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REVIEW ARTICLE

Syndecans: from peripheral coreceptors to mainstream regulators of cell behaviour

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SUMMARY

In the 25 years, as the first of the syndecan family was cloned, interest in these transmembrane proteoglycans has steadily increased. While four distinct members are present in mammals, one is present in invertebrates, including *C. elegans* that is such a powerful genetic model. The syndecans, therefore, have a long evolutionary history, indicative of important roles. However, these roles have been elusive. The knockout in the worm has a developmental neuronal phenotype, while knockouts of the syndecans in the mouse are mild and mostly limited to post-natal rather than developmental effects. Moreover, their association with high-affinity receptors, such as integrins, growth factor receptors, frizzled and slit/robo, have led to the notion that syndecans are coreceptors, with minor roles. Given that their heparan sulphate chains can gather many different protein ligands, this gave credence to views that the importance of syndecans lay with their ability to concentrate ligands and that only the extracellular polysaccharide was of significance. Syndecans are increasingly identified with roles in the pathogenesis of many diseases, including tumour progression, vascular disease, arthritis and inflammation. This has provided impetus to understanding syndecan roles in more detail. It emerges that while the cytoplasmic domains of syndecans are small, they have clear interactive capabilities, most notably with the actin cytoskeleton. Moreover, through the binding and activation of signalling molecules, it is likely that syndecans are important receptors in their own right. Here, an overview of syndecan structure and function is provided, with some prospects for the future.

Keywords
cytoskeleton, glycosaminoglycan, heparan sulphate, proteoglycan

Background

Syndecans comprise a small family of transmembrane proteoglycans. In mammals, there are four distinct genes, while all invertebrate members of the Bilateria possess one. They have, therefore, a long evolutionary history. With the cloning of the first member, syndecan-1, by Merton Bernfield's group in 1989 (Saunders *et al.* 1989), other members were cloned in the following few years and interest in them has steadily grown. It soon became apparent that they could support cell adhesion, and now it is known that all four mammalian members can interact with the actin cytoskeleton (Fig. 1). This was perhaps unsurprising, as heparan sulphate proteoglycans (HSPGs) had been proposed to be important in cell adhesion (e.g. Culp *et al.* 1986) long before the first syndecan was cloned and indeed even before integrins were identified (Tamkun *et al.* 1986; see also Hynes 2004). During the 1980s, it became apparent that there were two classes of HSPGs on many cell types, matrix proteoglycans with hydrophilic properties and cell surface HSPGs with hydrophobic characteristics (Woods *et al.* 1985). It is now appreciated that while syndecans are typical type I membrane proteins, a second family, the glypicans, are endowed with glycosylphosphatidylinositol (GPI) anchors. There are six mammalian glypicans and, apart from bearing heparan sulphate chains, are unrelated to the syndecans. So far, the glypicans are not widely implicated in cell adhesion, but rather appear to have major roles in the

binding of a wide range of growth factors, cytokines, chemokines and other polypeptide regulators and cooperate with high-affinity receptors (Filmus & Capurro 2014).

With the identification and cloning of syndecans, we became interested in how these HSPGs participate in adhesion and signalling. This work continues, but here, we summarize some of the historical aspects, syndecan signalling properties and what we have learned from genetic knockouts. Syndecans are frequently implicated in inflammation and tumour biology. Their pathogenesis also involves regulation of cell adhesion and important roles for heparan sulphate-interacting proteins. Therefore, we include a summary of recent work together with some future perspectives.

Syndecans too become associated with other receptors, notably integrins, and so came to be known as coreceptors. For a while, interest was not surprisingly focussed on integrins, as their involvement in focal adhesion formation and migration was quickly realized. Integrin knockout mice were shown to have, in most cases, profound developmental problems and often these resulted in embryonic or perinatal mortality. A side effect of the enormous interest in integrins was that syndecans were assumed to have minor supporting roles, rather than key functions.

Syndecan knockouts

The situation was not, perhaps, helped by the finding that syndecan-1 and syndecan-4 null mice had almost no developmental defects (Ishiguro *et al.* 2000; Stepp *et al.* 2002). As syndecan-1 is enriched in many epithelia, it was discovered that the proteoglycan had roles in post-natal repair, notably in the epithelial layers of the skin and cornea (Pal-Ghosh *et al.* 2008). The syndecan-4 null was also described to have defects in post-natal repair, involving in this case, granulation tissue angiogenesis and fibroblast migration (Echtermeyer *et al.* 2001). Long-term potentiation defects were noted in the syndecan-3 null mouse, the proteoglycan being notably enriched in neural tissue (Hienola *et al.* 2006). In *Caenorhabditis elegans*, syndecan is also widely present in the developing and adult nervous system, perhaps indicative that syndecan-3 is most closely related to the invertebrate members. The invertebrate syndecan is involved in diverse cellular processes during development.

