

Proteoglycans in cancer biology, tumour microenvironment and angiogenesis

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Abstract

Proteoglycans, key molecular effectors of cell surface and pericellular microenvironments, perform multiple functions in cancer and angiogenesis by virtue of their polyhedric nature and their ability to interact with both ligands and receptors that regulate neoplastic growth and neovascularization. Some proteoglycans such as perlecan, have pro- and anti-angiogenic activities, whereas other proteoglycans, such as syndecans and glypicans, can also directly affect cancer growth by modulating key signalling pathways. The bioactivity of these proteoglycans is further modulated by several classes of enzymes within the tumour microenvironment: (i) sheddases that cleave transmembrane or cell-associated syndecans and glypicans, (ii) various proteinases that cleave the protein core of pericellular proteoglycans and (iii) heparanases and endosulfatases which modify the structure and bioactivity of various heparan sulphate proteoglycans and their bound growth factors. In contrast, some of the small leucine-rich proteoglycans, such as decorin and lumican, act as tumour repressors by physically antagonizing receptor tyrosine kinases including the epidermal growth factor and the Met receptors or integrin receptors thereby evoking anti-survival and pro-apoptotic pathways. In this review we will critically assess the expanding repertoire of molecular interactions attributed to various proteoglycans and will discuss novel proteoglycan functions modulating cancer progression, invasion and metastasis and how these factors regulate the tumour microenvironment.

Keywords: perlecan • endorepellin • LG3 • VEGF • syndecan • glypican • heparanase • small leucine-rich proteoglycans • decorin • lumican

Introduction

Proteoglycans were found to be involved in cancer biology over 50 years ago. Initially, pathologists noted that some carcinomas induce a desmoplastic hyperproliferative-like reaction within the host stroma and the connective tissue surrounding the cancer cells. Often this reaction stains metachromatically with cationic dyes such as alcian blue indicating a high glycosaminoglycan, and

thus proteoglycan, content in the desmoplastic reaction. Given the fact that desmoplasia occurs in tumours with diverse histogenetic backgrounds [1], it seems plausible to consider this abnormal expression of proteoglycans a sort of general 'reaction to invasion' with agonistic and antagonistic elements embedded in the same stroma [2]. With the advancement of molecular and cellular biology,

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proteoglycans' specific roles in cancer biology are being better delineated. For example, we now know that some heparan sulphate proteoglycans (HSPGs) act as pro-angiogenic factors by binding several growth factors and presenting them to their cognate receptors [3]. In contrast, C-terminal fragments of other proteoglycans counteract the angiogenic process by blocking the activity of several integrins necessary for endothelial cell movement [4–6]. The structural and functional characterization of cell surface heparan sulphate proteoglycans over the last two decades has led to an understanding of the broad impact these molecules have on cell behaviour [7]. Both the syndecan and glypican family have been implicated as regulators of cancer progression and in addition may serve as biomarkers for early detection or severity of disease [8–10]. Interestingly, the emerging data indicate that cell surface heparan sulphate proteoglycans may play diverse roles in cancer and act as either inhibitors or promoters of tumour progression depending on the type and stage of cancer. In addition, the function of these proteoglycans can be altered by two classes of enzymes within the tumour microenvironment, those that can release proteoglycans from the cell surface (shedases) and those that can modify the structure of heparan sulphate chains (heparanases and endosulfatases) [11, 12]. Thus, the expression of these enzymes can act as a mechanism to dynamically control proteoglycan location and function and thus, tumour cell behaviour [9]. Another class of proteoglycans carrying primarily dermatan or chondroitin sulphate, the so-called small leucine-rich proteoglycans (SLRPs), act as tumour repressors by blocking the activity of receptor tyrosine kinases (RTKs) such as the epidermal growth factor receptor (EGFR) and Met [13]. In addition some of the SLRPs interact with integrins and Toll receptors and modulate adhesion and innate immunity [14, 15]. In this review we will discuss examples of both HSPGs and SLRPs to critically assess their roles in cancer biology, tumour microenvironment and angiogenesis.

Perlecan: a pro-angiogenic proteoglycan

Perlecan is a complex modular proteoglycan consisting of five domains with homology to growth factors, immunoglobulin and adhesion molecules [16–20] and encoded by a very large gene encompassing 95–97 exons [21]. Its biology is complicated by the large number of post-translational modifications including potential substitution with three heparan sulphate chains at the N-terminus, several O-linked oligosaccharides throughout the large protein core and the potential substitution with chondroitin sulphate [4, 22]. Its promoter region is quite complex [23] and is under positive and negative control by transforming growth factor (TGF)- β [24, 25] and interferon- γ [26], respectively. Perlecan is widely expressed [27] and targeted disruption of the perlecan gene *Hspg2* causes embryonic lethality at about day 12. This is primarily caused by intra-pericardial haemorrhage likely due to the lack of perlecan causing failure of the basement membrane upon increased haemodynamic pressure concurrent with the enhanced cardiovascular

circulation at that time [28]. The few surviving pups die very early from pulmonary insufficiency owed to severe cephalic and cartilage abnormalities [29]. It was soon realized, however, that about 75% of the *Hspg2*^{-/-} mice developed transposition of the great vessels, aorta and pulmonary artery, as well as abnormal position of coronary arteries [30, 31], thereby implicating perlecan in cardiovascular development. These mouse results correlate well with the phenotype obtained by morpholino-mediated knockdown of zebrafish perlecan [32]. In addition to a severe muscular phenotype, the perlecan morphants show a markedly reduced angiogenic sprouting especially prominent in the intersegmental vessels which emerge from the dorsal aorta during development [32].

