, suggesting that these subunits may not have a role in embryonic development, although there are some cardiovascular defects in adult male mice lacking β3 integrin [69]. Indeed, αvβ3 integrin has anti-angiogenic properties in the case of pathogenic vascular development, because mice lacking β3 and β5 integrins not only develop tumours but actually display enhanced angiogenesis and increased tumour growth, mediated by elevated VEGFR-2 signalling [70]. These findings raise questions as to precisely how αvβ3 integrin regulates both developmental and pathological angiogenesis, and the importance of the β3 subunit in these processes. One possibility is that the αv integrin subunit is more critical (it is promiscuous for

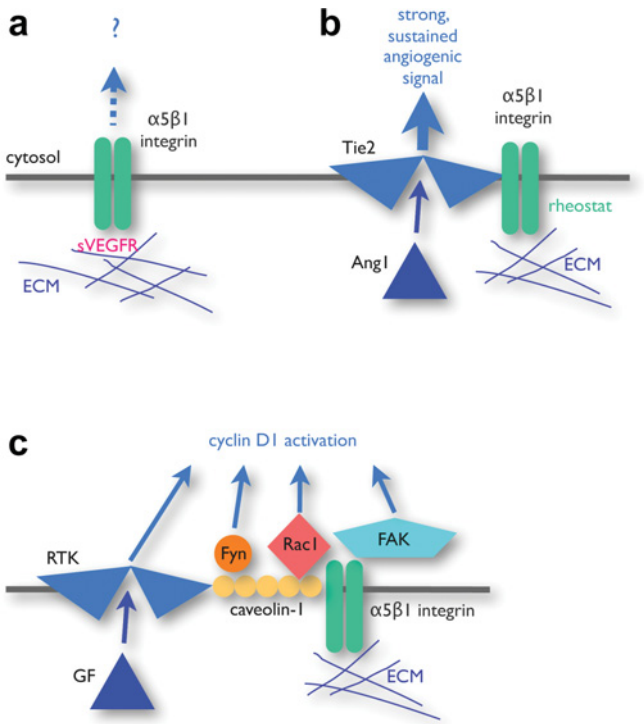

Figure 6 $\alpha5\beta1$ integrin co-operates with GFs

(**a**) A soluble VEGFR directly binds integrins. The signalling consequences of this interaction are not known. (**b**) $\alpha5\beta1$ integrin is a rheostat for Ang-1 signalling through Tie2. (**c**) Integrins collaborate with RTKs via caveolin-1 and lipid rafts, recruiting a variety of signalling enzymes that contribute to cell-cycle activation.

β integrins), because 30% of mice null for this subunit die at birth through vascular defects, although angiogenesis at early stages of embryonic development appears normal [71]. Nonetheless, the interdependency between $\alpha v\beta3$ and VEGFR-2 still exists, and it is bi-directional since VEGF-A induces $\alpha v\beta3$ expression while deletion of the $\beta3$ subunit elevates VEGFR-2 [70]. Maybe $\beta3$ integrins have more subtle roles than have previously been recognized, with both pro- and anti-angiogenic effects in order to prevent unrestrained vasculogenesis during developmental angiogenesis. An interesting concept is that the αv integrins have evolved as a second tier of adhesion receptors that are mainly utilized for repair processes such as wound healing and inflammation, while the $\beta1$ integrins have more fundamental roles in embryogenesis and tissue homoeostasis.

The role of $\alpha5\beta1$ integrin

$\alpha5\beta1$ integrin and its ligand, FN (fibronectin), have essential roles in vascular development, co-operating with GFs in three ways [72]. (i) A soluble form of VEGFR-1 that is deposited into the ECM, binds directly to $\alpha5\beta1$ integrin and promotes endothelial cell adhesion and migration, through a specific peptide sequence within its Ig-like domain II [73]. Although there is no lateral interaction between $\alpha5\beta1$ and transmembrane VEGFR-1 (Flt-1), the soluble VEGFR-1 may contribute to vessel development and angiogenesis via integrins (Figure 6a). (ii) $\alpha5\beta1$ forms a stable interaction with a separate class of angiogenic RTK, Tie2, which is a receptor for the angiopoietin, Ang-1 (angiopoietin-1) [74]. $\alpha5\beta1$ integrin is required for Ang-1-mediated blood vessel formation *in vivo*, and the co-receptor complex is enhanced when cells adhere to FN, correlating with increased endothelial cell migration. The association of integrin with Tie2 therefore acts like a rheostat to amplify both the strength and duration of angiogenic signals (Figure 6b). Mechanistically, it may be that optimal receptor output occurs when integrins recruit FAK and the RTK binds the PI3K (phosphoinositide 3-kinase) p85 subunit, providing synergy for efficient downstream Akt signals. (iii) $\alpha5\beta1$ integrin also co-operates with other RTKs, such as the receptors for bFGF, insulin and EGF, to activate cyclin D1 and thereby drive endothelial cell-cycle progression (Figure 6c) [75]. The $\alpha5\beta1$–RTK synergy occurs through a concerted activation of PI3K (probably via FAK) and Shc (Src homology and collagen homology), with consequent recruitment of the GEF (guanine-nucleotide-exchange factor), SOS (Son of sevenless), then activation of Rac1 and (unknown) downstream signals to cyclins. The link between receptors may depend on integrins binding to a phosphorylated form of a membrane adaptor, caveolin-1, which recruits Rac to lipid rafts [76]. Caveolin also binds Fyn and thereby Shc, and caveolin-1 can be visualized in focal adhesions, so representing an integrin-to-GF receptor node for activating multiple signalling enzymes [77].

The role of other $\beta1$ integrins

Other $\beta1$ integrins also contribute to angiogenesis, with the laminin receptor $\alpha3\beta1$ being negatively involved in tumour angiogenesis, and the collagen receptor $\alpha1\beta1$ restraining the activity of VEGFR-2 [56,78]. Interestingly, in the latter case, the suppression of signalling on collagen is via recruitment of the phosphatase, SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase 2), to VEGFR-2, which resembles the mechanism of inhibition of prolactin receptor signalling in mammary cells on the same ECM (see below) [79]. Finally, the $\alpha6\beta1$ laminin receptor may have a key role in switching the proliferation phase of angiogenesis to a morphological one where the cells form tubes, because it does not permit RTK-dependent cell-cycle signals in the way that $\alpha5\beta1$ does, but rather promotes differentiation [75].

