

## REVIEW

# Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor

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### Abstract

Extracellular matrices (ECM) are secreted molecules that constitute the cell microenvironment, composed of a dynamic and complex array of glycoproteins, collagens, glycosaminoglycans and proteoglycans. ECM provides the bulk, shape and strength of many tissues *in vivo*, such as basement membrane, bone and cartilage. *In vitro*, most animal cells can only grow when they are attached to surfaces through ECM. ECM is also the substrate for cell migration. However, ECM provides much more than just mechanical and structural support, with implications in developmental patterning, stem cell niches and cancer. ECM imparts spatial context for signalling events by various cell surface growth factor receptors and adhesion molecules such as integrins. The external physical properties of ECM may also have a role in the signalling process. ECM molecules can be flexible and extendable, and mechanical tension can expose cryptic sites, which could further interact with growth factors or their receptors. ECM proteins and

structures can determine the cell behaviour, polarity, migration, differentiation, proliferation and survival by communicating with the intracellular cytoskeleton and transmission of growth factor signals. Integrins and proteoglycans are the major ECM adhesion receptors which cooperate in signalling events, determining the signalling outcomes, and thus the cell fate. This review focuses on the emerging concept of spatial cell biology of ECM, especially the current understanding of integrins and heparan sulphate proteoglycans as the essential cellular machineries that sense, integrate and respond to the physical and chemical environmental information either by directly connecting with the local adhesion sites or by regulating global cellular processes through growth factor receptor signalling pathways, leading to the integration of both external and internal signals in space and time.

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### Extracellular matrix as structural support and binding platform

In order for cells to function, they must be properly supported, having contacts with neighbouring cells and/or the extracellular matrix (ECM). The ECM provides much of the structural support available to parenchymal cells in tissues. In the skin, it provides the dermis and the basement membrane, on which sit the basal cells that give rise to the stratified skin layers. All this provides the tensile strength and flexibility inherent to skin. In other tissues, basement membranes provide anchoring support to epithelial and endothelial cells. The ECM is produced by epithelial cells and stromal cells found within the matrix itself, including fibroblasts, osteoblasts and basal epithelial cells.

The primary proteins present in the ECM and indeed the entire body are the collagens. Collagens are a family of

proteins with at least 29 members; though not all are found in the ECM, they share a common structural motif of helical fibrils formed by three protein subunits. There are many types of collagens present in the ECM and basement membrane, including, but not limited to type I, III, IV, V and the glycosaminoglycan-containing type XI (Hulmes 2002). The primary function of the collagens is to act as the structural support and binding partners for other ECM proteins. Along with collagen, elastin is the major structural protein in the ECM (Kielty *et al.* 2002). Individual tropoelastin protein subunits are crosslinked together to give the mature elastin fibre. Elastin, along with fibrillin, is responsible for the flexibility inherent in many tissues.

The diverse array of ECM proteins not only support the physical structure of the cell but also various biological functions, largely through their ability to bind multiple interacting partners such as other ECM proteins, growth

factors, signal receptors and the adhesion molecules, which is mediated by the multiple, specific domains present within each protein. The best example is fibronectin (FN), which, like other ECM proteins, is produced by fibroblasts among other cell types. FN exists as a dimer and can bind to collagens and heparan sulphate proteoglycans (HSPGs) (Ruoslahti 1988), also see later section), thus contributing to the structural framework for many cell surface receptor systems. One of the major functional domains contained in FN is the FN type III (FNIII) domain. FnIII domains contain about 100 amino acids in two, anti-parallel  $\beta$ -sheets, which are also present in collagens, neural cell adhesion molecules (NCAMs) and some cytokine receptors. The FNIII domains contain the amino acid sequence responsible for integrin-binding (RGD motif) and heparin-binding domains, which are further discussed later. Two other domains of FN, the FNI and FNII domains, are smaller than the FNIII domains, with 45–60 amino acids, but they share the basic anti-parallel  $\beta$ -sheet structure stabilised by disulphide bonds. The FN domains are primarily responsible for the binding of FN to collagen, fibrin and other FN molecules and are often present in multiple copies within each protein (for example, there are 15 FNIII domains in FN), allowing for multiple interactions, thus providing a protein-binding platform (Ruoslahti 1988). Another well-known example would be laminins, which are mainly present in the basement membranes and partly responsible for providing the tensile strength of the tissue. Laminin consists of three subunits –  $\alpha$ ,  $\beta$  and  $\gamma$  – which come together to form a characteristic cross pattern that can bind to other laminins as well as proteoglycans and other ECM proteins (Colognato & Yurchenco 2000). Moreover, vitronectin can bind to and regulate components of the plasminogen activator signal complex, in addition to its cell adhesion duties (Preissner & Seiffert 1998).

HSPGs are proteoglycans found in ECM with multiple heparan sulphate (HS) side chains covalently coupled to the core protein. HSPGs present in the matrix include perlecan, agrin, collagen type XI, syndecans and glypicans. The perlecan, agrin and collagens are actively secreted into the ECM, while the syndecans and glypicans are cleaved from the cell surface by proteases and phospholipases respectively (Brunner *et al.* 1994, Manon-Jensen *et al.* 2010). Secreted HSPGs bind to almost all of the structural proteins in the ECM via both protein–protein interactions and HS–protein interactions. The cleaved HSPGs interactions are primarily, but not exclusively, via HS chains.