The initial report of perlecan involvement in cancer and angiogenesis described a 15-fold increase in perlecan mRNA in invasive melanomas which correlated with an abundant deposition of perlecan proteoglycan in the pericellular matrix of the tumour cells [33]. Concurrently it was discovered that perlecan promotes high-affinity binding of fibroblast growth factor (FGF)-2, an established pro-angiogenic factor [34], to cells lacking heparan sulphate and to soluble forms of FGF receptor [35]. Moreover, perlecan of both arterial and venous endothelial cells bind FGF-2 with a $K_D \sim 70$ nM [36] and enhances FGF-2-mediated angiogenesis and its activity is as powerful as that obtained with heparin [35]. At the same time, in a study using PC3 prostate carcinoma tumour xenografts in nude mice, it was observed that perlecan of human origin is deposited in the basement membrane of the tumour vessels which, in theory, should be of mouse origin [18]. Thus, it was suggested that perlecan could serve as an initial scaffold upon which the murine endothelial cells would migrate and deposit an appropriate vascular basement membrane [18]. This concept has been subsequently validated by a study using mouse tumour xenografts in immunocompromised rats which showed that perlecan distributed around the tumour neovessels was of both host and tumour cell origin [37].

The central role of perlecan in angiogenesis, as a large multi-modular heparan sulphate proteoglycan, has been confirmed by several independent studies using antisense RNA strategies in various tumour cells, both *in vitro* and *in vivo* [38]. For example, in both human colon carcinoma tumour xenografts and mouse melanoma tumour allografts, perlecan suppression caused substantial inhibition of tumour growth and angiogenesis [39]. Perlecan is required to inhibit thrombosis after deep vascular injury [40] and is essential to the strain-mediated effects on endothelial cell growth control [41]. Moreover, suppression of endogenous perlecan in mouse fibroblasts and human melanoma cells blocks both autocrine and paracrine functions of FGF-2 [42], further stressing a role for perlecan as co-receptor for FGFs. Knockdown of perlecan in melanoma cells has also been linked to a suppression of proliferation and invasion [43] and to a reduced responsiveness to both FGF-2 and vascular endothelial growth factor A (VEGFA) in human prostatic carcinoma cells [44]. In contrast, fibrosarcoma cells devoid of perlecan grow better, form more aggressive tumours and do not depend on FGF-2 for their proliferation, suggesting that cellular context is important [45]. We should also point out that perlecan, either *via* the protein core or the heparan sulphate chains, also binds platelet derived growth

factors AA and BB [22, 46], FGF-1 [22], FGF-7 [47–49], FGF-9 [22], FGF-18 [50, 51], hepatocyte growth factor (HGF) [22] and FGF-binding protein [52] which could also modulate angiogenesis. Furthermore, perlecan binds progranulin [53] and extracellular matrix protein (ECM)1 [54], both of which have been involved in promoting angiogenesis [5], and is involved in regulating the Sonic Hedgehog pathway during development [55] and in prostate cancer [56, 57].

A central role for perlecan as a regulator of VEGFA/VEGFR2 axis has been recently reported in the zebrafish perlecan knockdown [58]. In this model system, the perlecan morphants show a surprising increase of VEGFA, which is also abnormally distributed in the embryos suggesting that perlecan is required for the proper localization of VEGFA. Moreover, a significant proportion of the perlecan morphants could be rescued by intra-embryonic injections of human VEGFA, suggesting that perlecan acts upstream of VEGFA/VEGFR2 axis [58]. This is interesting because the vascular abnormalities observed in the perlecan morphants are similar to those observed in VEGFA morphants [59], null mutants of VEGFR2 [60] or phospholipase C γ 1 [61], a downstream effector of VEGFR2 [62, 63]. In human brain microvascular endothelial cells, VEGFA stimulates the synthesis of perlecan [64]. Notably, in human umbilical vein endothelial cells (HUVECs), addition of exogenous perlecan enhances VEGFA-induced phosphorylation of VEGFR2 [58] suggesting a positive feedback loop controlling VEGFR2 bioactivity. These findings further suggest that perlecan could be a key player in regulating not only the geographic distribution of various heparan sulphate-binding growth factors, but also the specific interaction with RTKs such as FGF receptors (FGFRs) and VEGFRs.

The pivotal role of perlecan heparan sulphate chains has been corroborated by transgenic animals lacking part of domain I where the heparan sulphate chains are attached [65]. These mice show impaired tumour growth and angiogenesis induced by FGF-2 [66]. In hepatoblastoma xenografts treated with a VEGF trap, consisting of soluble domains of both VEGFR1 and VEGFR2, there is an initial collapse of the tumour vasculature followed by a recovery. Strikingly, during the recovery phase there is a concurrent increase in both VEGFA and perlecan mRNA expression with abundant overlapping deposits of VEGFA protein and perlecan around the tumour vasculature [67]. Concomitantly, there is vascular expression of VEGFR2 and sustained VEGFR2 activation, suggesting that, like in the zebrafish model described above, perlecan might represent a potent reservoir of growth factors that can be presented to and utilized by VEGFR2 [67]. Indeed VEGF anchored to the extracellular matrix activates VEGFR2 in a different and more protracted way than soluble VEGF, in terms of receptor recruitment, phosphorylation kinetics and activation of downstream signalling [68]. This concept is further supported by the discovery that heparan sulphate proteoglycans in *trans* can potentiate VEGFR2 signalling and thus angiogenesis by evoking a sustained signal transduction due to heparan sulphate-dependent trapping of the active VEGFR2 signalling complex [69].

Thus, whole perlecan can be a pro-angiogenic factor by virtue of two main mechanisms. The first is by directly interacting with the VEGFR2 through its heparan sulphate chains as co-factors for

VEGF and by binding to adjacent integrin receptors through the C-terminal domain V (see below) (Fig. 1). The second involves the activity of heparanase which would liberate the VEGF bound to the heparan sulphate chains located at the N-terminus (Figs 1 and 2). Indeed, heparan sulphate from perlecan has been shown to be sensitive to heparanase [70, 71], and during branching morphogenesis of the salivary gland, heparanase-mediated cleavage of perlecan heparan sulphate chains modulates FGF-10 bioactivity [72]. Of course, other heparan sulphate-binding growth factors would be equally presented to their cognate receptors by the enzymatic activity of heparanase [73], which generally cleaves the heparan sulphate chains into large fragments [12]. An additional mechanism involves the partial proteolytic cleavage of perlecan protein core mediated by various matrix metalloproteinases (MMPs) [70, 74], which together with heparanase, could generate soluble bioactive fragments [75].