Summary

The formation of blood vessels is a relatively simple programme in development involving the proliferation, migration and cellular reorganization of endothelial cells (and pericytes). Although RTK ligands provide a direct activation mechanism for angiogenesis, co-ordinating these three different types of cellular responses to create hollow tubes within a complex ECM environment requires numerous positive and negative signals. Integrins are central in this process, controlling and co-ordinating the outcome of extracellular GF signals.

Major gaps in knowledge

Major gaps in knowledge include: (i) the molecular details of precisely how the two receptor systems interact, both within the plane of the membrane and at the level of signal integration; (ii) the details of how different α integrin subunits direct the phenotypic endpoints, i.e. proliferation compared with tubular morphogenesis; and (iii) differences of integrin–RTK interactions in normal and pathological angiogenesis.

$\alpha6\beta4$ integrin co-operates with RTKs in normal epithelial function

Hemidesmosome architecture

The LM (laminin) receptor $\alpha6\beta4$ is the classic core component of hemidesmosomes [80]. In normal epithelial cells, especially those prone to mechanical shear stresses such as epidermal keratinocytes, $\alpha6\beta4$ performs adhesive functions by interacting

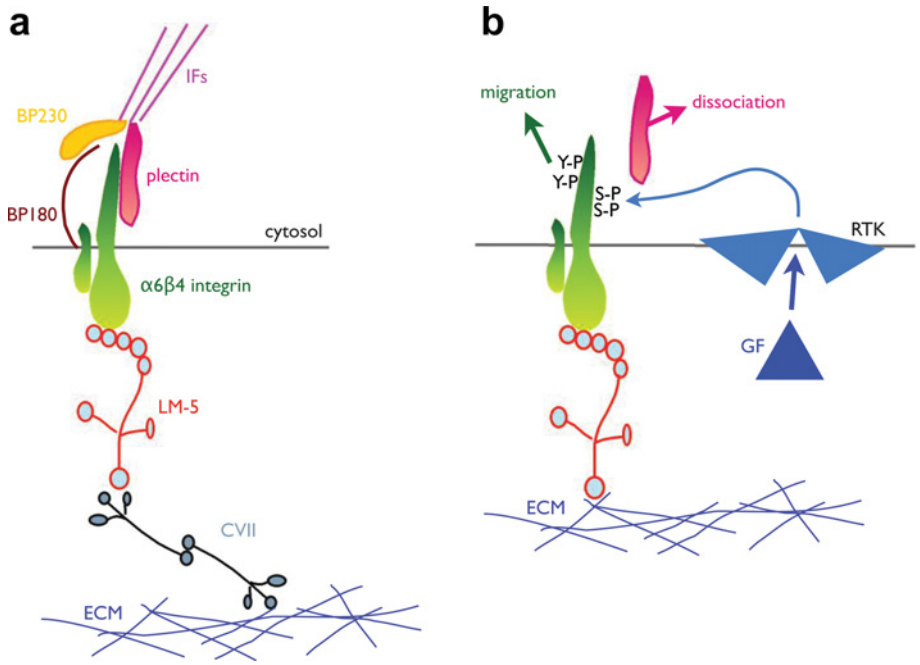

Figure 7 The dynamics of $\alpha 6\beta 4$ integrin

(**a**) Schematic diagram of hemidesmosomal components which provide a continuous mechanical link between the ECM and intermediate filament network. Lateral clusters of these units are the building blocks of hemidesmosomes, which are mechanical adhesive devices for cells. CVII, collagen VII; IF, intermediate filaments. (**b**) RTK signalling causes the phosphorylation of serine residues on $\beta 4$ integrin, leading to dissociation of plectin and disassembly of hemidesmosomes. $\alpha 6\beta 4$ integrin may be converted into a migration machine via tyrosine phosphorylation. S-P, phosphorylated serine; Y-P, phosphorylated tyrosine.

with LM-5 (laminin332) in the basement membrane, which binds collagen VII then collagen I within the dermis. On the inside of the cell it binds to plectin which directly links to the keratin cytoskeleton, and together with BP180 and BP230, this multiprotein complex forms massive adhesion plaques that stabilize tissue architecture (Figure 7a) [81]. The importance of $\alpha 6\beta 4$ is demonstrated by skin defects in humans with $\beta 4$ integrin mutations, and epidermal blistering and postnatal death in mice lacking the $\beta 4$ cytoplasmic tail [82,83].

However, the enormous strength of these adhesions means that they need to be undone at certain times. In order for epidermal cells to undergo cytokinesis, enter the supra-basal cell layer during stratification, or to migrate laterally in a wound-healing response, hemidesmosomes become destabilized, and this occurs through RTK signalling to $\alpha 6\beta 4$ integrin.

The $\beta 4$ integrin subunit is unique by virtue of a long cytoplasmic tail. This region provides a variety of binding sites for cytoskeletal and signalling proteins, making it distinct from other integrins. The N-terminal half of this segment contains the binding domain for plectin, whereas the distal half has multiple serine and tyrosine phosphorylation sites. Targeted deletion of the phosphorylation domain (C-terminal to the plectin-binding region) has no effect on the formation and stability of hemidesmosomes, in contrast with mice lacking the entire $\beta 4$ cytoplasmic tail, indicating that phosphorylation sites are not required for epidermal adhesion [84].

RTKs alter the dynamics of $\alpha 6\beta 4$ integrin

Dynamic processes such as wound healing are necessarily accompanied by destabilization of cellular adhesions. Under these conditions, $\beta 4$ integrin redistributes from hemidesmosomes to actin-based structures at leading edges of cells, where it actively promotes migration and proliferation in conjunction with GFs secreted at the wound [85]. The disassembly of hemidesmosomes is mediated by GFs, e.g. EGF, which induces the phosphorylation of three highly conserved serine residues on $\beta 4$ integrin via PKCα (protein kinase Cα), and possibly PKA (protein kinase A) [86]. These modifications destabilize the interaction between plectin and $\beta 4$ and thereby break down adhesion complexes (Figure 7b). The phosphorylation region of $\beta 4$ integrin is required for keratinocyte migration and signalling because its deletion suppresses wound closure (and also inhibits proliferation and enhances apoptosis in cultured cells) [84].

One possible link between $\beta 4$ and migration lies in the association of non-hemidesmosomal $\alpha 6\beta 4$ integrin with a fraction of EGFR. This interaction occurs within lipid rafts and leads to the activation of Fyn, which can phosphorylate tyrosine residues in the $\beta 4$ integrin tail, causing recruitment of Shc and activation of downstream enzymes [51]. One of these is Rac, which is downstream of both $\beta 4$ integrin and EGFR, and has a central role in directional cell migration [87,88].