Syndecan has been genetically linked to slit/robo signalling, in both *Drosophila* and *C. elegans* where it is responsible for cell migration and axon guidance during development. Mutants defective in syndecan display phenotypes such as inappropriate axon crossover at the CNS midline (Fig. 2), defects in myotube formation and mispositioned cell bodies; these phenotypes can be rescued by expressing syndecan in the affected cells and not the surrounding tissue, suggesting that syndecan acts as a cell autonomously (Steigemann *et al.* 2004; Gysi *et al.* 2013). The question of whether syndecan acts as a coreceptor or as a simple ligand gatherer is debated. In one study, it was shown that slit and its robo receptor specifically bind to

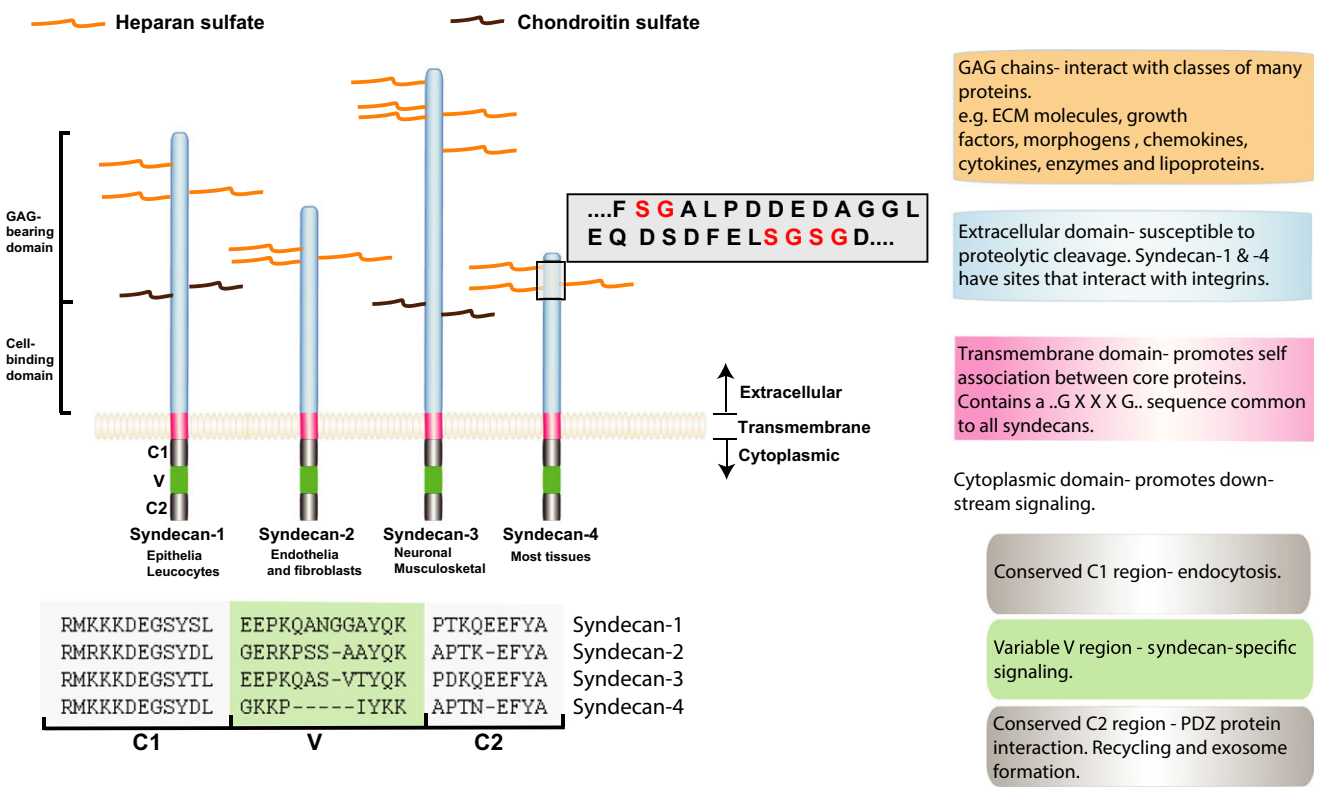

Figure 1 Schematic of the mammalian syndecans, illustrating structure and interactions.