Endorepellin, a C-terminal fragment of perlecan with anti-angiogenic activity

The discovery of endorepellin occurred by serendipity during a yeast two-hybrid screening utilizing the C-terminal domain V of perlecan as bait. Domain V of perlecan bound with high affinity to the non collagenous NC1 domain of collagen XVIII [76] which includes the well-established angiogenic inhibitor endostatin [77]. Soon, however, it was realized that perlecan domain V was as anti-angiogenic as endostatin and it was renamed endorepellin to signify its anti-endothelial nature [76]. Endorepellin binds with high affinity to the intercalated I domain, also known as von Willebrand/Integrin A domain, of the α 2 β 1 integrin, a key receptor involved in angiogenesis [78–80] in both arterial and microvascular endothelial cells [81] as well as in platelets [82]. In endothelial cells, endorepellin evokes a signalling cascade that leads to disruption of the actin cytoskeleton thereby immobilizing endothelial cells and preventing them to establish capillary morphogenesis in either a collagen type I sandwich or Matrigel and in a number of angiogenic assays [5, 83, 84]. These functional studies correlate with the proteomic identification of at least five different proteins differentially regulated by exposing endothelial cells to recombinant endorepellin [85]. These proteins include β -actin, HSP60 and calreticulin (down-regulated), and vimentin and protein disulfide isomerase (up-regulated), all of which have been involved in angiogenesis. Notably, systemic delivery of recombinant human endorepellin to mice carrying two tumour xenografts, squamous cell carcinomas and lung carcinomas, caused a marked reduction of tumour growth, tumour metabolism and angiogenesis [86]. Further genetic experiments utilizing mice lacking the α 2 β 1 integrin have revealed that this receptor is necessary for endorepellin-evoked anti-angiogenic activity insofar as microvascular endothelial cells isolated from the α 2 β 1^{-/-} integrin mice do not respond to exogenous endorepellin in contrast to α 2 β 1^{+/+} mice [87]. Moreover, Lewis lung carcinoma allografts in the α 2 β 1^{-/-} mice fail to respond to systemic delivery of