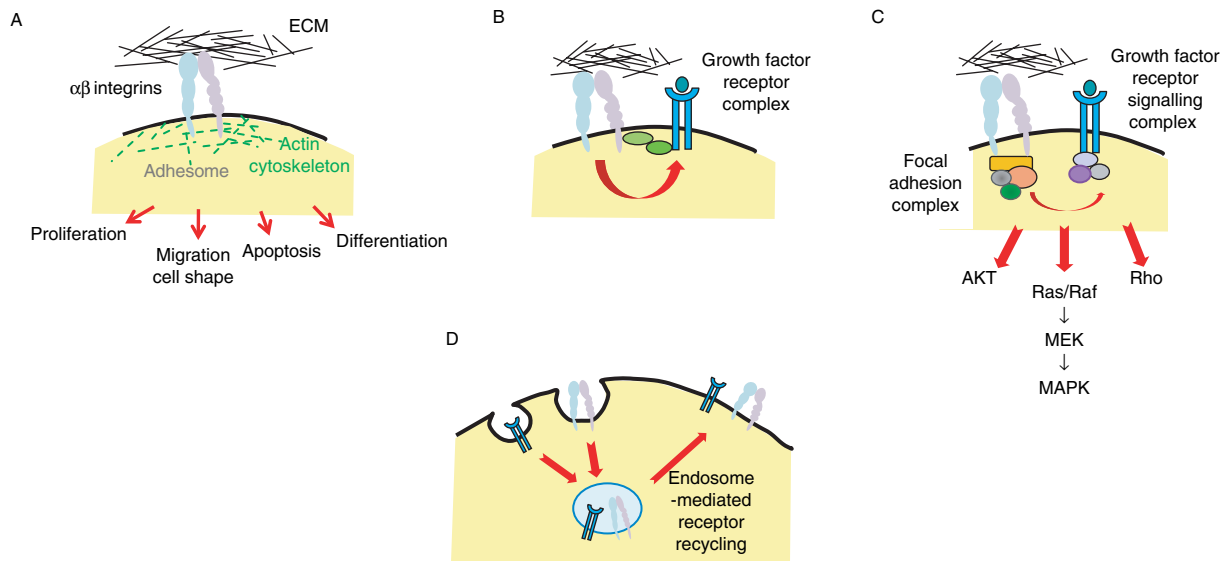
#### *Features of the physical adhesion surface and cell signalling*

Studies using fabricated inert matrix substrates such as polyethylene glycol have suggested that the binding interaction of cell surface receptors to specific adhesion ligands can be purely dependent on the mechanical attributes of the surface (Marastoni *et al.* 2008), and the matrix stiffness has profound effects on cell fate and behaviour (Discher *et al.*

2009). For example, mesenchymal stem cells differentiate into specific cell types only when grown on the appropriate physiological stiffness; thus, matrix elasticity can direct stem cell lineage specification (Engler *et al.* 2006). Matrix stiffness also has effects on cell migration, proliferation and survival (Wells 2008), and focal adhesions can form and grow only if they experience pulling forces through their cytoskeleton. It is known that growth on soft substrates leads to smaller focal adhesions, containing less phosphotyrosine, and reduced cytoskeletal organisation (Pelham & Wang 1997); how changes in focal adhesion size and composition as well as other tension-dependent mechanisms drive the genetic programs responsible for the differential responses is under investigation. Through the recent progress in nanotechnology, it is now possible to engineer specific nanopatterned surfaces, providing new insights into the mechanical properties of ECM (Geiger *et al.* 2001).

#### **Integrins, the inside-out and outside-in signalling**

Cells respond to the mechanical and biochemical changes in ECM through the crosstalk between integrins and the actin cytoskeleton. Integrins are heterodimeric transmembrane receptors composed of eighteen  $\alpha$  subunits and eight  $\beta$  subunits that can be non-covalently assembled into 24 combinations. The integrin dimers bind to an array of different ECM molecules with overlapping binding affinities, as summarised in a review by Alam *et al.* (2007). Therefore, the specific integrin expression patterns by a cell dictate which ECM substrate the cell can bind (Hemler & Lobb 1995) and the composition of integrin adhesomes determines the downstream signalling events, thus the eventual cell behaviour and fate. Integrins have unique ability to respond to the molecular composition and physical properties of the ECM and integrate both mechanical and chemical signals through direct association with the cytoskeleton, which also determines the selection of specific integrin species to be involved (Fig. 1). Integrin recognises and binds to the Arg-Gly-Asp (RGD) motif, which was first discovered in FN but later found in many other ECM proteins including laminin, tenascin, vitronectin and thrombospondin, to name a few (Ruoslahti 1996). The evolutionarily conserved three residue motifs, Arg-Gly-Asp, efficiently serve as the attachment site for integrin-mediated cell adhesion. Both the  $\alpha$  and  $\beta$  subunits of integrins bind to RGD sequences and the specificity of integrin binding to different matrix proteins is determined, in part, by other amino acids surrounding the RGD sequence (Ruoslahti 1996). It has been shown that a sufficiently high density of RGD motif that allows a precise spatial distribution pattern of integrins seems to be required to initiate an optimal cellular response (Chen *et al.* 1997), and spacing between adhesive ligand molecules in a 10–200 nm range seems to mimic physiological properties at focal adhesions (Jiang *et al.* 2004). Not surprisingly, the short synthetic peptide containing the RGD sequences has been



**Figure 1** Mechanisms proposed for growth factor signalling regulation by integrins. (A) The repertoire of integrins expressed on a particular cell can specify the signal pathway due to the differential binding affinity of ECM ligands for the integrins. The tissue stiffness and matrix composition can initiate specific signalling pathways regulating cell behaviour. Clustering and activation of the integrins/actin complexes strengthen the focal adhesion interaction and initiate the framework for cell signalling through assembly of adhesomes. (B) Integrins, via their cytoplasmic domain, recruit specific adaptors to the plasma membrane, which regulate the growth factor receptor signalling. For example,  $\beta 1$ c integrin recruits Gab1/Shp2 and presents Shp2 to IGF1R, resulting in dephosphorylation of the receptor (Goel *et al.* 2005). (C) In response to growth factor stimulation, integrins co-localise at focal adhesion with the growth factor receptors and their associated signalling molecules, such as Src and FAK, as well as with cytoskeletal molecules such as paxillin, talin, and vinculin. For example, the  $\alpha 2\beta 1$  integrin co-localises with EGFR (Yu *et al.* 2000), which eventually regulate the activity of the downstream effectors such as ERK, AKT, JNK, and the Rho GTPases. The details of these downstream pathways can be found in other recent reviews (Legate *et al.* 2009). (D) Integrins can change the rate of growth factor receptor internalisation and degradation. One such example is the interaction of EGFR1 and  $\alpha 5\beta 1$  integrin, which is co-ordinately recycled to the plasma membrane, regulating the protein kinase B (PKB) signalling (Caswell *et al.* 2009). Integrin-mediated cell adhesion can also alter PDGFR rate of degradation via ubiquitination (Baron & Schwartz 2000).