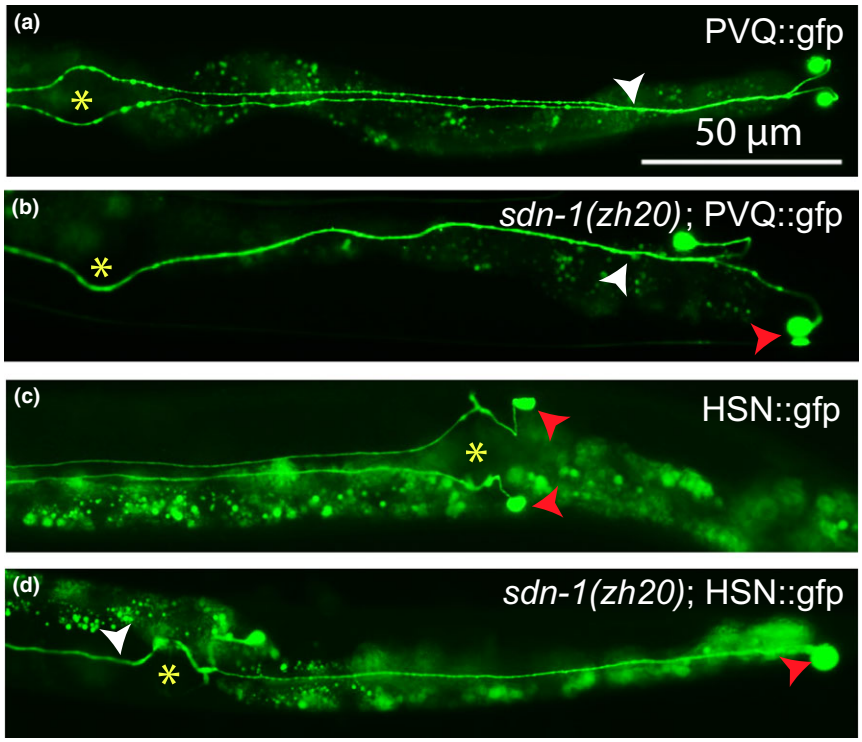

Figure 2 Migration and axon guidance defects in *sdn-1 (zh20)* null mutants of *C. elegans*. Genetic null mutants of SDC1, the sole syndecan gene in *C. elegans*, show migration and axon guidance defects in the hermaphrodite-specific neurons (HSNs) and the PVQ interneurons. In the figure, posterior is to the right and anterior to the left. GFP is expressed in the PVQ neurons (a–b) and in the HSNs (c–d). Wild-type PVQ neurons are born in the tail of the animal and extend their axons from tail to head. Axons extend to each side of the ventral nerve cord (see white arrow). In mutant worm, one of the PVQ neuronal cell bodies is mispositioned (red arrow) and axons fail to extend to each side of the ventral nerve cord (white arrow). Wild-type HSNs are born in the tail and neuronal cell bodies migrate towards the midbody of the animal (red arrow), Axons then extend to the head of the animal on each side of the ventral nerve cord. In mutant animal, one of the neuronal cell bodies failed to migrate to the midbody (red arrow) and axons fail to extend to each side of the ventral nerve cord (white arrow). Yellow stars denote position of vulva.

syndecan suggesting that syndecan acts as a coreceptor (Johnson *et al.* 2004). However, in another study was shown that the extracellular domain of syndecan is sufficient to mediate slit signalling and surprisingly only the chondroitin chains were needed for signalling (Chanana *et al.* 2009). Along similar lines, a third study showed that the cytoplasmic domain was dispensable for migration and fusion of dorsal branch cells in *Drosophila* (Schulz *et al.* 2011).

Together with the invertebrate glypican (lon-2), sdn-1 is responsible for correct guidance of D-type motor axons (no other HSPGs involved) – connecting syndecan with the unc-6/netrin signalling pathway in *C. elegans* (Gysi *et al.* 2013). Unexpectedly, an enhancement of the defect was observed when combining sdn-1 mutation (no enhancement combined with lon-2) with mutants of the heparan sulphate modifying enzyme machinery, suggesting that the sulphation pattern of the syndecan was not important for correct axon guidance. Furthermore, in a screen for genetic enhancers of the ventral-to-dorsal distal tip cell migration defect, seen in netrin mutants, an allele of sdn-1 was identified. The migration defect was partially reversed when mutation in Wnt and FGF genes was introduced, suggesting that syndecan could affect netrin signalling through dysregulation of growth factor pathways (Schwabiuk *et al.* 2009).