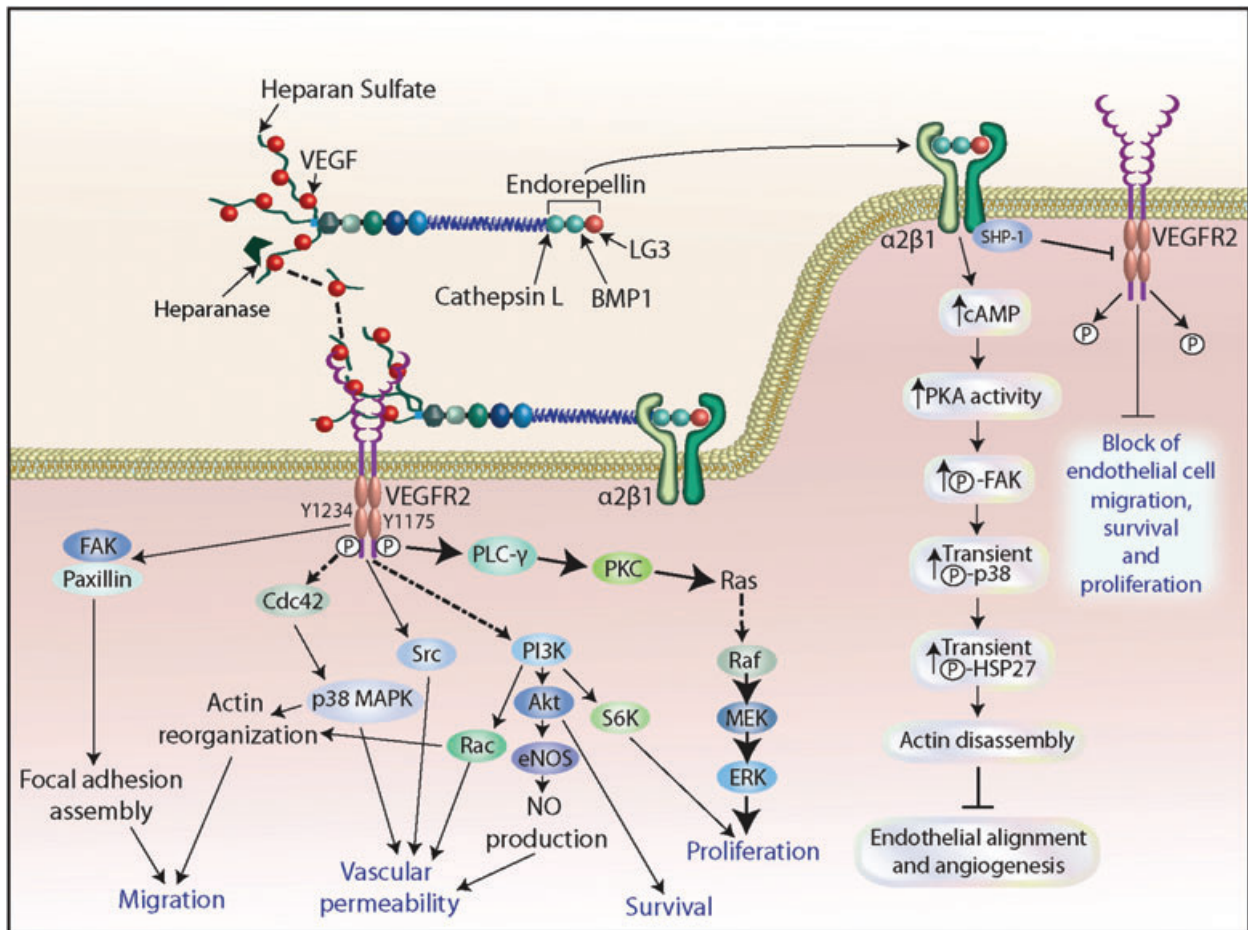


Fig. 1 Schematic representation of the dual activity of perlecan. Perlecan can act as a powerful pro-angiogenic factor by either directly presenting VEGFA (and other heparan sulphate growth factors) to the VEGFR2, or indirectly following partial heparanase cleavage of the heparan sulphate side chains. Both can trigger VEGFR2 signalling with stimulation of migration, vascular permeability, survival and proliferation. Perlecan can also act as a powerful anti-angiogenic factor following cathepsin L or BMP-1/Tolloid-like protease cleavage of endorepellin and LG3, respectively. These C-terminal fragments bind with high affinity to the $\alpha_2\beta_1$ domain of the $\alpha_2\beta_1$ integrin triggering a signal cascade that leads to disruption of endothelial cytoskeleton and endothelial cell motility. Endorepellin also activates the tyrosine phosphatase SHP-1 which is bound to the cytoplasmic domain of the $\alpha_2\beta_1$ integrin. SHP-1 then dephosphorylates a number of RTKs including VEGFR2, thereby blocking endothelial cell migration, survival and proliferation.

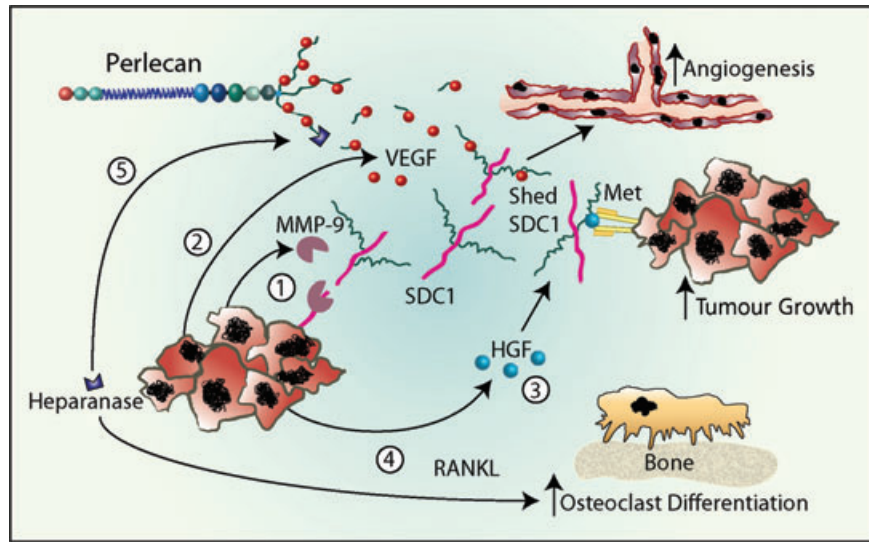
endorepellin, whereas $\alpha_2\beta_1^{+/-}$ mice did [87]. In support of a central role for the $\alpha_2\beta_1$ integrin in perlecan-mediated pro- and anti-angiogenic activity are the recent results obtained with knockdown of the $\alpha_2\beta_1$ integrin in zebrafish. In this case, reduced levels of $\alpha_2\beta_1$ integrin evoke a vascular phenotype that is reminiscent of the perlecan morphants [88] with blunted intersegmental vessels which are largely non-functional by live video microscopy.

Endorepellin can be liberated by cathepsin L [89] and its terminal LG3 domain can be also specifically liberated by BMP1/Tolloid-like proteases [90] releasing a laminin-like globular domain (LG)3 domain that is biologically active [81, 91]. Perlecan-derived LG3 has been found in the amniotic fluid of women with premature rupture of foetal membranes [92], or mothers carrying Down Syndrome fetuses [93] and in the urine of patients with end-

stage renal disease [94] and chronic allograft rejection [95]. Importantly, circulating levels of perlecan LG3 are decreased in women with breast cancer, suggesting that circulating LG3 titres could be a biomarker for breast cancer [96]. Interestingly, intact endorepellin has been found in the upper proliferative zone of cartilage foetal growth plate [97] suggesting that cartilage-derived endorepellin might be one of the key factors able to counteract invasion of blood vessels into cartilage. We note that the concept that certain tissues, such as cartilage, are not invaded by vascularized mesenchyme has been proposed nearly 40 years ago by Eisenstein *et al.* [98], implying that soluble factors from cartilage might prevent invasion by blood vessels.

Regarding the molecular mechanism of action of endorepellin, a recent study utilizing antibody arrays recognizing multiple

Fig. 2 Heparanase enhances gene expression and syndecan-1 (SDC1) shedding which together enhance tumour progression. Upon up-regulation of heparanase expression by the tumour cell, expression of syndecan-1, MMP-9, VEGF, HGF and receptor activator of nuclear factor κ -B ligand (RANKL) are elevated and released into the tumour micro-environment. MMP-9 acts as a 'sheddase' to increase release of syndecan-1 from the tumour cell surface (1). Shed syndecan-1 binds to VEGF (2) to promote angiogenesis and to HGF (3) to stimulate tumour growth. RANKL (4) activates differentiation of osteoclast precursors to accelerate bone turnover leading to release of factors that further drive tumour progression. Heparanase also cleaves the heparan sulphate chains of perlecan (5) thereby liberating additional VEGF that further stimulates angiogenesis.



tyrosine phosphorylated RTK has shown that endorepellin causes a global dephosphorylation of several RTKs including VEGFR2 [99]. This is mediated by the Src homology-2 protein phosphatase-1 (SHP-1) which binds to the intracellular domain of α_2 subunit of the $\alpha_2\beta_1$ integrin and upon endorepellin engagement dephosphorylates several RTKs including VEGFR1, VEGFR2, EGFR, Tie2 and other receptors involved in angiogenesis, migration and growth control. Notably, endorepellin-mediated effects on SHP-1 require the $\alpha_2\beta_1$ integrin insofar as human endothelial cells depleted in the receptor α_2 subunit *via* siRNA-mediated strategies or endothelial cells derived from the $\alpha_2\beta_1^{-/-}$ mice do not respond to endorepellin [99].