explored to regulate integrin-mediated cell migration, growth, differentiation and apoptosis, as a new therapeutic agent for thrombosis, osteoporosis and cancer (Ruoslahti 1996).

#### Diverse molecular interactions at the adhesion sites

Integrins are the main cell adhesion receptors involved in focal adhesion formation and are required for cell migration. The scaffolding interactions of integrins to the actin cytoskeleton involve actin polymerising and actin linking molecules, whose interactions regulate, and are regulated by, the associated adhesion signalling molecules (Mittra *et al.* 2005). When inactive, integrins are unable to bind to ECM or other receptors, which is important for circulating cells such as lymphocytes. The initial assembly of the nascent adhesion is mediated by anchoring proteins called talins, whose amino terminus interacts with the cytoplasmic tail of the  $\beta$  integrin subunit, while the carboxyl termini of talins bind actin. However, to achieve maximal integrin activation, the cooperation of another anchoring protein, kindlin, which binds to  $\beta$  integrin tails distinct from those used by talin is required (Moser *et al.* 2008). The synergistic effect of talin and

kindlin on the activation and the assembly of adhesion structures is enhanced by the binding of activated vinculin to talin, which triggers the clustering of activated integrins/actin complexes, further strengthening the focal adhesion interaction (Galbraith *et al.* 2002). The progression from the rather unstable nascent adhesions to dot-like focal complexes upon recruitment of vinculin leads to the formation of larger focal adhesion assembly and the conformational transition of the entire  $\alpha$ - $\beta$  integrin dimers to their active state capable of high-affinity interactions with ECM ligands, eventually leading to maturation of fully activated streak-like fibrillar focal adhesions which can transmit the signal (Tadokoro *et al.* 2003).

The turnover of focal adhesions is essential for cell motility, and the formation of the actin stress fibre usually occurs underneath the lamellipodia at the leading edge of the cell protrusion. Lamellipodia are the thin, flat extensions of plasma membrane formed by a dynamic network of actin polymerisation mediated by the actin-related protein 2/3 (Arp2/3) complex, and generates mechanical forces that alter local cytoskeletal dynamics. These physical cues, in turn, cause changes in cell shape and motility and eventually gene transcription, regulating

cell migration and differentiation (Geiger *et al.* 2001, 2009, Humphries *et al.* 2007, Choi *et al.* 2008).

Database mining, combined with an extensive literature search, recently identified a large number of molecules that comprise the 'integrin adhesome' (Zaidel-Bar *et al.* 2007). The integrin adhesome consists of up to 156 distinct components, most of which are a fundamental part of the adhesion sites, while others may only transiently associate with the adhesion site to influence the structures or signalling activity. As the cytoplasmic domains of integrins have no catalytic activity of their own, they must recruit accessory molecules, which contribute to the actin cytoskeletal reorganisation and endow catalytic activity to the focal adhesion. The integrin adhesome-associated proteins include a diverse range of kinases, phosphatases and adaptor proteins, which contribute and initiate cascades of signalling events (Fig. 1). Several tyrosine kinases and phosphatases are shown to be localised to focal adhesions, which are necessary for the regulation of mechano-sensory activity of focal adhesions; these include focal adhesion kinase (FAK), Src, receptor type tyrosine protein phosphatase  $\alpha$  (RPTP $\alpha$ ) and SH2 domain-containing protein tyrosine phosphatase 2 (SHP2; Tilghman & Parsons 2008).

#### *Src-FAK: the focal point for integrin signalling*

One of the earliest signalling events upon the integrin ligation and clustering, thus transducing extracellular cues to the cytoskeleton, is the activation of the tyrosine kinases Src and FAK. FAK is a non-receptor tyrosine kinase which gets activated upon integrin binding to autophosphorylate Y397 – this induces subsequent binding of Src by the SH2 domain, leading to stable and increased activation of Src-FAK complex. Src further phosphorylates FAK and several downstream binding partners (Brown *et al.* 2005). Src is a non-receptor tyrosine kinase associated with the cytoplasmic tail of  $\beta 3$  integrins via its SH3 domains. The Src kinase family consists of nine members including Src, Fyn and Yes, and the phosphorylation of Src leads to activation of JNK (Arias-Salgado *et al.* 2003). One of the targets of the FAK is Rho (Hanks *et al.* 1992). The Rho family of GTPases, including Rac, Cdc42 and RhoA, cycles between an active GTP-bound form and an inactive GDP-bound form and link the actin polymerisation at focal adhesions to the global actin dynamics (Jaffe & Hall 2005). FAK activation also leads to the recruitment of PI3 kinase to focal adhesion, leading to activation of AKT, a ubiquitously expressed serine/threonine kinase that regulates integrin-mediated cell survival. Moreover, FAK represents a crosstalk point for growth factor receptor pathway, since the signals from integrins-Src-FAK complex can be integrated with that of growth factors and be transmitted through the same Ras-MEK-MAPK pathway, which modulates both focal adhesion dynamics and cellular functions (Kim & Kim 2008). Although growth factors can activate the Ras pathway independently of adhesion, studies have shown that activation of MEK1 and Raf1 by

adhesion-mediated signalling through Src-FAK is necessary for the signal to proceed to MAPK activation. Thus, MEK1 and Raf1 are critical convergence points between growth factor and integrin signalling (Slack-Davis *et al.* 2003, Edin & Juliano 2005; Fig. 1).