Syndecans are commonly substituted with heparan sulphate chains, in the case of syndecan-4, three are present. In some cases, syndecans may additionally or alternately carry chondroitin/dermatan sulphate chains (Deepa *et al.* 2004). Glypicans also predominantly bear heparan sulphate chains. Many genetic experiments in invertebrates and the mouse show that heparan sulphate is required in development. For example, the Ext1 and 2 proteins, which, in combination, comprise the major polymerase responsible for heparosan synthesis, were shown to be essential (Lin *et al.* 2000; Stickens *et al.* 2005). Deletion of the heparan sulphate 2-*O*-sulphotransferase leads to renal agenesis in the mouse (Bullock *et al.* 1998). The absolute requirement for heparan sulphate is not surprising, given the wide range of growth factors and morphogens that bind this glycosaminoglycan. However, these data highlight a sharp contrast with the HSPG core proteins, where in the mouse at least, no single knockout has a severe developmental phenotype. It is certainly true that knockouts of glypican-3 or glypican-6 core proteins have clear phenotypes, and there are known

mutations in these genes in humans giving rise to disease (Pilia *et al.* 1996; Campos-Xavier *et al.* 2009). For syndecans, however, no mutations with human disease relevance are known.

Lower vertebrates have more marked phenotypes where syndecans are repressed by morpholinos, but here, at least in the bony fish, there appears to have been a secondary loss of the syndecan-1 gene (Chakravarti & Adams 2006). Perhaps the further loss of a syndecan by morpholinos in a background of only three family members has more pronounced effects. It does, however, suggest that there may be redundancy across the syndecan core proteins, and perhaps even that glypicans may substitute for syndecans. However, the latter seems less likely given their quite different protein structure and lack of cytoplasmic domain. It is certainly the case that in tissue culture at least, most cell types possess more than one syndecan. Syndecan-4 is most widespread, being present in mesenchymal cells, epithelia, endothelia and cells of the immune system. The other three syndecans appear to be more tissue restricted, but most data suggest they are more abundant in developing tissues where they may have redundant functions explaining the lack of phenotypes when knocked out. Double syndecan knockouts in mice have not yet been reported but are probably imminent. These may be very helpful to address functional relationships and potential redundancy between family members. For now, the molecular basis for redundancy has not been explained and it is the case that while many years of research implicate syndecans in a wide variety of diseases, including vascular disease, arthritis and cancer (Echtermeyer *et al.* 2009; Iozzo & Sanderson 2011; Herum *et al.* 2013), the molecular basis for their functions has only slowly revealed themselves.

Syndecan signalling

The cytoplasmic domains of syndecans are small, some 40 amino acids or less (Fig. 1). Unsurprisingly, therefore, they have no intrinsic enzymatic activity. Some years ago, we suggested a nomenclature to partition the cytoplasmic domains into conserved and variable regions. The membrane-proximal region (C1 region) is highly conserved across all syndecans and has been shown to participate in linkage to the actin-associated cytoskeleton. Interactions with ezrin–radixin–moesin (ERM) proteins, tubulin, cortactin and Src have been noted (Kinnunen *et al.* 1998). The recent work of Chen and Williams (2013) reveals an interesting role of the MKKK sequence immediately adjacent to the transmembrane domain. It alone is required for clustering and endocytosis of syndecan-1 from membrane lipid rafts. Extracellular-regulated signal kinase (ERK) activation moves syndecan-1 into rafts where interactions with tubulin are lost, but cortactin interactions, subsequent to syndecan tyrosine phosphorylation, are gained. Endocytosis then follows.

A second highly conserved region of syndecan cytoplasmic domains, the C2 region, is located at the C-terminus and comprises a hydrophobic motif that interacts with PDZ domain containing proteins. These can include syntenin (also known as MDA-9), synectin (also known as GIPC1) and Ca^{2+}/calmodulin-associated serine/threonine kinase (CASK; Multhaupt *et al.* 2009). PDZ proteins are numerous, and several different types may coexist in the same cells, but how interactions with receptor targets, such as syndecans, are regulated is poorly understood. Syntenin has many receptor targets other than syndecans, and little is known about how interactions are controlled. Work with syntenin suggests that interactions are important for syndecan trafficking and recycling (Zimmermann *et al.* 2005), but most recently, an interesting new concept has emerged (Baietti *et al.* 2012; Ghossoub *et al.* 2014). Syndecan–syntenin–ALIX complexes have been shown to regulate exosome formation. These signalling particles are thought to be important in cellular communication and are attracting much attention, for example in tumour–host crosstalk. More recently, exosome formation by tumour cells has also been shown to be accelerated by heparanase activity, which cleaves heparan sulphate chains (Thompson *et al.* 2013). In turn, this may impact exosome composition as well as secretion rate. Roles for the small G protein Arf6 and its target phospholipase D are now also implicated in syntenin-based exosome biogenesis (Ghossoub *et al.* 2014).