In summary, C-terminal fragments of perlecan, either endorepellin or its terminal LG3 module, can act as a powerful anti-angiogenic factor by interacting with the α_2 domain of the $\alpha_2\beta_1$ integrin. This unique interaction triggers a signal cascade that leads to disruption of endothelial cytoskeleton and endothelial cell motility (Fig. 1). Endorepellin also activates the tyrosine phosphatase SHP-1 which is bound to the cytoplasmic domain of the $\alpha_2\beta_1$ integrin. SHP-1 then dephosphorylates a number of RTKs including VEGFR2, thereby blocking endothelial cell migration, survival and proliferation.

Syndecans in cancer biology

The syndecans are a family of transmembrane proteoglycans that bear predominantly heparan sulphate glycosaminoglycan chains [100, 101]. The core proteins consist of a short intracellular domain, a transmembrane domain that is highly conserved, and an ectodomain that is divergent in amino acid sequence among the four syndecan family members [100]. Syndecans are expressed ubiquitously throughout embryonic development and

adulthood, although the individual syndecans exhibit differing patterns of expression. Importantly, the pattern of expression and/or the structure of heparan sulphate is altered in response to certain pathophysiological stimuli and this in turn can regulate tumour progression [7, 102]. As cellular components, syndecans can function both intracellularly and at the cell surface. In addition, *via* protease shedding that occurs at the cell surface, syndecans can be shed into the extracellular milieu where they can diffuse away from the cell, become part of the extracellular matrix or remain soluble and influence surrounding cells as well as cells distal to their original source. On the cell surface, the core protein and heparan sulphate chains of syndecans play a number of roles in promoting cell-cell and cell-ECM interactions and in regulating cell survival, adhesion and migration [103]. Syndecans also bind to numerous soluble molecules (*e.g.* cytokines, chemokines) *via* their heparan sulphate chains thereby facilitating downstream signalling events [104, 105]. Additionally, syndecans signal through their protein cores, largely by direct interaction with other molecules that mediate signalling cascades [68]. Thus, due to their diversity in localization and function, syndecans are well positioned to regulate tumorigenesis and tumour progression.

Syndecan-1 is highly expressed at the basolateral membrane of simple epithelial cells, where it promotes adhesion to the ECM and regulates cell shape by interacting with the actin cytoskeleton [100]. Transformed epithelial cells often lose expression of syndecan-1 and E-cadherin which together appear to be key steps in regulating epithelial to mesenchymal transition [106, 107]. This likely explains why loss of syndecan-1 expression is associated with accelerated tumour progression and reduced patient survival in some types of carcinomas [108, 109]. Conversely, in other tumour types such as breast carcinoma and haematological malignancies, syndecan-1 is often highly expressed and associated with increased tumour invasiveness and poor prognosis [110–114]. For example, in myeloma, expression of syndecan-1 regulates cell

growth, adhesion and migration [9]. Myeloma cell lines expressing low levels of syndecan-1 grow poorly *in vivo*, whereas their counterparts expressing elevated levels of syndecan-1 grow very aggressively and form large, highly vascular tumours [115]. In addition to its expression by tumour cells, syndecan-1 expressed by stromal cells can also impact tumour behaviour. In breast cancer syndecan-1 is present in high levels in the stroma of some patients and stromal expression has been correlated with poor prognosis [116, 117]. Moreover, studies in mouse models have demonstrated that stromal syndecan-1 released into the tumour microenvironment enhances breast tumour growth and angiogenesis [118, 119].

In addition to regulating tumour growth and metastasis, studies with syndecan-1 null mice indicate that this proteoglycan may also be critical for initial steps leading to initiation of tumours. Crossing *Wnt* transgenic mice that normally have a high incidence of spontaneous breast tumour formation with syndecan-1 null mice results in a dramatic reduction in tumour formation [120]. Further studies probing the mechanism of syndecan-1 activity in tumorigenesis indicate that the proteoglycan may be required for the activation of tumour precursor cells that arise within the stem/progenitor cell compartment [121, 122].

Because heparan sulphate is known to have multiple roles in regulating tumour growth, much of the work on syndecans in cancer has focused on heparan sulphate. However, recent data indicate that a region within the extracellular portion of the core protein of syndecan-1 binds to and activates signalling of both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins present on mammary carcinoma cells [123]. This syndecan-1 facilitated activation of the integrins enhances the invasive phenotype of the carcinoma cells and a peptide mimic of the region of syndecan-1 responsible for this activation can block integrin activation and tumour growth *in vivo*. Also, it has been found that the cytoplasmic domain of syndecan-1 interacts with $\alpha_6\beta_4$ integrin and regulates ErbB2 activation by the integrin [124]. Interestingly, although it has been known for some time that syndecan-1 promotes tumour cell binding to type I collagen and inhibits its invasion into collagen gels [125], a series of recent studies identify syndecan-1 as a co-receptor for $\alpha_2\beta_1$ integrin-mediated adhesion to collagen [126, 127]. These studies reveal that even when the collagen receptor $\alpha_2\beta_1$ integrin is present, suppression of syndecan-1 in carcinoma cells diminishes cell adhesion to collagen and enhances cell motility and invasion. Although the heparan sulphate chains were required for the effects of syndecan-1 on carcinoma cell behaviour, neither syndecan-2 nor syndecan-4 could substitute for syndecan-1 indicating that the core protein of syndecan-1 may participate in $\alpha_2\beta_1$ integrin signalling. Also supporting this notion is the recent finding that shedding of syndecan-1 from the surface of injured lung epithelial cells weakens the affinity state of $\alpha_2\beta_1$ integrin resulting in increased cell migration [128].