Further insights into how these signalling molecules are assembled within the spaces of the plasma membrane structure have emerged from the studies on lipid rafts. Integrin clustering induces reorganisation of the plasma membrane into a highly ordered specific subclass of lipid rafts – caveolae – around the focal adhesion, which effectively concentrate the multi-protein signalling complex. It has been shown that integrins associate with Src and FAK within caveolae (Park *et al.* 2011). Furthermore, caveolin-1, the main component of caveolae, gets phosphorylated during focal adhesion signalling in migrating cells and integrin-regulated caveolae trafficking (Salanueva *et al.* 2007). Any changes in integrin ligand binding can also effectively turn off the entire signalling modules by rapid internalisation at the caveolae (del Pozo *et al.* 2004). Growth factor receptors often localise to caveolae and are known to be regulated by receptor trafficking and internalisation. Further investigation on how the assembly of integrin complexes, the plasma membrane order and the processes of endocytosis are coordinated in a three-dimensional environment, thus providing the spatial regulation of signal transduction pathways, is awaited (Fig. 1).

#### **ECM as the organising centre for growth factor receptor signal regulation**

ECM proteins play crucial and complex roles during cell surface receptor signalling. First of all, ECM serves as a reservoir for growth factors. ECM-bound growth factors could be released locally and bind to their canonical receptors. Many ECM proteins have binding sites for both cell adhesion and growth factors, allowing local concentration of the growth factors near to their cell surface receptors and cell adhesion sites. This localisation of growth factors, and thus their signalling, by the ECM probably contributes to the establishment of gradients of the soluble, diffusible growth factor morphogens, which play vital roles in patterning in developmental processes (Kirkpatrick *et al.* 2004, Kreuger *et al.* 2004). Such examples include fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs), both of which bind to HSPG and can be cleaved off from the glycosaminoglycan components of HSPG by the enzyme heparanase and released as soluble ligands (Patel *et al.* 2007), which are discussed in detail later. However, ECM-bound growth factors do not have to be released in soluble form to function. In fact, it is well established that FGFs actually bind to their receptors with HS as a cofactor, with the HSPG 'presenting' these ligands during signalling. Other examples are FN and vitronectin, both of which bind to hepatocyte growth factor (HGF) and form complexes with Met (the HGF receptor) and integrins, either positively or negatively

regulating the functions of the diffusible morphogen HGF. In this regard, ECM can also function as an organising centre of the signalling complex on the cell surface.

The binding of ECM with growth factors often involves specific domains of ECM proteins and results in modulation of signalling activities. For instance, FnIII domains in both FN and tenascin-C bind to VEGF, which potentiates the VEGF-mediated signalling through its receptor VEGFR2 (Wijelath *et al.* 2006). FnIII domains of ECM-associated protein anosmin-1 are shown to bind to FGFR1 ectodomains and function as a co-ligand for FGFR1 signal complex, enhancing or inhibiting the activity (Hu *et al.* 2009). Similarly, *Drosophila* collagen IV binds to Dpp, a bone morphogenetic protein (BMP) homologue, and enhances interactions with BMP receptors (Wang *et al.* 2008). Collagen II, the major collagen of cartilage, contains a chordin-like VWC domain, which binds to transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) and BMP-2, acting as a negative regulator for these essential chondrogenic growth factors (Garcia *et al.* 2002). On the other hand, some intrinsic domains within ECM proteins can act as non-canonical ligands for the receptors. Many ECM proteins including laminin, tenascin, thrombospondin and fibrillin contain epidermal growth factor (EGF)-like domains which can directly bind to EGF receptor as soluble ligands and modulate its signalling (Schenk *et al.* 2003). FnIII domains of NCAM bind directly to FGFR1 and can induce ligand-independent receptor phosphorylation (Kiselyov *et al.* 2003). However, further investigation is needed to determine if there is any difference between the ECM-associated growth factor-like ligands (either acting from the anchored solid-phase or as released soluble form) and the canonical ligands in terms of the specific signalling outcome.

#### *Integrin and growth factor receptor signalling*

Integrins can activate several signalling pathways independently (Assoian & Schwartz 2001), but more frequently they act synergistically with other growth factor receptors (Alam *et al.* 2007). Such examples include insulin receptor (Schneller *et al.* 1997); type 1 insulin-like growth factor receptor (IGF1R); Zhang & Yee 2000); VEGF receptor (Ruoslahti 2002), TGF- $\beta$  receptor (Scaffidi *et al.* 2004), HGF receptor (c-Met) (Sridhar & Miranti 2006), platelet-derived growth factor- $\beta$  (PDGF- $\beta$ ) receptor (Schneller *et al.* 1997) and EGF receptor (Bill *et al.* 2004). The functional activities of these growth factor receptors are dynamically and reciprocally controlled by integrins. The fact that growth factor signalling requires the presence of specific integrin subunits and that different cell types express different profiles of integrins may constitute the 'cellular context' determining the outcome of the growth factor signal (Alam *et al.* 2007).

#### *Role of proteoglycans in signal regulation*

Traditionally, there were two functions ascribed to HSPGs in the matrix (David 1992, Hardingham & Fosang 1992).

The first was to contribute to the organisation of the matrix by binding to the many core matrix molecules via HS chains (e.g. laminin, FN and collagen). The second was to act as an extracellular storage mechanism for heparin-binding growth factors such as FGFs secreted by stromal cells. The HSPGs would control the diffusion of the growth factors to establish protein gradients, such as during development, or act as a repository of growth factors that could be sequestered from cells and released at an appropriate time, such as wound healing (Zcharia *et al.* 2005). More recently, however, it has been identified that the HS chains play a more proactive role.