Re-epithelialization of wounds is also accompanied by cytokines such as macrophage-stimulating protein, which contributes to hemidesmosome disassembly via its receptor Ron [89]. Ron activation results in serine phosphorylation of $\beta 4$ integrin, which presumably has a similar effect to EGFR in displacing plectin. However, in addition, 14-3-3 proteins bind the modified serine residues of $\beta 4$ leading to the formation of a $\beta 4$–14-3-3–Ron complex, which relocalizes to lamellipodia and causes accelerated wound healing *in vivo*. This cross-talk is reciprocal, since integrins trigger Ron phosphorylation and kinase activity, most likely through Src/FAK signalling [90].

Summary

Although $\alpha 6\beta 4$ integrin is conventionally thought of as being a receptor for stable adhesions, it's role is much more

sophisticated. Thus, in normal wound repair, multiple RTKs switch the function of this core hemidesomsomal component from a mechanical adhesive assembly into a migration and signalling ECM receptor (Figure 7). Interestingly, it seems that serine phosphorylation destabilizes the hemidesmosomes, whereas tyrosine phosphorylation promotes migration.

$\alpha 6\beta 4$ integrin co-operates with RTKs in epithelial cancer

Although the above provides an elegant mechanism for creating stable epithelial tissues, with the option of dynamic repair when the situation arises, it is susceptible to abuse. $\beta 4$ integrin has a central role in the progression of several tumour types, where it combines with mutated or amplified RTKs, including EGFR, ErbB2 and c-Met [91–93]. These RTKs inappropriately phosphorylate tyrosine residues within the distal half of the $\beta 4$ cytoplasmic tail, thereby preventing the assembly of hemidesmosomes and promoting invasion instead [43].

$\alpha 6\beta 4$–RTK co-operation in migration and survival

A link between the $\alpha 6\beta 4$ and ErbB2 receptors was reported initially in breast carcinoma cell lines where their association enhanced migration [94]. The invasive effects of this receptor pairing are mediated in part by the PI3K/Akt pathway, which activates Rac1. ErbB3, which is a strong activator of PI3K/Akt, might be involved as well, since $\alpha 6\beta 4$ integrin elevates the translation of ErbB2 and ErbB3, and so potentially transactivates both EGFR/ErbB2 and ErbB2/ErbB3 [95,96]. The mechanism of receptor synergy between $\alpha 6\beta 4$ and RTKs in cancer cells has not been fully resolved, but their physical association may promote tyrosine phosphorylation on the $\beta 4$ tail: in addition to altering hemidesmosome stability, this also provides SH2-binding sites for several signalling proteins including the p85 subunit of PI3K. As mentioned above, this may occur via Fyn in the context of EGFR activation. Ligation of $\alpha 6\beta 4$ promotes an interaction with the adaptor protein, IRS (insulin receptor substrate), which itself binds and activates PI3K [97].

$\alpha 6\beta 4$ integrin is also implicated in cancer-cell invasion mediated by another RTK, c-Met (the receptor for HGF, originally known as scatter factor). A constitutively active form of Met, which is present in some cancer cell lines, physically associates with $\alpha 6\beta 4$ integrin. In overexpression studies, Met induces $\beta 4$ integrin tyrosine phosphorylation and recruitment of Shc and hence PI3K, promotes transformation and tumorigenesis of cell lines, and both receptors are required for HGF to cause cell invasion [98]. However, it is not certain whether this integrin–RTK interaction really occurs *in vivo* because other studies indicate that Met and $\alpha 6\beta 4$ have separate and independent roles in cancer-cell invasion, and the $\beta 4$ intracellular domain by itself can not enhance HGF signalling [99,100].

$\alpha 6\beta 4$ also mediates anchorage-independent survival of breast cancer cells. This occurs through Akt, and via a mechanism involving Rac, PAK (p21-activated kinase) and NF-κB (nuclear factor κB) [101,102]. Confirmation of the role for $\beta 4$ integrin in cancer-cell survival comes from shRNA (small hairpin RNA) studies, where $\beta 4$ knockdown promotes apoptosis [103].

$\alpha 6\beta 4$–RTK co-operation in tumorigenesis

Whether any of these are *bone fide* mechanisms in human tumours is not yet known. In the MMTV-Neu mouse model for breast cancer (which carries an activated form of ErbB2), targeted deletion of the $\beta 4$ cytoplasmic domain suppresses tumour onset and invasive growth, arguing for a functional association. In primary cells isolated from these tumours, $\beta 4$ integrin complexes with ErbB2 and enhances signalling by activating STAT3 (signal transducer and activator of transcription 3), nuclear translocation of JNK and phosphorylation of c-Jun [104].

However, contrasting with this, in one study of human breast cancer, $\beta 4$ integrin expression was strongly associated with 'basal' tumours, but not with ErbB2, and in another study there was no association between $\beta 4$ and ErbB2. Instead, all of the tumours with elevated ErbB3 also had a higher level of $\beta 4$ integrin [29,105]. Thus while there is compelling evidence that the $\beta 4$ integrin has an important role in cancer progression, its mechanism of involvement is not certain.

Major gaps in knowledge

Major gaps in knowledge include whether or not $\alpha 6\beta 4$–RTK interactions actually occur during tumorigenesis *in vivo*, and if so which residues are phosphorylated and what the *trans*-phosphorylation mechanism might be. $\beta 4$ integrin may be a good new therapeutic target for aggressive cancers, but chiselling out the mechanism of its involvement will be necessary to develop strategies which prevent tumour invasion and at the same time do not perturb either normal hemidesmosomes or wound repair.

$\beta 1$ integrins, RTKs and the cell cycle

One further example of integrin–RTK cross-talk is worth mentioning because it provides some additional clues about the mechanisms of synergy. $\beta 1$ integrins co-operate with EGFR (ErbB1) in several cell types in the context of classical signalling output via the ERK (extracellular-signal-regulated kinase) and Akt pathways, and this has important implications for regulation of diverse aspects of cell fate, including proliferation [106].