Syndecan-2 expression is up-regulated in a number of cancers where it plays an important role in mediating cell shape, adhesion and migration. Syndecan-2 functions in this manner by regulating organization of the actin cytoskeleton and activation of focal

adhesion kinases through the interaction of its cytoplasmic domain with ezrin, radixin and moesin family members [129]. Co-dimerization of syndecan-2 with $\alpha_5\beta_1$ integrin in Lewis lung carcinoma cells induces actin stress fibre formation [130]. Syndecan-2 overexpression in highly metastatic variants of Lewis lung carcinoma cells inhibited their metastasis to lung possibly *via* down-regulation of the activation of the pro-metastatic protease MMP-2 [131]. Similarly, overexpression of syndecan-2 by osteosarcoma cells reduces their migration and enhances their apoptosis *in vitro* as compared to controls expressing lower levels of syndecan-2 [132, 133]. However, in contrast, syndecan-2 can promote tumour progression of other cancer types. In colon cancer, syndecan-2 expression enhances cell adhesion, spreading and proliferation indicating that it is important in colon cancer survival and perhaps initial tumorigenesis and metastasis [134, 135]. These adhesion and migration functions of syndecan-2 appear to be, at least in part, due to its functioning as a co-receptor for $\alpha_2\beta_1$ integrin because knockdown of the α_2 integrin chain on HCT116 colon carcinoma cells diminished syndecan-2-induced cell migration [136]. Also, a recent report indicates that the cell migration promoted by syndecan-2 occurs through Tiam1-mediated activation of Rac [137].

In addition to its effects in tumour cells, syndecan-2 may also enhance angiogenesis. Syndecan-2 is highly expressed in the microvessels of mouse glioma tumours and when syndecan-2 expression is knocked down in brain microvascular endothelial cells both cell migration and *in vitro* capillary tube formation are inhibited [138]. Additional evidence of a role for syndecan-2 in angiogenesis has come from knockdown experiments in zebrafish where it appears that syndecan-2 may act as a co-receptor for VEGF [139].

Syndecan-4 is reportedly expressed at some level by most tumours and also within the tumour microenvironment. In contrast to syndecan-2 which enhances migration of tumour cells, syndecan-4 is similar to syndecan-1 in that it mediates tumour cell adhesion to extracellular matrix and inhibits cell invasion in some cancers. Syndecan-4 mediates adhesion and spreading of breast cancer cell lines [140] and is down-regulated on invasive colon carcinoma cells [135, 141]. Diminished expression of syndecan-4 in melanoma cells resulted in down-regulation of FGF-2 signalling leading to an increase in tumour cell motility and decreased adhesion to fibronectin [142]. In contrast, a different study reports that diminishing syndecan-4 expression caused a decrease in melanoma cell invasion by decreasing Wnt5A signalling [143]. This suggests that syndecan-4 can play differing roles in tumour invasive potential depending on the factor(s) that are present to regulate motile behaviour. Also, complexing of syndecan-4 with FGF-2/FGFR-1 promotes pro-angiogenic signalling events through the protein kinase C_α pathway [144]. Disruption of interactions between syndecan-4 and fibronectin by the extracellular matrix molecule tenascin-C decreases focal adhesions on tumour cells leading to increased tumour cell proliferation [145]. Thus, regulation of cell adhesion *via* syndecan-4 is critical in controlling both the migration and proliferation of some tumour types.

Glypicans and the control of cancer growth

Glypicans share similarities to the syndecans in that they are expressed in cell-, tissue- and development-specific patterns, interact with a multitude of extracellular matrix proteins, including chemokines, growth factors/morphogens and their receptors and regulate cell-signalling events during morphogenesis, adult physiology and carcinogenesis [146–148]. Glypicans differ from syndecans in their mode of interaction with the cell membrane which occurs *via* a glycosyl–phosphatidyl–inositol (GPI) anchor. The six-member family of glypicans is characterized by having protein cores of 60–70 kD and containing 14 conserved cysteine residues. All glypican C-terminal regions have attachment sites for heparan sulphate chains, as well as the signal sequence for the GPI anchor. In addition to their heparan sulphate chains, glypican core proteins can also mediate important cellular functions such as stimulating apoptosis and supporting Wnt signalling [149–151].

Within the context of human malignancies, glypican-3 has been studied most extensively within the glypican family members [146, 149, 152]. Glypican-3 expression and function correlate with tumour type and cell of origin and thus analysis of glypican-3 can aid in the diagnosis and prognosis of some cancers. In cancers of the breast [153], lung [154], ovary [155] or mesothelium [156], glypican-3 expression is often down-regulated during tumour progression. Down-regulation of glypican-3 could enhance growth of cells by indirectly causing an up-regulation of cell signalling through such mechanisms as competing with Patched, the receptor for Hedgehog, for binding to Hedgehog [157]. In contrast, in neoplasms originating from tissues that express glypican-3 only in the embryo, its expression reappears during malignant transformation as an oncofetal protein [158, 159]. An example of this is hepatocellular carcinoma (HCC) where soluble glypican-3 is detected in serum of 40–53% of HCC patients, but is not present in serum of healthy individuals [160, 161]. In HCC, glypican-3 promotes Wnt signalling as well as oncogenesis *via* the IGF signalling pathway [162]. Interestingly, a recent study demonstrated that overexpression of soluble glypican-3 inhibits HCC growth at least in part by blocking Wnt signalling [163].

Studies have also revealed the potential of glypican-3 as an immunotherapeutic target in tumours that overexpress the proteoglycan. Preclinical studies have demonstrated that the antigenic peptide (aminO acids 144–152 of glypican-3) induces peptide-reactive cytotoxic T lymphocytes that reduce tumour mass without inducing autoimmunity [164]. A protective effect was also achieved by the transfer of glypican-3 peptide pulsed dendritic cells or glypican-3-reactive cytotoxic T lymphocytes in a model of colon carcinoma [165].