The ability of heparin (a highly sulphated form of HS found in connective tissue mast cells and often used as the source of HS in experiments) to bind to many proteins has been known for many years. Heparin can bind to many different classes of proteins, ranging from growth factors (FGFs, VEGFs, PDGF, glial cell-derived neurotrophic factor (GDNF), and HGF) to cytokines (interleukins, interferon, PF4, and RANTES), metabolic enzymes (lipoprotein lipase) and structural proteins (laminin and FN), among many others (Kjellen & Lindahl 1991, Casu & Lindahl 2001). HS-binding domains in these proteins are not usually defined by their structural motifs, but rather typically characterised by a collection of basic residues, such as arginine and lysine, that are in close proximity either in the linear amino acid sequence as found in FGFR or through protein folding as found in FGF-1 (Kan *et al.* 1993, Wong *et al.* 1995, Blaber *et al.* 1996). As such there is no general consensus sequence for identifying HS-binding domains in proteins. In the past, HS-binding sequences have been identified empirically using mutation of lysine and arginine residues, structural studies such as nuclear magnetic resonance (NMR) and X-ray crystallography and by protease protection assays, where the region of the protein bound by heparin is protected from protease digestion and the resulting peptide fragments can be identified by mass spectrometry (Ori *et al.* 2009).

Most of the protein-binding activity of HSPGs is contributed to the HS chains. The average HS chain is 50–200 repeating disaccharide units in length, which is synthesised by the sequential addition of a xylose, galactose, galactose and D-glucuronic acid motif on a serine residue of the core protein. The next sugar to be added is N-acetyl glucosamine. Following this, two enzymes, EXT1 and EXT2, polymerise the chain with the addition of alternating D-glucuronic acid and N-acetyl glucosamine. There is then a series of sequential modification reactions carried out by families of sulphotransferase enzymes that generate the diversity of HS seen in tissues (Lindahl *et al.* 1989, Turnbull & Gallagher 1990, Esko & Selleck 2002). It is important to note that these enzyme reactions do not go to completion and that any given cell may express only a subset of the available biosynthetic enzymes, thus generating the structural diversity of HS. Other factors include posttranscriptional and posttranslational regulation of the enzymes (Pinhal *et al.* 2001, Grobe & Esko 2002, Nagai *et al.* 2004) and the action of

extracellular Sulf enzymes, which remove 6-O sulphates on HS after it has been synthesised (Lamanna *et al.* 2006).

The sulphate residues and the carboxylic acid group are largely responsible for the ionic interactions between HS and proteins, but hydrogen bonding and van der Waals interactions also make a significant contribution to the binding energy (Faham *et al.* 1996). However, binding and regulation of proteins by HS is not solely down to the linear sequence of the sulphates present. The conformation of the HS is also suspected to determine its protein-binding properties (Skidmore *et al.* 2008). There are several ways that a certain conformation can be achieved. The presence or absence of specific sulphate groups and the identity of the uronic acid affect the conformation of the sugar residues and thus the overall conformation of the molecule (Rudd *et al.* 2008). The conformation can also be affected by the associated cation present with the molecule, which in turn affects the biological activity of HS (Rudd *et al.* 2008, Guimond *et al.* 2009).

Perlecan is a major HSPG found in the basement membrane-like structure around chondrocytes, and binds to many growth factors, including FGFs and VEGFs (see below), thus involved in regulating proliferative and migratory signals in response to growth factor signals during differentiation, cancer and angiogenesis. Perlecan is shown to have both pro-angiogenic and anti-angiogenic capacities (Whitelock *et al.* 2008).

The other major proteoglycan components of the ECM are the chondroitin sulphate proteoglycans (CSPGs). Unlike the HSPGs, there are a large number of CSPG core proteins secreted directly into the matrix, including aggrecan, versican, brevican and the small, leucine-rich proteoglycans such as decorin and biglycan among others (Kresse & Schonherr 2001). Some of the CSPGs can be quite large (MW > 10 000 000) and bind to many different components of the ECM, effectively crosslinking them. CSPGs also bind to and regulate a number of growth factors, such as members of the TGF family (Hildebrand *et al.* 1994).

Hyaluronic acid is a glycosaminoglycan consisting of alternating glucuronic acid and *N*-acetyl glucosamine sugars. Hyaluronic acid is different from the other glycosaminoglycans in that it is synthesised on the cell surface and not covalently linked to a core protein during synthesis. It is very hydroscopic and is responsible for the gel-like consistency of tissues such as cartilage (Day & Sheehan 2001).

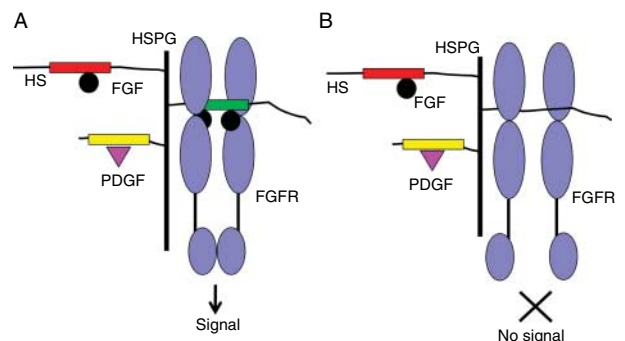
#### Role of HSPG in FGF and VEGF signalling

The binding specificity of FGFs to FGFRs is partly dictated by the receptor isoform (Ornitz *et al.* 1996). Notably, these FGF/FGFR binding interactions were all determined in the presence of heparin. HS can influence the FGF binding specificity and activation of FGFR, and indeed particular HS structures can block or inhibit the signalling process. It must be noted that the ability of HS to bind a protein does not necessarily support a positive biological activity; for example,

structures have been identified that can bind to FGF-2 but not support its signalling through FGFR1 (Turnbull *et al.* 1999, Guimond *et al.* 2006). The potential mechanisms of HS-mediated regulation of FGF signalling are illustrated in Fig. 2. Three different types of HS structures characterised by the presence of different sulphate groups are depicted as three different coloured boxes (red, yellow, or green). Only one of these HS structures (represented by the green box) can allow FGF binding as well as activate the FGFR, while another HS structure (represented by the red box) can bind to FGF, but cannot support its binding to FGFR. PDGF binding to a third type of HS sequence (yellow box) is not affected and may have a different regulatory role in PDGF signalling activity (Fig. 2).