All adherent cells require ECM adhesion to progress through the cell cycle. Thus RTK activation is not sufficient for proliferation and integrin signalling is needed as well. It has been established for some time that integrin adhesion is required in fibroblasts for the activation of MEK [MAPK (mitogen-activated protein kinase)/ERK kinase] downstream of Raf, translocation of ERK into the nucleus and phosphorylation of Elk-1 [ETS (E twenty-six)-like kinase 1], transcription of an EGF-responsive gene Erg-1, degradation of p27 via the ubiquitin ligase Skp-2 (S-phase kinase-associated protein 2), expression of cyclin D1 and progression through G_1/S-phase [107–111] (Figure 8a).

The mechanism for this requirement of integrins involves both a direct cross-talk with RTKs such as EGFR, and RTK-independent signalling from adhesomes. However, integrins are not able to activate RTK sufficiently to trigger the cell cycle without GFs. Thus the cell cycle progresses through a combination of GF–RTK, integrin–RTK and direct-integrin signals (Figure 8b). These distinct modes of signal integration by extracellular ligands may be necessary to ensure that both the early and late stages of the cycle are accomplished in order to pass through the restriction point and enter S-phase [112].

$\beta 1$ integrin activation of EGFR

In primary fibroblasts and ECV304 endothelial cells, integrin-mediated adhesion results in rapid tyrosine phosphorylation and kinase activation of the EGFR [48,113]. This occurs in the absence of any RTK ligands, and in cells that have moderate levels of EGFR ($> 3 \times 10^4$ receptors/cell), adhesion induces ShcA phosphorylation and ERK kinase activity via the EGFR. This is a direct activation mechanism since a fraction of EGFR associates with $\beta 1$ integrins in a complex with Src and p130Cas, and both the $\beta 1$ integrin cytoplasmic domain and Src/Cas are required

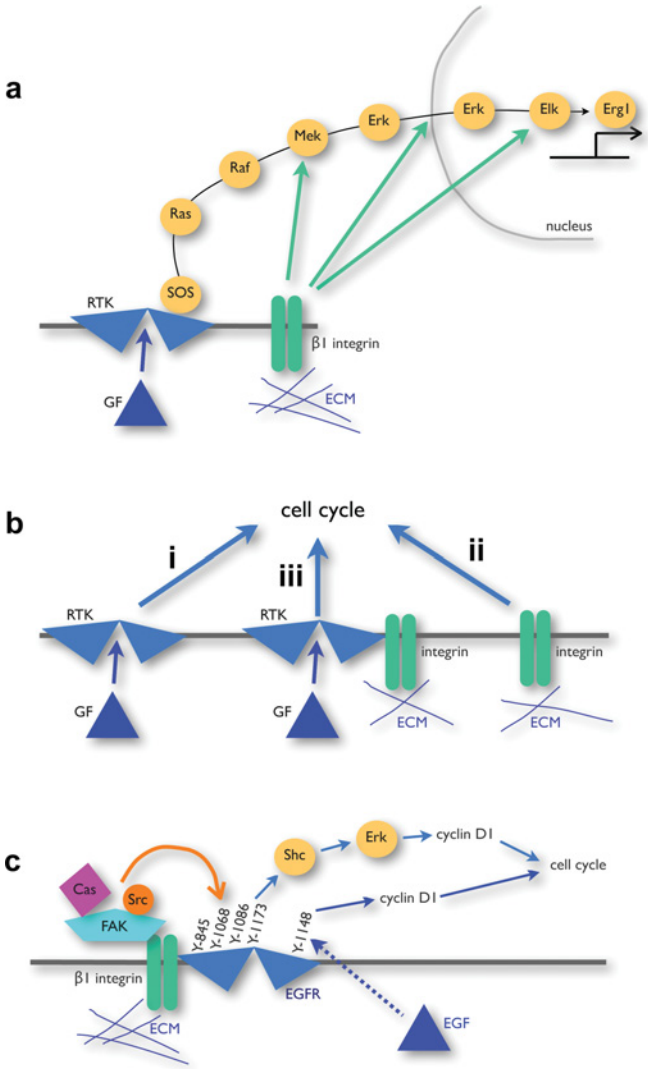

Figure 8 β**1 integrins and cell-cycle control**

(**a**) Integrins regulate many of the steps in the GF-to-cell cycle pathway. (**b**) The cell cycle requires separate signals from (i) GFs, (ii) integrins and (iii) integrin–RTK interactions. (**c**) One mechanism of integrin–RTK cross-talk is via direct association between the receptors and indirect phosphorylation of several tyrosine residues and thereby ERK activation, whereas EGF causes phosphorylation of a separate residue, Tyr^{1148}. The outputs are different, but both are required for cell-cycle progression.

for EGFR phosphorylation. Moreover, integrin activation leads to phosphorylation of a distinct subset of tyrosine residues to EGF, leaving the major EGF-responsive Tyr^{1148} unphosphorylated. Thus integrins directly affect the tyrosine phosphorylation of RTKs, thereby influencing their signalling output and phenotypic endpoints (Figure 8c).

This model has been extended with the use of COS-7 and CV1 cells, and primary prostate epithelial cells and keratinocytes, to show that integrins cause EGFRs to recruit a variety of adaptors independently of EGF, which stimulate the ERK and Akt pathways, then cyclin D1 synthesis and Cdk4 (cyclin-dependent kinase 4) and Rb (retinoblastoma) phosphorylation [114]. Separate ligand-induced activation of the EGFR is required for p27 down-regulation, Cdk2 activation, Myc and cyclin A induction and cell-cycle progression (Figure 8c).

Integrin activation of PDGFR

The mechanism of integrin activation of RTKs also involves proteins identified for cross-talk with a different receptor, PDGFRβ [115]. In MEFs (mouse embryonic fibroblasts), integrin adhesion to FN promotes the association of SHP-2 with PDGFRβ, leading to a positive effect on signalling. SHP-2 dephosphorylates the tyrosine residue that would otherwise bind RasGAP, with the consequence that Ras and ERK activation are sustained and more cells enter the cell cycle. SHP-2 may also increase tyrosine phosphorylation on SIRPα (signal regulatory protein α), which further contributes to adhesion-mediated ERK activation [116]. Through these mechanisms, and most likely Src-family kinases as well, β1 integrins alter the ability of RTKs to recruit adaptors, thereby changing their signalling output and phenotypic endpoints.