Glypican-1 is reportedly up-regulated in pancreatic and breast cancers and is also frequently overexpressed in human gliomas, where it enhances FGF-2 signalling [166, 167]. Down-regulation of glypican-1 in the human pancreatic cancer cell line PANC-1 prolonged doubling times and decreased anchorage-independent growth *in vitro* and tumour growth, angiogenesis and metastasis

in vivo [166, 168]. Release of glypican-1 from the surface of PANC-1 or COLO-357 pancreatic cell lines by treatment with phosphoinositide-specific phospholipase-C, which cleaves the GPI anchor of glypican-1, inhibited the mitogenic responses of these cells to FGF-2 and heparin binding EGF, two growth factors that are commonly overexpressed in pancreatic cancer [169]. Similarly, glypican-5 increases tumour proliferation in rhabdomyosarcomas by potentiating the action of FGF-2, HGF and Wnt1A [170].

Role of heparanase and proteoglycan remodelling in cancer

Enzymatic modification of heparan sulphate proteoglycans has emerged as an important mechanism of regulating proteoglycan function in cancer. These modifications consist primarily of three types: cleavage of the extracellular core protein by sheddases which can release soluble domains of the proteoglycans from the surface of cells, trimming of heparan sulphate chains by heparanase and removal of 6-O sulphates from heparan sulphate chains by extracellular sulfatases. These modifications to proteoglycan structure can dramatically impact the function of the molecule and lead to changes in tumour cell behaviour. The action of the sulfatases in cancer will not be considered here but are the subject of several recent reviews [171, 172].

It has become increasingly clear that shedding of proteoglycans from the surface of cells plays an important role in regulating proteoglycan localization and function [11]. Shedding occurs through the activity of various MMPs which clip the core proteins of proteoglycans and release functional ectodomains [173]. These ectodomains can then diffuse into the microenvironment where they may impact other cells or they can become inserted into the insoluble extracellular matrix. In addition, they can diffuse into the serum where they can be detected and used as a marker for cancer. For example, elevated levels of soluble syndecan-1 have been detected in the serum of patients with Hodgkin's lymphoma [174] and lung cancer [175, 176]. Syndecan-1 is also found in patients with multiple myeloma where high levels of circulating proteoglycan are associated with high tumour burden and a poor prognosis [113, 177]. Further analysis utilizing animal models of myeloma indicates that soluble syndecan-1 is not only a marker of poor prognosis but actually participates in driving the progression of this cancer by stimulating tumour growth, angiogenesis and metastasis [178]. In myeloma, shed syndecan-1 appears to be derived predominantly from the surface of the tumour cells themselves. However, in an animal model of breast cancer, syndecan-1 shed into the tumour microenvironment was found to originate predominantly from stromal cells adjacent to the tumour cells [119]. This shed syndecan-1 stimulates tumour cell proliferation *via* activation of FGF-2. A role for stromal cell derived syndecan-1 in breast cancer is also supported by the finding that syndecan-1 is highly expressed in the stroma of many breast cancer patients

[116]. In addition to studies on syndecan-1, syndecan-2 was found to be highly expressed on endothelial cells in a murine glioma model and its inhibition leads to reduced tubulogenesis *in vitro*, consistent with its role in cell adhesion and migration [138]. In this same model, addition of exogenous MMPs or pro-angiogenic factors (*e.g.* VEGF, FGF-2) leads to increased syndecan-2 shedding and promotion of endothelial cell migration on Matrigel. Also, shedding of both syndecan-1 and glypican-1 may be important in the progression of pancreatic cancer [169, 179].

Interestingly, recent findings implicate heparanase as an important regulator of syndecan shedding. Heparanase is an endo- β -D-glucuronidase that cleaves heparan sulphate chains into fragments 5–7 kD in size. Through both enzymatic and non-enzymatic activities, heparanase enhances tumour progression by degrading the extracellular matrix, stimulating cell signalling and by up-regulating expression of genes that promote an aggressive tumour phenotype (reviewed in [180]). Heparanase is up-regulated in myeloma patients and high levels of heparanase expression correlate with enhanced angiogenesis and poor prognosis [181, 182]. These findings in part have recently been linked to the fact that heparanase enhances shedding of syndecan-1 from the surface of myeloma cells [182, 183]. Mechanistically, this occurs *via* heparanase stimulation of ERK signalling with a resulting increase in expression of MMP-9, a protease that acts as a sheddase for syndecan-1 [184]. Up-regulation of heparanase expression in myeloma cells also leads to enhanced expression of VEGF and HGF, two factors that bind to the shed syndecan-1 and form complexes that stimulate angiogenesis and tumour growth [185, 186] (Fig. 2). Thus, the dual effects of heparanase (*i.e.* stimulation of both syndecan-1 shedding and growth factor expression) together condition the tumour microenvironment thereby establishing an aggressive tumour phenotype. Additionally, heparanase within the tumour extracellular matrix may act to release heparin binding growth factors from matrix bound heparan sulphate proteoglycans such as perlecan (Fig. 2). These multifunctional activities of heparanase are consistent with its known roles in regulating tumour growth, angiogenesis and metastasis.

Decorin and growth control

Decorin is a prototype member of the SLRPs, an expanding family of about 18 homologous genes that encompass five distinct classes [14, 187, 188]. Decorin received its eponym because of its ability to 'decorate' collagen fibres [189] at specific sites [190] and to regulate fibrillogenesis both *in vitro* [191] and *in vivo* [192]. Decorin is also known to regulate growth factor bioactivity [193, 194]. The decorin-null animals are fertile and viable but show abnormal collagen architecture, most pronounced in the dermis, which causes a decrease tensile strength of the skin with a consequent skin fragility phenotype [192]. The initial observation linking decorin to growth control derives from studies using