VEGF is another example of essential growth factor, whose spatially restricted distribution during development is mediated by the binding of HS. The precise deposition and expression of specific isoform of VEGF with a differential binding affinity for HSPG regulate endothelial cell proliferation, migration, differentiation and survival, which is necessary for appropriate pattern formation and tissue morphogenesis in mice (Ruhrberg *et al.* 2002).

Perlecan co-localises with FGF2 and acts as a mechanotransducer and mitogen for chondrocytes through the activation of MAP kinases (Vincent *et al.* 2007). Interestingly, perlecan can be substituted not only with HS but also with chondroitin sulphate. Nonetheless, HS chains of perlecan seem to favour FGF/FGFR interaction, while chondroitin sulphate chains in perlecan act as a negative regulator of FGF/FGFR by sequestering the FGFs from their cognate receptors (Whitelock *et al.* 2008). It has also been shown that



**Figure 2** Different HS structures support different biological activities. (A) Heparan sulphate that has different cell- and tissue-specific structures (denoted schematically by red, yellow and green bars) with binding domains for different growth factors. Here, green represents structures that are capable of binding to both FGF and FGFR, thus supporting ligand-induced dimer formation and consequent signalling in the FGFR complexes. Yellow structures cannot bind to FGF or FGFR, but are capable of binding to PDGF and regulating its activity. Red structures can bind to FGF, but cannot support subsequent binding to FGFR. (B) Heparan sulphate that lacks the required structures (green) cannot support FGF signalling through FGFR, although the presence of other structures (red) still allows it to bind to FGF without supporting its activity. Other structures (yellow) may bind and regulate PDGF, mediating alternative signal pathway.

different isoforms of VEGFs exhibit differential binding to the HS side chains of perlecan, thus affecting the VEGF signalling outcome as well as creating differential gradient of the ligand at the cell surface (Robinson & Stringer 2001, Whitelock *et al.* 2008). VEGF has also the ability to displace FGF from the ECM, potentially inducing a synergy between the VEGF and FGF pathways during angiogenesis (Kamei *et al.* 2010).

## ECM proteases and heparanase

Cell movement and tissue remodelling are important both physiologically (e.g. during development) and pathologically (e.g. wound healing and cancer metastasis). In order for these processes to occur, the ECM must be degraded to allow the free movement of cells or the processing and deposition of new matrix. These processes are accomplished by proteases and also the HS-degrading enzyme heparanase. There are two well-known families of proteases that are involved in the biology of the ECM, the matrix metalloproteinase (MMP) and the A disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) families.

### Matrix metalloproteinases

MMPs are a family of 24 proteins in mammals (Yong 2005, Shiomi *et al.* 2010). Six of these are associated with cell membranes via glycosylphosphatidylinositol anchors (MMP17 and 25) or protein transmembrane domains (MMP14, 15, 16, and 24) and the rest are secreted. Others can be associated with cells via interactions with other proteins, such as MMP9 with CD44 and MMP7 with HSPGs and integrins (Yu *et al.* 2002). It is the cell-associated MMPs that are responsible for the majority of ECM degradation. MMPs can also cleave other proteins such as growth factors and cell adhesion molecules, in some cases releasing active molecules (Mott & Werb 2004). The activity of the MMPs is highly regulated since inappropriate degradation of matrix would compromise the integrity of tissues (Overall & Lopez-Otin 2002). The first point of regulation is at the level of transcription. Secondly, the MMPs are made as inactive zymogens that require processing before they can degrade matrix. Related to this is the fact that some MMPs, such as MMP14 and tissue inhibitors of metalloproteinase-2 (TIMP-2), must be associated with cell surface proteins prior to activation (Apte *et al.* 1997). In addition, there are a family of inhibitors of MMPs, including TIMPs that regulate the activity of the active enzyme (Brew & Nagase 2010). Due to their ability to degrade matrix and activate proteins such as growth factors, the MMPs are involved in a number of disease processes, particularly in the CNS, including inflammation, neuronal regeneration and stroke (Yong 2005). The expression of several MMPs, such as MMP 1, 2 and 3, increases in models of multiple sclerosis (Weaver *et al.* 2005). MMP7 expression also increases during spinal cord injury (Wells *et al.* 2003).

### A disintegrin and metalloproteinase with thrombospondin motif

There are 19 members of the ADAMTS family, which are related to the ADAM transmembrane proteases responsible for processing membrane-bound precursor proteins such as Notch, Delta and HB-EGF (Tang 2001, Shiomi *et al.* 2010). ADAMTS family shares a common domain structure, including, but not limited to, the Zn<sup>2+</sup>-dependent catalytic domain, a disintegrin domain, a thrombospondin repeat domain and cysteine-rich domains. These proteases bind to both the cell surface and ECM proteins via a spacer domain between the thrombospondin repeat domains and the C-terminal domain of the protein. Similar to the MMPs, they can be inhibited by the TIMPS and made as inactive zymogens that must be proteolytically cleaved to become active.