Between the two conserved regions (C1 and C2) is a variable (V) region that is distinct to each syndecan. Nevertheless, the sequence for each syndecan V region is conserved across species, for example zebrafish, avian and mammalian syndecan-4 V regions are highly homologous (Whiteford *et al.* 2008). On the basis of sequences, it would be expected that C1 and C2 functions are common to all syndecans, while V region interactions and functions are distinct to each syndecan. However, V region functions have been difficult to ascertain for all syndecans except that of syndecan-4, where quite extensive progress has been made. Widely used techniques such as yeast 2-hybrid analyses have yielded PDZ protein interacting partners for syndecan C2 regions, but little for V regions. In the light of recent work, however, required phosphorylation events in syndecan cytoplasmic V regions may explain these results. There are highly conserved tyrosine residues in syndecan cytoplasmic domains, and Tyr180 in syndecan-4, for example, determines the proteoglycan's roles in integrin internalization and function (Morgan *et al.* 2013). Similarly, syndecan-4 V region has a conserved serine residue immediately adjacent to tyr180, which is potentially phosphorylated by protein kinase Cδ (Murakami *et al.* 2002). This influences not only cell adhesion and migration, but also alters the conformation of the entire cytoplasmic domain (Koo *et al.* 2006). In so doing, affinity for inositol phospholipid and protein kinase Cα is reduced.

In 1997, we demonstrated that syndecan-4 V region could bind and activate PKCα, and in the following year showed that this interaction was dependent on phosphatidylinositol 4, 5 bisphosphate (Oh *et al.* 1997, 1998). Substrates for this

kinase in the context of syndecan-4's role in cell adhesion have been proposed and include p190RhoGAP and RhoGDI (Bass *et al.* 2008; Dovas *et al.* 2010). These events may link PKC to Rho G proteins, known to be essential in focal adhesion and microfilament bundle assembly (Nobes & Hall 1999; Dovas *et al.* 2006), which is logical given data suggesting that syndecan-4 is a promoter of these structures (Couchman 2010).

The V region of syndecan-4 has also been shown to interact with a protein called syndesmos (Denhez *et al.* 2002), although beyond interactions with the focal adhesion protein paxillin, its role is presently uncertain. Lastly, α-actinin interacts directly with syndecan-4 V region, and syndecan-4 null fibroblasts show disrupted α-actinin patterns in concert with a loss of large microfilament bundles and fewer focal adhesions (Okina *et al.* 2012). It has been suggested that serine179 phosphorylation favours interactions with α-actinin at the expense of interactions with PKCα (Chaudhuri *et al.* 2005), while other data do not support this hypothesis (Okina *et al.* 2012).

There still remains a considerable challenge with respect to the V regions of other syndecans, including those of invertebrates. It is interesting that the sequences of the V regions of *C. elegans* and *Drosophila* are not very homologous, suggesting that their binding partners may be distinct. The structure of the syndecan cytoplasmic domains is also unknown for all except syndecan-4, which has a twisted clamp motif involving two parallel peptides interacting with each other. Whether this is the same for all syndecans needs to be addressed. Finally, the V region of syndecan-4 binds an inositol phospholipid, which by extrapolation suggests that the cytoplasmic domain lies along the inner face of the plasma membrane rather than projecting into the cytosol. This has not been experimentally verified, and whether other syndecans are similarly organized is unknown, but so far, none has been shown to interact with phospholipids in the same way. Their sequences are not, perhaps, consistent with lipid interactions.

Glypicans, as they are not transmembrane, do not have direct linkage to the cytoskeleton and may occupy distinct microdomains on the cell surface, as is seen with GPI anchored molecules. It is perhaps unlikely that they can signal independently, but they have important roles in development and in some rare genetic diseases (Pilia *et al.* 1996). Moreover, glypican-3 may be a key cell surface receptor in cancers such as hepatocellular carcinoma (Filmus & Capurro 2013).

Overall, the current data suggest that the C1 and C2 domains common to all syndecans are involved in trafficking, while the V regions have distinct functions. The ancestral syndecan appears to have functions in the nervous system, mirrored perhaps by mammalian syndecan-3. However, the V regions of these syndecans have largely unknown interactions. Given the power of the invertebrate genetic models, it seems likely that deeper understanding will come from that quarter.

Syndecans and inflammation

For some years, there has been interest in syndecan involvement in disease. While no mutations in human syndecans have been described with disease relevance, inflammation has become an important focus (Götte 2003; Alexopoulou *et al.* 2007; Teng *et al.* 2012). As with many other aspects of syndecan biology, most attention has been paid to syndecan-1. Several models of acute and chronic inflammation have been studied, and it appears that a major function of syndecan-1 is to negatively regulate the adhesion and migration of leucocytes. Endothelial syndecan-1 can serve in this capacity (Teng *et al.* 2012; Zhang *et al.* 2013). A recent report suggests that syndecan-1 as a component of the endothelial glycocalyx of arteries is an important mechanosensor, and consistent with other reports, its absence leads to increased leucocyte adhesion (Voyvodic *et al.* 2014). In studies of aortic aneurysm, on the other hand, macrophages bearing syndecan-1 had an important role in damping the inflammatory environment (Xiao *et al.* 2012).