RTK-independent integrin signalling

In cell types with very low levels of EGFR (e.g. 3T3 fibroblasts) the adhesion-to-ERK signalling is autonomous of RTKs, and can be triggered directly through adhesome enzymes, e.g. FAK/Src and/or GEFs/Rac [48]. Indeed, Rac1 is a key mediator of integrin signals for proliferation, via PAK1, and its activity is directly dependent on signals induced through the β1integrin tail and integrin recruitment to membrane microdomains [117–119]. Further complexity is revealed through the use of β1 integrin-rescued β1-null GD25 cells, where the Akt pathway is triggered by separate mechanisms downstream of integrins and EGFR [120–122]. β1 integrin activates Akt through a novel mechanism that is independent of EGFR and FAK/Src, Abl or other Src-family kinases, but involves an unknown PP2 (protein phosphatase 2)-inhibitable enzyme. Moreover EGFR can separately cause the phosphorylation of Akt on Ser^{273}/Thr^{308} and it uniquely triggers a Src-dependent Tyr^{326} phosphorylation.

Integrin suppression of EGFR

In addition to activating RTKs, integrins also have a role in suppressing their activity. An example of this involves the PTP (protein tyrosine phosphatase), TCPTP (T-cell PTP), which binds specifically to the collagen receptor $\alpha1\beta1$ integrin, but not other collagen-binding integrins, as well as to EGFR [123]. $\alpha1\beta1$ integrin adhesion activates the phosphatase activity of TCPTP and inhibits ligand-induced EGFR phosphorylation. Although TCPTP does not adversely affect EGF-induced ERK activation, it does prevent EGFR interactions with ShcA-Grb2, and therefore has quite specific effects [124]. A challenge will be to identify how this and other inhibitory PTPs are involved with integrin–RTK cross-talk, as this may be crucial to limit proliferation and promote morphogenesis, particularly in three-dimensional developmental situations. Moreover, defects in such interactions could promote tumorigenesis, and it is of interest to note that ShcA is essential for tumour formation in an ErbB2 model of breast cancer [125].

β1 integrin–EGFR cross-talk in tumours

A different mechanism of functional interplay between β1 integrin and EGFR may be important in some transformed breast epithelial cell lines, and be of relevance to tissue organization and possibly cancer progression. A normal breast line adapted to culture in the presence of EGF becomes tumorigenic after a few generations in the absence of EGF, due to the acquisition of an extra chromosome 7p harbouring the EGFR gene [126]. In comparison with the parental cells, which form hollow acini in three-dimensional culture, the tumorigenic cells form

disorganized cellular aggregates. This is caused by acquisition of *de novo* integrin–EGFR interactions, because function-blocking antibodies to either β1 integrin or EGFR: (i) inhibit EGFR-to-MAPK signalling, (ii) revert the disorganized morphology to normal acinar appearance with hollow lumens, and (iii) reduce tumour incidence *in vivo* [127]. There is a related restoration of 'normal' three-dimensional architecture of some breast cancer lines (Hs578T and MB-MDA-231) by anti-β1 integrin antibodies [128]. However, this may largely be due to apoptosis induced by the antibody, and both anti-integrin antibodies and EGFR antagonists cause mammary epithelial cell apoptosis [129,130]. The mechanism of communication between β1 integrin and EGFR in breast tumour cells appears indirect, although it has not been fully determined.

Interestingly, the cross-talk only occurs in three-dimensional cultures, and is apparently not related to the loss of tissue organization and altered polarity seen in three-dimensional cultures of breast epithelial cells overexpressing ErbB2 [131]. Since β1 integrins have crucial roles in the formation of primary tumours, as well as metastatic dissemination and preventing tumour cell senescence in mouse models of breast and pancreatic cancer (respectively), it will be important to determine whether β1 integrin antagonists provide a useful therapeutic approach for human cancer, particularly when used in combination with anti-EGFR strategies [132,133].

Summary

Two of the central signalling modules (i.e. ERK and Akt) ensuring that all of the components for cell-cycle commitment are in place, are co-ordinately regulated by β1 integrins and EGFR (as well as other RTKs). Integrins have a further role in cell-cycle regulation through both the Src family kinase and Rac signalling cassettes, and here GFs also contribute to the signalling output [134]. Although not discussed in the present review, integrin–RTK coupling also strongly influences survival and migration, both of which are fates that are linked to spatial and temporal aspects of cell behaviour.

In the bigger picture, integrins and RTKs are no longer seen as individual receptors binding either GFs or ECM proteins, but rather as joint sensing apparatus for cells to detect where they are and how they should respond to instructive signals from other cells (Figure 4). Importantly, the ability of integrins to activate a variety of RTKs indicates that this is a widely used device to enhance the cellular effects of GFs, providing a switch (or turning up the rheostat) that allows GF signalling to be sustained when cells are located within an appropriate ECM (Figure 5a). A consequence is that dysregulation of these systems can have profound effects on the progression of morphogenetic diseases such as cancer.

Major gaps in knowledge

Major gaps in knowledge include: (i) a deep understanding of how the two different receptor systems are spatially configured within the plasma membrane *in vivo* in order to allow physical interactions and signalling cross-talk; (ii) knowledge of the specificities of β1 integrin–RTK interactions in different cell types, and two-dimensional compared with three-dimensional compared with *in vivo*; and (iii) elucidation of how their positive interactions are restrained during developmental switches from proliferation to differentiation.

In addition, there are several key questions that still need to be evaluated. (i) A crucial part of integrin function during cell migration is related to endocytic cycling [34]. Since Rab11 controls β1 integrin recycling, the same GTPase is also involved with modulating EGF-induced cell cycle, and Ras/ERK signalling can continue on 'signalling endosomes', do early or sorting endosomes have a central role in co-regulation of cell cycle by integrins and RTKs such as EGFR [135,136]? (ii) Some adhesome proteins [e.g. ILK and NEDD9 (neural-precursor-cell-expressed developmentally down-regulated 9)] are found at centrosomes and are involved with mitotic spindle assembly [137,138]. Are integrins or integrin–RTK interactions involved directly with the later stages of mitosis, in addition to the early signalling events? (iii) There are mechanical connections between the ECM and nucleus through integrins, actin/keratin cytoskeletons and the nuclear envelope [139,140]. Do such links directly affect the cell cycle?