While the MMPs are generally broad-spectrum proteases, some of the ADAMTS play specific roles in the processing of the ECM. ADAMTS2 cleaves pro-collagen to collagen to allow fibril formation (Colige *et al.* 1999); hence, mutations of ADAMTS2 are responsible for a subtype of Ehlers–Danlos syndrome. ADAMTS4 cleaves aggrecan in cartilage (Tortorella *et al.* 1999), and has a role in the development of osteoarthritis. Other ADAMTS proteins have been implicated in inflammation (Tang 2001).

### Heparanase

Besides the degradation of the protein component of the ECM, HS can also be degraded. The enzyme heparanase is an endoglucuronidase that cleaves between the uronic acid and glucosamine of HS (Hulett *et al.* 1999, Vlodaysky *et al.* 1999). The cleavage of HS accomplishes three objectives: the removal of a vital crosslinker of matrix proteins, the release of growth factors bound to HS that can subsequently act on cells and the release of oligosaccharides of HS that can regulate protein–protein binding, all of which are crucially important for cell migration and tissue remodelling during development. However, these very same properties can contribute to pathologic conditions such as angiogenesis during wound healing (Dempsey *et al.* 2000) and inflammation (Li & Vlodaysky 2009), where heparanase has been investigated as a therapeutic target for new treatments.

## Role of ECM in endocrine disease and cancer

Recent studies have revealed the role of ECM in many pathologic conditions; defects in ECM assembly can have a direct or an indirect effect as a consequence of mis-regulated receptor signalling. The progressive changes in ECM composition and organisation in the diseased tissue can induce a variety of genetic and biological alterations, including amplification or inactivation of specific signal pathways, expression of disease markers and aberrant cell and

tissue architecture. When epithelial cells are detached from ECM, they undergo cell death, namely anoikis or autophagy, again supporting the notion that cell adhesion to ECM is critical for cell survival and growth (Lock & Debnath 2008, Marastoni *et al.* 2008).

#### *ECM and the endocrine system*

Many aspects of endocrine system development and pathology have interdependent relationships with the ECM. Sertoli cells and testicular peritubular cells cooperate in the synthesis and deposition of ECM components in the formation of the basal lamina of the seminiferous tubule (Skinner *et al.* 1985). FSH increases the activity and biosynthesis of ECM-associated protease, tissue-type plasminogen activator, during the initial granulosa cell development in the ovary (Knecht 1988). Stress hormones such as norepinephrine and epinephrine can contribute to ovarian cancer progression by activating FAK, which protects the cancer cells from anoikis (Sood *et al.* 2010). Pancreatic fibrosis, which is characterised by stromal expansion and the excessive deposition of ECM replacing pancreatic tissue, underlies many major endocrine diseases, including pancreatic cancer, chronic pancreatitis and type 2 diabetes mellitus. The detailed molecular mechanisms are still unclear, but recent evidence indicates that ECM-dependent EGFR signalling may regulate pancreatic fibrogenesis *in vivo* (Blaine *et al.* 2009). The importance of optimal ECM re-establishment for human pancreatic islet culture has been recently highlighted. The interactions between islet cells and ECM are essential for normal islet physiology; thus, the biggest challenge for the researchers is how to overcome apoptosis (anoikis) caused by the loss of the ECM upon islet cell isolation and subsequent *in vitro* culture condition. A recent study demonstrated that the various ECM components, including collagen I and IV, FN and laminin can exert different effects on human islet adhesion, survival and functionality (i.e. structural integrity, insulin gene expression and release and glucose metabolism-related gene expression) (Daoud *et al.* 2010).

ECM also plays key roles in the reproductive function. In most of the mammals, large amount of hyaluronan is synthesised by the cumulus cells before ovulation and organised into ECM, a process called cumulus expansion, which provides an essential microenvironment for oocyte fertilisation. Interestingly, RhoA activation induces resistance of the cumulus to hyaluronidase and sperm penetration, indicating integrin and consequent ECM remodelling through the Rho are required for successful fertilisation (Yodoi *et al.* 2009).

Renal hypertrophy and abnormal ECM deposition are hallmarks of diabetic nephropathy and it has been proposed that MMP-mediated breakdown and turnover of ECM in the glomerulus underlies the renal pathology (Thraill *et al.* 2009).

#### *ECM as tumour microenvironment*

The role of ECM in tumorigenesis has been intensively studied. It has become increasingly clear that cancer does not result from unregulated growth of a single cell, but rather involves multiple dysfunctions of interactions between various cell types and malignant alteration of the tissue microenvironment. The recent identification of cancer stem cells and the epithelial–mesenchymal transitions have brought further support to this concept. Metastatic tumours arise when the cancer cells gain the capacity to degrade the normal tissue architecture and migrate out into interstitial spaces and eventually to different sites after breaking away from the surrounding ECM boundaries (Wiseman & Werb 2002). The microenvironment surrounding the malignant cells is called the tumour stroma, which contains the tumour-derived non-malignant cells, including endothelial cells, pericytes, fibroblasts, tumour-associated macrophages and immune cells (CD4+ and CD8+ T lymphocytes, B lymphocytes and Natural Killer cells), with a multitude of growth factors and angiogenic factors like VEGFs embedded in the ECM. Both the stromal and cancer cells produce proteases that continuously remodel the ECM (Kalluri & Zeisberg 2006), and a five-step process of ECM remodelling concomitant to the tumour cell migration has been proposed (Friedl & Wolf 2009). In fact, almost all aspects of cancer formation (e.g. growth, differentiation, motility, and apoptosis) and their signal transduction networks can be regulated by physical interactions between ECM and the cells. For instance, normal function of epithelia is critically dependent on the basement membrane, a highly specialised ECM which contains type IV collagen, laminin, types XV and XVIII collagens, perlecan and nidogen (Kalluri 2003). Progressive loss of an intact basal lamina is the first stage of the neoplastic disorganisation and the breakdown of boundaries during metastatic invasion (Ingber *et al.* 1981). In a rat model, chemical carcinogens must be applied both on the basement membrane and the epithelium to induce mammary gland cancer, suggesting that alteration of the stroma is essential for carcinogenesis and mutations in the epithelium alone is not sufficient (Maffini *et al.* 2004). Interestingly, overexpression of the ECM-degrading enzyme MMP3/stromelysin-1 in transgenic mice induces the transformation of normal breast epithelium into cancerous tissue, which exhibits genomic abnormalities; therefore, structural or mechanical changes in the tissue microenvironment may contribute to the genetic reprogramming of the cancer cells (Sternlicht *et al.* 1999). Conversely, stroma from healthy animals can restore normal characteristics of epithelial cancer cell implants (Maffini *et al.* 2005), and integrin-blocking antibodies were shown to revert the malignant phenotype of human breast cells (Weaver *et al.* 1997). This is somewhat controversial, since it has long been believed that cancer is an irreversible disease that results from accumulated gene mutations and chromosomal abnormalities. However, it seems possible for cancer to be induced to become quiescent or revert to a normal state, if provided with