Studies of inflammation, with the attendant increase in local proteinase expression, are inextricably linked to the shedding of syndecans from the cell surface. All syndecans appear to be exquisitely sensitive to cleavage, particularly in a membrane-proximal region of the core protein (Manon-Jensen *et al.* 2013). In addition, syndecan core proteins are sensitive to a large number of proteinases, notably the metalloproteinases. Therefore, an important consideration in the role of syndecans in inflammation is that shedding will likely occur, to release a large portion of the core protein with glycosaminoglycan chains attached. Whether this truncates signalling through the core protein is not confirmed but likely. Further processing of the remnant core proteins in their transmembrane domains by presenilin/γ-secretase has been shown for syndecan-3 (Schulz *et al.* 2003). The released ectodomain can serve as a competitive inhibitor of cell surface proteoglycan but may also diffuse into the local environment where it can sequester a number of inflammatory factors, many of which are heparin binding (Elenius *et al.* 2004; Sarrazin *et al.* 2011). This is, to a large extent, an anti-inflammatory response (Teng *et al.* 2012). However, with respect to cancer, syndecan-1 can support the pathogenic process by amplifying growth factor and cytokine signalling.

In the mammalian genome, there is a single heparanase gene, encoding an enzyme that specifically cleaves heparan sulphate chains (Fux *et al.* 2009; Peterson & Liu 2013). The mechanisms of cleavage and substrate specificity in terms of heparan sulphate fine structure are under investigation. It appears that the enzyme does not have precise substrate specificity, but is influenced by the sulphation in the vicinity of the cleavage site (Peterson & Liu 2013). Such cleavage can, however, leave significant portions of the polysaccharide attached to the core protein. Heparanase is now intensively studied because it is implicated in the pathogenesis of several diseases. In addition, however, it has also been demonstrated that heparanase cleavage of the syndecan hep-

aran sulphate chains can engender enhanced sensitivity of the core protein to shedding (Ramani *et al.* 2012).

The closest homologue to syndecan-1 is syndecan-3, which is enriched in neural tissue but also in the musculoskeletal system. A recent study has shown up an interesting dichotomy of roles across a range of chronic inflammatory diseases. In a murine model of rheumatoid arthritis and using genetic deletion of syndecan-3, the proteoglycan was shown to be pro-inflammatory in the joint where it enhanced chemokine signalling and leucocyte recruitment. By contrast, in CXCL1-induced skin and muscle inflammation, syndecan-3 had an opposite anti-inflammatory role (Kehoe *et al.* 2014). Tissue context is clearly important in the pathobiology of syndecans.

While syndecan-2 has received little attention with respect to inflammation, perhaps because a knockout mouse has not yet been reported, syndecan-4 has been examined with respect to skeletal and vascular disease in particular. Expression of syndecan-4 is rapidly upregulated in inflammation, at least in part because the gene has an NF-κB response element. Therefore, tumour necrosis factor-α, interleukin-1β and lipopolysaccharide all trigger substantial increases in syndecan-4 expression (Strand *et al.* 2013). Equally, syndecan-4 can be shed in the inflammatory milieu, much as syndecan-1. In a model of pulmonary inflammation, syndecan-4 expression was shown to be anti-inflammatory (Tanino *et al.* 2012) and a similar conclusion was reached in a study of myocardial infarction (Xie *et al.* 2012). Indeed, therapeutic potential for syndecan-4 in suppressing inflammation and fibrosis, promoting neovascularization and improved cardiac function was suggested (Xie *et al.* 2012).

In a study of osteoarthritis, it was noted that the syndecan-4 null mouse was protected from cartilage degeneration. In part, this was ascribed to decreased aggrecanase (ADAMTS-5) activity at the cell surface, but also indirect regulation of MMP-3 expression (Echtermeyer *et al.* 2009). ADAMTS-5 was shown to interact with the heparan sulphate of syndecan-4, in which wild-type animals were upregulated in arthritic disease. This study utilized both null animals, but also injected 'blocking' antibodies against syndecan-4. This is one of many reports using such an approach, yet in some ways, this is a mysterious phenomenon. ADAMTS-5 interacts with the heparan sulphate chains, but core protein-directed antibodies are shown to 'block'. How does this happen? In an immunocompetant animal, perhaps the antibody–syndecan complex is removed by leucocytes or the antibody cross-links the syndecan and the patched receptor is then internalized. In the Echtermeyer study, however, there is a possible clue. The antibodies were prepared against a membrane-proximal core protein region, so perhaps shedding was targeted and blocked. It would be interesting to establish the underlying mechanism.