SIGNAL INTEGRATION CONNECTING INTEGRINS AND CYTOKINE RECEPTORS

The STAT family of proteins are activated downstream of cytokines to regulate cell-fate responses such as proliferation and differentiation. Cytokine receptors for IL (interleukin), prolactin and IFN (interferon) lack intrinsic kinase activity, and transmit intracellular signals by interacting with protein tyrosine kinases belonging to the JAK (Janus kinase) family [141]. Ligand binding leads to the ligation of two receptor molecules, which brings receptor-associated JAKs in close enough proximity to allow *trans*-phosphorylation and thus activation of the kinase domain. Activated JAKs then phosphorylate the receptors, which provide specific sites for the recruitment of SH2-domain signalling components, including the STATs, and rapid propagation of downstream signals. Tyrosine phosphorylation of STATs by JAK family members leads to nuclear translocation where they bind to specific promoter elements and control expression of target genes. Fine-tuning of this pathway is achieved through regulation by phosphatases including SHP-1, SHP-2 and PTP1B (which exert their effects both positively and negatively), nuclear export of STATs and the SOCS (suppressors of cytokine signalling) proteins [142]. In addition, several of the cytokine receptors and/or JAKs activate other signalling cassettes initiated by Src family kinases and GEFs, e.g. Vav [143], adaptor proteins, such as SH2B1 which can recruit Rac and IRS [144], or adaptor/kinases such as IRAKs (IL-1-receptor-associated kinase), which are involved with innate immune responses [145].

There is a critical dependence on integrins for cytokines to activate intracellular signalling and dictate cell-fate responses in adherent cell types.

Integrins regulate IL-receptor signalling

Signal co-operativity between integrins and IL receptors occurs in a variety of cell systems, with a range of responses.

The epithelial cells lining the intestinal tract are involved in immune responses by producing pro-inflammatory cytokines in response to IL-1β. Cell–ECM interactions regulate this pathway, because the interaction of Caco-2 cells (IEC) with FN promotes AP-1 (activator protein 1) and NF-κB activation, and cytokine production, whereas the basement membrane ligand, LM-5 and its cognate receptor $\alpha 3\beta 1$ integrin suppress it [146]. Thus cells in their native environment (i.e. contact with basement membrane) are refractory to IL-1β, whereas those sensing an altered ECM environment, e.g. following FN exposure in the event of wound healing, might be stimulated to synthesize pro-inflammatory cytokines. The detailed mechanism of how this cross-talk works is not yet known.

The link between IL-3 receptor and β1 integrin is better understood, and resembles integrin interactions with some RTKs.

IL-3 acts as a pleiotropic factor in regulating endothelial cell biology, controlling proliferation, migration and survival. In breast cancer for example, IL-3 secretion by infiltrating T-cells promotes angiogenesis. IL-3 drives primary endothelial cell proliferation via the JAK2/STAT5 pathway and c-Fos gene transcription, but integrin-mediated adhesion is a key element in this pathway because: (i) signalling only proceeds when cells are attached to the ECM ligands FN or LM; (ii) activating anti-(β1 integrin) antibodies trigger IL-3 signalling in the absence of cytokine; and (iii) β1 integrin, activated by ECM adhesion or Mn^{2+}, physically associates with the IL-3β receptor and directly activates JAK2/STAT5 signalling [147]. The two different receptors have separate roles because integrin activation alone stimulates this cytokine pathway only transiently, but addition of IL-3 promotes a much more sustained response and a higher proportion of cells entering the S-phase of the cell cycle (Figure 9a).

Thus, in cells that are naturally adherent, there is an elegant interplay between integrins and cytokine receptors that is required in order for the cytokine to deliver a maximal response. Here, cell adhesion provides a spatial checkpoint to prime the IL-3 signalling axis, which only becomes fully active at times when IL-3 is present.

Integrins regulate the prolactin signalling cascade

A similar situation occurs for prolactin signalling, where cells need to be in contact with the correct ECM environment in order to respond to instructive signals from this endocrine hormone. The mammary gland develops in a temporal and spatially regulated manner such that, during pregnancy, epithelial cells proliferate and then differentiate, but only produce their tissue-specific products (milk) at the right time and place, i.e. during lactation and in epithelial cells within acini [148]. β1 integrin adhesion to LM provides the spatial signals controlling this cell-fate process, and prolactin provides the temporal signals [22,149]. Here, both negative and positive influences from the ECM control cytokine signalling.

Prolactin activates the JAK2/STAT5 cascade when mammary cells adhere to LM, but not to collagen I, being inhibited on the latter ECM by a PTP [150], which is likely to be SHP-2 [151]. This PTP can be suppressed chemically or with constitutively activated Rac (V12Rac), restoring prolactin-dependent STAT5 signalling. The mechanism by which V12Rac regulates SHP-2 is unknown, but one possibility is via ROS (reactive oxygen species), which inhibit PTPs.

Active integrin signalling is also essential for driving lactational differentiation, since ablation of β1 integrin both *in vivo* and in a primary three-dimensional culture model inhibits the ability of prolactin to activate STAT5 and induce milk protein synthesis [152,153]. Genetic knockout approaches have demonstrated that the adhesion complex protein ILK, via its adaptor function rather than kinase activity, acts downstream of β1 integrin to regulate this process [154]. Importantly, not all adhesion complex proteins have equal roles in this aspect of integrin signalling, because although another integrin signalling protein (FAK) is located in the same complex, it does not have a role in mammary differentiation. This suggests that the adhesome circuitry is wired specifically for different cell-fate decisions.

Further on in the pathway (in mammary cells), Rac1 serves as a nodal point integrating signals between integrins and cytokine receptors, since expression of V12Rac in β1 integrin-null acini rescues JAK activation and the lactational defect [151]. The link between ILK and Rac is not known, but is presumably an adhesome-recruited GEF. Rac has a further role in JAK/STAT signalling via its effector PAK, which regulates lactational