the correct set of ECM signals, similarly to the normal embryogenesis (Kenny & Bissell 2003).

Collagen is the major structural component in the formation of solid tumours and collagen fibre density can contribute to tumour initiation, invasion and metastasis (Gao *et al.* 2010, Kakkad *et al.* 2010). It has been shown that the increased expression of lysyl oxidase (LOX), an enzyme that covalently crosslinks collagen and elastin, is responsible for excess collagen deposition and hypoxia-induced metastasis of lung cancer through induction of  $\beta 1$  integrin signalling (Gao *et al.* 2010). Kakkad *et al.* (2010) also report that hypoxia causes reduction of collagen I fibre density and restructuring of the ECM, thus contributing to the tumour cell dissemination.

A wealth of information has been generated on the integrin family playing a critical role in tumorigenesis and metastasis by modulating the activities of growth factor receptors, extracellular proteases and chemotactic molecules, which can be found in other excellent reviews (White & Muller 2007, Desgrosellier & Cheresch 2010) and such importance of integrins in tumour progression of multiple cell type has made them an attractive anti-cancer target.

#### *HSPG in disease mechanisms*

There is a growing body of evidence that HS changes naturally during many physiologic processes. During normal mouse brain development, there are changes in chain length and 6-O sulphation that occur as the neural epithelium develops (Brickman *et al.* 1998). These changes have functional consequences on the ability of the HS to promote FGF activity (Guimond & Turnbull 1999). Phage display antibodies to HS (van Kuppevelt *et al.* 1998) with different levels of sulphation or natural HS protein ligands have been used to identify spatial and temporal differences in HS expression during development (Friedl *et al.* 2001, Allen & Rapraeger 2003). Differences in HS within tissues are not restricted to physiologic processes. Because of the ability of HS to regulate the activities of many growth factors, alterations in HS structure could be a contributing factor in the progression of many tumours. For example, removal of 6-O sulphates can reduce tumour growth *in vivo* (Narita *et al.* 2006), and HSPGs from normal versus malignant epithelial cells show differences in their ability to support FGF-2/FGFR complex formation (Mundhenke *et al.* 2002). Stromal fibroblasts expressing high levels of syndecan-1, a HSPG core protein, enhanced the attachment of breast carcinoma cells and the establishment of cancer microenvironment permissive to the cancer cell migration and invasion (Yang *et al.* 2011). Basement membranes of renal cell carcinomas show altered ability to bind to members of the FGF family in an HS-dependent manner (Friedl *et al.* 1997). It is not known whether any or all such changes are a consequence of the progression of a cell from normal to cancerous, but the ability of HS to regulate many proteins involved in cell proliferation and migration makes it a potential target for future therapeutics.

#### **Implications for future research and ECM as a therapeutic target**

Despite the recent growth of information, many questions still remain, and continued research is required to build on and refine our current knowledge of the ECM, especially utilising the rapidly improving new technologies. The molecular nature of ECM-induced receptor complexes in the membrane and their movement upon activation can be monitored by methods such as single-molecule tracking using nanoparticles, fluorescence energy transfer technique or high-resolution live cell imaging. These studies will reveal more potential therapeutic opportunities in ECM remodeling. The caveat might be finding specific targets from the unique microenvironment of the diseased cells or tissues. Further investigations of the roles of ECM proteins in regulating signalling events would provide additional clues. However, multiple cell types and pathways within ECM are functionally interdependent and crosstalk to each other as described earlier, making it difficult to devise specific therapeutics that do not interfere with other pathways (Drucker *et al.* 2004). Nonetheless, multiple integrin antagonists have been tested as anti-tumour agents in clinical trials. Examples include  $\alpha v \beta 3$  and  $\alpha v \beta 5$  inhibitor cilengitide, which is currently being tested on glioblastoma patients in a phase III trial (Desgrosellier & Cheresch 2010). An excellent summary of recently developed novel agents targeting the tumour microenvironment can be found elsewhere (Hanna *et al.* 2009). ECM structural defects seem more difficult to treat, although the regenerative capacity of stem cells are being explored in different tissues; the problem encountered in recent attempts is that the injured microenvironment loses conformity with fibrotic scarring, thus resulting in a non-inducing environment where the stem cells cannot remodel (Berry *et al.* 2006). HSPGs should provide an interesting axis for novel approaches, since they act as master regulators of multiple ECM proteins and signalling pathways. In conclusion, ECM components are major molecular switches and crucial therapeutic targets to prevent abnormal growth factor receptor activities and to intervene in multiple signalling pathways, thus further investment could be made to aid the treatment and management of degenerative diseases, inflammation and cancer.

#### **Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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