Lastly, in a more recent study of bone fracture repair, syndecan-4 null mice were shown to have delayed repair, with the finger of suspicion pointed at an elevated inflammatory response (Bertrand *et al.* 2013). This study also sheds light on possible redundancy between syndecans in development. As has been noted many times previously, development in the single null mouse is not impaired, but post-natal repair can be compromised. Here, the authors show that in the absence of syndecan-4 and syndecan-2 is upregulated in the developing cartilage. However, it is important to note in conclusion that apart from the potential for compensatory expression of alternate syndecans in development, post-natal repair inevitably involves inflammation that, as summarized here, influences both syndecan expression and function.

With the advent of sophisticated immune systems and a closed vasculature, vertebrates have clearly expanded the roles of syndecans, which may be related to the two rounds of gene duplication events that took place at the invertebrate–chordate boundary (Chakravarti & Adams 2006). Moreover, vascular tissues and many leucocytes are rich sources of syndecans. Although unclear at the molecular level today, it will be interesting to understand the regulatory mechanisms that extend from roles in invertebrate neural development, on the one hand, to the complex processes of acquired and innate immunity in vertebrates.

Syndecans and tumour biology

Syndecans have prominent roles in cell–cell and cell–ECM interactions as well as in regulating cell adhesion and motility, processes which are central to cancer progression (Beauvais & Rapraeger 2004). In addition, syndecan ectodomains can be shed into the ECM by proteases (Manon-Jensen *et al.* 2010) and in soluble form could compete with growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) for binding to cell surface receptors (Fears & Woods 2006). As proteinases including metzincins are frequently upregulated in tumour cells, syndecan shedding has received increasing attention. This feature of syndecans was shown to facilitate myeloma tumour progression (Yang *et al.* 2002).

Syndecan-1 has long been suggested to be a prognostic marker for some tumour types. Given that loss of syndecan-1 is often associated with loss of E-cadherin, it is thought to be a regulator of epithelial to mesenchymal transition in transformed epithelial cells with considerable alterations in cell morphology, growth and motility (Iozzo & Sanderson 2011; Kato *et al.* 1995; Sun *et al.* 1998). This is a likely explanation as to why low syndecan-1 expression associates with higher tumour grade and poor patient survival as well as poor prognosis in head and neck, lung and colorectal cancer (Teng *et al.* 2012). In contrast, high levels of syndecan-1 in breast cancer and particularly in the tumour stroma, myeloma, pancreatic and lung cancer increase tumour aggressiveness and forecast a poor outcome (Teng *et al.* 2012). In myeloma, syndecan-1 enhances tumorigenesis through the regulation of cell survival, adhesion and migration (Khotskaya *et al.* 2009; Yang *et al.* 2002). Consistent with this, syndecan-1 depletion in myeloma cells led to growth arrest and apoptosis (Khotskaya *et al.* 2009). Furthermore, neo-angiogenesis and disseminated growth of myeloma cells were commensurately inhibited when the

mice were injected with syndecan-1 depleted myeloma cells, indicating that syndecan-1 is important to trigger the tumour metastasis. Conversely, head and neck squamous cell carcinoma (HNSCC) cells expressing high levels of syndecan-1 exhibited reduced motility and invasiveness in collagen I matrices compared with HNSCC cells expressing low levels of syndecan-1 (Ishikawa & Kramer 2010). Thus, roles for syndecan-1 as a stimulatory or inhibitory factor are clearly specific to each cancer type, but precisely why there are such tumour-specific differences remains to be elucidated.

Accumulating evidence also suggests that syndecan-2 modulates several key cellular processes in tumourigenesis, such as cell adhesion, migration, apoptosis and metastasis (Beauvais & Rapraeger 2004; Iozzo & Sanderson 2011). Similar to syndecan-1, syndecan-2 also has dual-function properties: either acting as a tumour suppressor or tumour promoter depending on a cancer type (Fears & Woods 2006; Iozzo & Sanderson 2011). Syndecan-2 is reported to be upregulated in colon cancer, Lewis lung carcinoma, ovarian tumours, prostate cancer, melanoma, osteosarcoma and glioma, where it may regulate cell shape, adhesion and migration. Syndecan-2 functioning as a tumour suppressor is best illustrated in Lewis lung carcinoma cells (Munesue *et al.* 2002, 2007). We have very recently provided evidence that syndecan-2-regulated breast cancer cell morphology is highly dependent on Rho-GTPases (Lim & Couchman 2014). A crosstalk between syndecan-2 and p190ARhoGAP in regulation of breast cancer cell actin cytoskeleton and cell migration has been identified. Syndecan-2 appears to be a novel regulator of p190ARhoGAP activity and distribution, which, in turn, regulates localized RhoA activation. On the other hand, syndecan-2 suppresses roles of syndecan-4 in regulating the distribution of p190ARhoGAP in these tumour cells, highlighting the specific effects of syndecan-2 and syndecan-4 in the regulation of cytoskeletal dynamics. These data suggest that at least in some cells, syndecans can establish a hierarchy, one member suppressing the function of another.