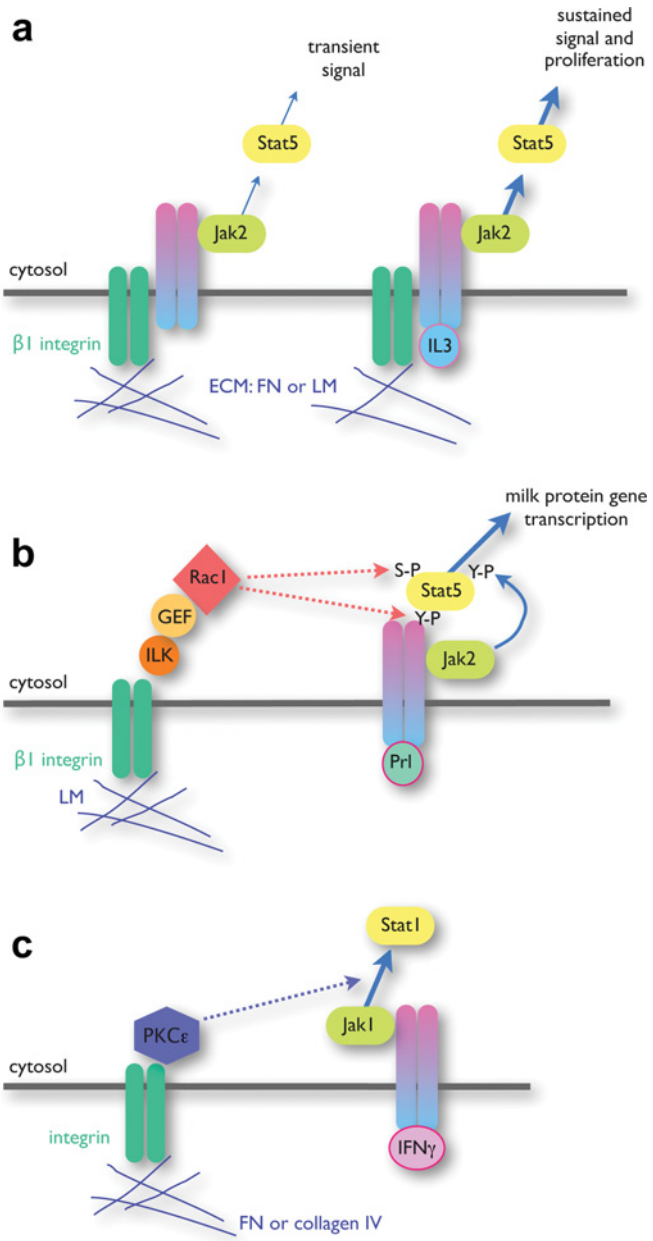

Figure 9 Integrin connections with cytokine receptors

(**a**) β1 integrins promote IL-3 signalling, but the signal is sustained only in the presence of both ligand and integrin–ECM interactions. (**b**) β1 integrins are required for prolactin signalling, via ILK and Rac1. The link to the prolactin pathway is indirect, and involves intermediates to promote both tyrosine phosphorylation of the receptor and JAK2 activation, as well as full STAT5 activation. S-P, phosphorylated serine; Y-P, phosphorylated tyrosine. (**c**) Integrins are required for maximal interferon signalling, via cross-talk with PKC.

differentiation in part by phosphorylating STAT5a on Ser^{779} [155]. We view the adhesion complex as a structural framework for localized GEF activation, which in turn activates Rac1 in a spatially restricted manner, leading to transmission of highly localized signals, e.g. prolactin receptor activation in this cell system (Figure 9b). Recent studies have shown that, in the absence of LM, prolactin does trigger its signalling pathway but only transiently and not enough to influence gene expression, whereas the LM–cell interaction enables sufficiently sustained activation of STAT5 for milk protein synthesis [155a]. This means

that LM adhesion acts as a classic rheostat to promote strong prolactin receptor signals, analogous to the integrin–RTK receptor integration (Figure 5b).

Integrins regulate IFN responses

Integrin-mediated adhesion also regulates IFN responses, which in this case is via an indirect mechanism involving PKCε (Figure 9c). In cells responsive to IFNγ, signalling to STAT1 is much stronger in cells that are attached to either FN or the basement-membrane collagen IV than in detached cells or those adhering to fibrillar collagen I [156]. PKCε is also required for the ability of IFNγ to activate STAT1, with the input occurring at a point downstream of JAK1/2. Since integrin-mediated adhesion leads to the phosphorylation and activation of PKCε, it appears that PKCε facilitates the coupling of JAK1/2 to STAT1. It is not clear how integrins directly regulate PKCε activation, but other members of the family, e.g. PKCα, associate with active β1 integrins, and roles for both members of the PKC family in integrin trafficking have been proposed [157,158]. There may also be a negative regulation of the IFNγ pathway in the absence of appropriate ECM signals, since PP2A phosphatase and PDK1 (phosphoinositide-dependent kinase 1) are recruited to form a complex with PKCε in cells cultured on fibrillar collagen, thereby dephosphorylating and inactivating PKCε and consequently preventing transmission of IFN-γ signals [156].

Adhesion signals relayed by Pyk2 (a homologue of FAK) also contribute to IFN-α and -γ-mediated STAT1 phosphorylation. In this case, the signals may converge at the level of JAK1 by forming a complex, although there is some controversy about the mechanism because in one study Pyk2 binding to JAK1 modulated its phosphorylation status [159], whereas in the other, JAK1 phosphorylation was unaffected [160]. FAK also mediates STAT1 activation upon cell adhesion to FN, but this occurs independently of the JAKs [161].

Although these studies provide tantalizing glimpses of an adhesion checkpoint for IFN signalling, more needs to be learnt about the detailed mechanisms whereby integrins facilitate the pathway, and whether this is a universal mechanism that applies to all cell types.

Summary

A new view of cytokine signalling emerges that, rather than being simple effectors of one-dimensional pathways, as is commonly perceived, in many cell types there is an absolute requirement for integrins in order for cytokines to activate efficient downstream signals. The formation of distinct adhesion signalling platforms, which are likely to be cell type- or ECM-dependent, then influence the intracellular response to hormonal stimulation, as for example in the rheostat model (Figure 5). Identifying the spatial localization of both adhesion and cytokine signalling components in adherent cell types is a major gap in our knowledge, yet fundamental in understanding how they interconnect to regulate various cellular responses. In most of the cases mentioned above, the exact details of the integrin connections with cytokine receptors still need to be worked out.

SIGNAL INTEGRATION BY INTEGRINS AND SYNDECANS

Syndecans are a family of transmembrane proteoglycans that are comprised of a protein core with covalently attached heparan sulfate sugar chains [GAGs (glycosaminoglycans)]. One of their key roles is bind GFs through their GAGs, which trap specific ligands that are either dilute or distant from their specific receptors. Cellular responses to FGF-2 are blocked when the syndecan GAGs are disrupted, so they are essential co-receptors for GFs and are therefore a part of the integrin–GF receptor system [162] (Figure 10a).

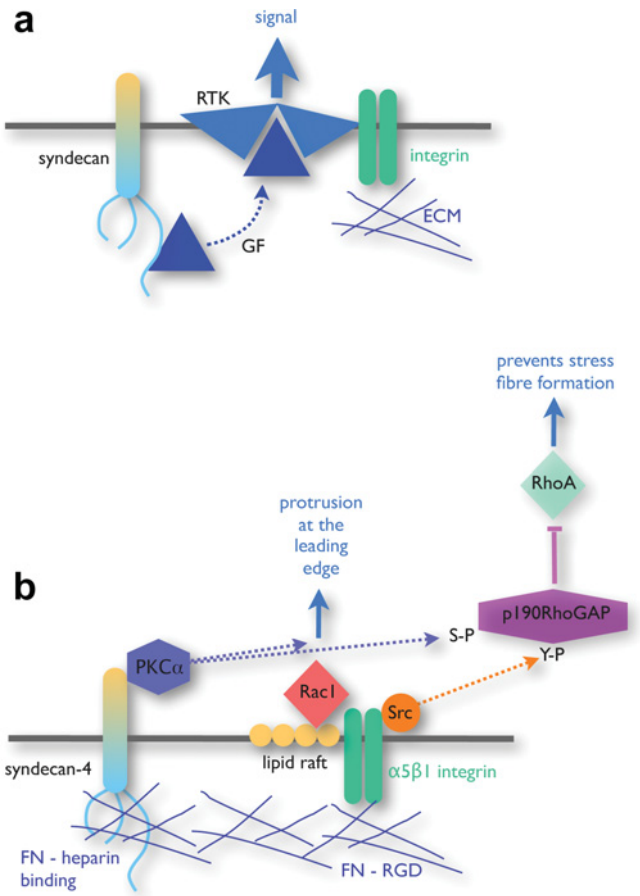

Figure 10 Integrin connections with syndecans

(**a**) Syndecans present GFs to their receptors, and are therefore part of the integrin–GF system. (**b**) In the initial stages of cell spreading, which is analogous to membrane protrusions at the leading edge of migrating cells, syndecan and integrin collaborate to promote Rac activation and inhibit Rho. S-P, phosphorylated serine; Y-P, phosphorylated tyrosine.

Syndecans also act as adhesion receptors by engaging with FN, LM or VN [163]. Intracellularly, their short cytoplasmic domain is composed of two conserved regions that bind Src/Fyn and PDZ-domain-containing-proteins respectively, whereas an intervening region is variable and unique to each syndecan. For syndecan-4, the variable region binds both the inositol lipid PtdIns(4,5)P_2 and PKCα, activating the latter in nascent focal adhesions [164]; it also connects to the actin cytoskeleton via α-actinin and enhances focal complex assembly [165].

Taken together, these properties suggest that syndecan behaves in a similar way to integrins. However, it is established that syndecans can co-operate synergistically with integrins to regulate adhesion-complex formation, cell spreading and directional migration. Indeed, there is co-operation between syndecans and VN-binding (αvβ3, αvβ5) or LM-binding integrins (α2β1, α6β4), and syndecan-4 synergizes with the FN-specific α5β1 integrin. In addition, syndecans can activate integrins, for example syndecan-1 regulates the migratory function of αvβ3 integrin [166]. Thus individual combinations of syndecan–integrin pairs influence the type of signalling proteins recruited at adhesion complexes, and consequently the physiological response elicited.

Syndecan-4 collaboration with $\alpha5\beta1$ integrin

As a specific example, syndecan-4 collaboration with $\alpha5\beta1$ integrin jointly influences cell adhesion and directed migration on FN [167]. Here, engagement of $\alpha5\beta1$ integrin to the central-cell binding domain of FN induces the attachment and spreading of fibroblasts [168]. However, focal adhesions only form when the heparin-binding region of FN binds syndecan-4 and activates PKCα. Indeed, syndecan-4-null cells can display impaired adhesions and stress-fibre formation in culture and a delayed wound-healing response (indicating migratory defects) *in vivo*, and moreover PKCα is required for syndecan-induced migration [169,170].

The mechanism for this co-ordinated receptor signalling occurs through a localized activation of Rho family GTPases, which are instrumental in cell spreading and directional migration on ECM substrates, processes requiring both focal adhesion assembly and actin polymerization [8].

Rac1 is transiently activated early in the process of cell spreading on FN, when RhoA is suppressed, and is then followed by Rho activation. This allows protrusions to form at the front of a moving cell, succeeded by retraction at the rear of the cell via stress fibres. However, the initial Rac1 activation does not occur during adhesion via $\alpha5\beta1$ integrin only (i.e. to the 50K fragment of FN), but also requires syndecan-4–ligand interactions (i.e. with the separate heparin-binding domain of FN), and moreover syndecan-4-null fibroblasts are unable to regulate Rac1 activity during spreading on complete FN [171,172]. It seems likely that integrins recruit Rac1 to the plasma membrane at lipid rafts, whereas syndecan-4, via PKCα, localizes the activation of Rac1 to the leading edge of migratory cells [76,172].

At the same time, RhoA is inactivated to prevent stress-fibre formation and to allow the leading edge of cells to move forward, and this is also accomplished by combinatorial signals from $\alpha5\beta1$ integrin and syndecan: here, receptor-derived signals from Src and PKCα (respectively) converge on p190RhoGAP to inactivate Rho [173] (Figure 10b). Subsequently, the maturation of focal complexes into focal adhesions, and stress-fibre formation, becomes dependent on RhoA activation, which also requires co-ordinated activation of $\alpha5\beta1$ integrin and syndecan-4 [174,175].

Together this discrete, and temporally regulated, interplay of ligands, receptors and signalling co-ordinates the complex events of cell migration, and is also important in determining which way a cell will move, i.e. its directional migration.

Summary

Cellular events that were once thought to be largely driven by integrins turn out to be much more complex, although maybe it is not surprising that different receptor systems are needed for processes that are as sophisticated as assembling adhesions and co-ordinating migration. The combinatorial integrin–syndecan engagement with the ECM enables environmental sensing, which activates internal signalling cascades to promote cell phenotype responses. Importantly the adhesion receptors also serve to restrict signalling to specific locations within the cell, and thereby control responses very precisely.

OVERALL CONCLUSIONS

The drivers of cell-fate decisions originate from outside the cell and work in concert to control transcription or other responses. It is now seen that RTK and cytokine receptors, and well as ECM adhesion receptors, do not signal in isolation, but operate in a co-ordinated fashion. Future studies will need to decipher how altering or combining environmental cues has an impact on signal output, and to identify the exact points of convergence within the signalling networks that control cell function. It also remains to be seen what contribution cell–cell adhesion plays in spatially regulating these signalling processes, and precisely how neighbouring cell types (e.g. stromal cells) can influence non-stromal cell types. Future directions might include the use of proteomics and systems biology to dissect the complexity of such signalling networks, which in turn will yield new therapeutic agents for targeting adhesion-related diseases.

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