Syndecan-4 is the only ubiquitously expressed syndecan in mammals. Although its expression is relatively low compared to the other syndecans, its role is pivotal and diverse in cancer biology. Aberrant expression of syndecan-4 is reported in breast cancer, melanoma, hepatocellular carcinoma and malignant mesothelioma. Syndecan-4 functions in breast carcinoma are currently unclear. It may, in fact, be a good prognostic marker in patients positive for oestrogen and progesterone receptors (Lendorf *et al.* 2011), where more treatment options are available. In this study, expressions of syndecan-4 and syndecan-1 were shown to be independent indicators for prognosis in breast carcinoma. Patient tumour tissue immunohistochemistry showed that syndecan-4 was expressed mostly in the cytoplasm but could also be found in the nucleus as well as the tumour stroma. In addition, association of syndecan-4 with growth factors such as FGF2 has been implicated in melanoma progression. FGF2 signalling is downregulated in the absence of syndecan-4 leading to an increase of melanoma cell motility and defects in fibronectin adhesion (Chalkiadaki *et al.* 2009).

Transport of receptors to the nucleus is certainly not unknown, but syndecan translocation to this site has been somewhat controversial. While there are now several reports that this may occur (Kovalszky *et al.* 2014; Stewart & Sanderson 2014), further detailed and careful analysis is required. What still remains completely unclear is the pathway that transports syndecans to the nucleus and whether truncated or whole proteoglycans reach this site. If these early reports are verified, the most important aspect to be resolved is what role it may have, bearing in mind that knockouts of syndecans are mild. It would be interesting if it transpires that nuclear translocation is a corollary of pathogenesis.

Future perspectives

Since 1989, there have been over 2500 publications concerning the structure, distribution and function of syndecans in vertebrate cells and tissues, as well as genetic analyses in invertebrates and vertebrates. They are now implicated in many diseases, though whether they contribute to, or are a consequence of, pathogenesis is for the most part unclear. In the main, however, it is clear that syndecans are more than ligand gatherers. Apart from many studies that show ternary complexes between ligand, heparan sulphate and high-affinity receptors, syndecans have the capacity to regulate cell function through their cytoplasmic domains. The differences in actin cytoskeletal architecture between syndecan-4 null and matching wild-type cells are clear and readily corrected by transfection of wild-type syndecan-4 cDNA into the null cells. However, cytoplasmic truncation mutant cDNAs will not do so (Gopal *et al.* 2010; Okina *et al.* 2012). Moreover, to date, it appears that syndecan signalling does not impact transcription significantly, but more the morphology and behaviour of cells. The concept of dual regulation then emerges where ligands promote syndecans to control the behavioural response of cells, while the same ligands interacting with specific high-affinity receptors drive transcriptional regulation.

There is much to be learned. Syndecans have been somewhat enigmatic and intractable, but it is likely that a combination of genetics and molecular cell biology and more attention from investigators across the spectrum of biomedicine will reveal much important information on this major group of cell surface proteoglycans. The cytoplasmic domain functions of syndecans remain mostly unclear, with the possible exception of syndecan-4. Redundancy between vertebrate syndecan core proteins is also not well understood although it is very apparent. We are now investigating a possible common signalling mechanism shared by all syndecans, including those of invertebrates. A second shared property of all syndecans is the ease with which they are shed from the cell surface by a wide array of proteinases. It is not yet fully apparent whether this property is important and what roles it may have. Structural analysis of the core proteins is still primordial, but undeniably complicated by

the glycosaminoglycan chains which are both heterogeneous yet an aid to retaining solubility in biochemical preparations. Are the heparan sulphate chains distinct in terms of fine structure when expressed on different syndecan core proteins *in vivo*, or does redundancy extend to the polysaccharide also? This is a difficult question, made even more complex by postexpression modifications by sulphatases and heparanase. Heparanase is implicated in the pathogenesis of several diseases, including tumour progression. This argues that syndecans may not be bystanders but important players. Syndecans are potentially unique in that not only do they have important roles as synthesized and transported to the cell surface, but also new properties can emerge from partial catalysis at the cell surface or in recycling pools. Sulfatases, heparanase and sheddases may all impart modified functions to syndecans but the biological rationale that underlies these events remains to be fully understood. Much more needs to be understood about how syndecans function in inflammation, proliferation, apoptosis and even epigenetic events if nuclear targeting of syndecans is relevant to disease.

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