Chinese Hamster Ovary (CHO) cells which are unique because their growth is dependent on TGF- β secreted as an autocrine factor. These decorin-expressing CHO cells formed a more orderly monolayer and grew to a lower saturation density than control cells lacking decorin, and these changes correlated with the levels of decorin expression [195]. It was subsequently found that decorin secreted by the transgenic CHO cells bound with high affinity to TGF- β thereby blocking the growth-stimulatory activity of the growth factor [196]. The second observation linking decorin to cell growth emerged from studies focusing on the identification of differentially expressed genes in quiescence *versus* growing cells. These studies showed that the mRNA levels of decorin, one of the eight most up-regulated genes, increased several folds during quiescence [197]. *In vitro* transcriptional analysis indicated enhanced transcriptional activity in quiescent fibroblasts when compared to cells harvested in their logarithmic growth phase [198]. The third line of evidence for a direct involvement of decorin in growth control is based on the fact that *de novo* expression of decorin suppresses the malignant phenotype in a wide variety of transformed cells [199, 200] and the effects are mediated by decorin-evoked induction of the cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} (p21) [201]. Induction of endogenous p21 has now been recorded in a number of experimental settings utilizing recombinant decorin, adenoviral gene delivery or stable transfection of decorin, both in normal and malignant cells [202–208]. It became apparent, however, that the growth of several of the tumour cell lines that responded to decorin did not depend on the presence of TGF- β or its downstream signalling like the original CHO cells mentioned above. A search for specific receptors for decorin revealed that decorin suppresses tumour cell growth by interacting with the epidermal growth factor receptor (EGFR) [209, 210]. Notably, the EGFR could be identified as an interacting partner for decorin using interaction cloning and the yeast two-hybrid system [211]. Decorin binds to a narrow region of the EGFR that is partially overlapping but distinct from the EGF-binding domain [211]. Decorin/EGFR interaction leads to a sustained down-regulation of the EGFR [212] and, in mammary carcinoma cells, decorin also interacts with ErbB4 and indirectly down-regulates ErbB2, presumably by down-regulating of ErbB2/ErbB4 heterodimers [213]. Decorin evokes protracted internalization of the EGFR *via* caveolar endocytosis rather than clathrin-mediated endocytosis as EGF usually does [214]. This leads to a profound physical down-regulation of the EGFR with consequent reduction of receptor number rather than affinity [212].

Biologically active decorin is likely to act as monomer in solution [215] and adenoviral gene delivery of decorin has been shown to inhibit *in vivo* tumorigenicity and metastatic spreading [205, 216–220]. Analogously, systemic delivery of decorin proteoglycan or decorin protein core inhibits tumour growth in various xenografts models, including squamous, breast and prostate carcinomas [208, 221, 222]. The *in vivo* effects of decorin, when tested, show a concomitant down-regulation of the EGFR in the tumour xenografts and induction of apoptosis, predominantly through activation of caspase-3 [221].

Collectively, these studies indicate that decorin is a component of the circuit that regulates growth of eukaryotic cells. The reported overexpression of decorin in the stroma of a variety of solid tumours [1, 223, 224] could indeed represent a protective mechanism, a sort of soluble tumour repressor, that could counteract *in vivo* tumour cell growth [225]. The recent report of reduced plasma decorin in oesophageal cancer patients suggests that plasma decorin could represent a novel biomarker for cancer recognition and prognosis [226].

Genetic evidence for a role for decorin in carcinogenesis

The human decorin gene is quite complex with two alternatively-spliced exons, potentially two distinct promoters [227–229] and its expression in colon cancer is regulated by methylation of its control regions [1, 230]. The original decorin null mice were generated in a mixed genetic background of 129/Sv and BL/Swiss [192] and did not develop spontaneous tumours. However, if decorin possesses anti-tumorigenic properties, lack of decorin would be permissive for tumorigenicity in a suitable background which is already predisposed to spontaneous tumour formation. To test this hypothesis, double-knockout mice were generated in which the genes encoding decorin and the tumour suppressor p53 were ablated [231]. Double-deficient mice showed a faster rate of tumour emergence, and succumbed very early with a mean survival age of ~4 months *versus* 6 months for the p53 null mice. Moreover, the double-deficient mice presented with very aggressive thymic lymphomas positive for CD4 and CD8 markers [231]. Mice harbouring one decorin allele and no p53 gene developed the same spectrum of tumours as the double knockout mice but had a survival rate similar to that of singly p53 null mice. These results provide strong genetic evidence for a cooperative action of germ-line mutations between decorin, a secreted proteoglycan, and p53 an endogenous controller of cell growth. The absence of decorin is permissive for lymphomagenesis in an animal model predisposed to cancer and suggests that this cooperation may lead to a more aggressive phenotype perhaps by shortening tumour latency [231].

To investigate the potential role of decorin in oncogenesis and to eliminate the potential confounding effects of mixed genetic backgrounds, the original decorin null mice were backcrossed for 14 generations into C57BL/6 mice [232]. Notably, targeted inactivation of decorin was associated with the development of spontaneous intestinal tumours, mostly adenomas, in ~30% of the mice studies. However, when mice were subjected for 36 weeks to a high risk western diet, high in fat and low in calcium and vitamin D, the animals developed malignant colon cancer that in some cases was invasive [232]. Moreover, it was observed that there was a concurrent disruption of intestinal epithelial cell maturation and concurrent up-regulation of β -catenin and down-regulation of p21 in the intestinal tumours [232], as well as the isolated epithelial cells.

Collectively, these two studies provide strong genetic support for a role of decorin in oncogenesis and suggest that pharmacological or dietary enhancement of decorin gene expression in the intestinal tract could be beneficial for prevention of colorectal cancer.

Mechanism of decorin action: suppression of β -catenin and Myc levels

The complex binding repertoire of decorin suggests that this proteoglycan might interact with other RTKs in addition to EGFR and ErbB4 [188]. Using an antibody array detecting 42 Tyrosine phosphorylated RTKs it was recently found that exogenous decorin binds to Met, the HGF receptor, and stimulates a process that leads to receptor down-regulation and shedding, both contributing to attenuation of Met signalling pathway [233]. Of note, human recombinant decorin proteoglycan and protein core induce a protracted suppression of intracellular β -catenin, a known downstream effector of Met, and concurrently inhibit cell growth. A recent study has shown that decorin blocks several biological activities mediated by the Met signalling axis, including cell scatter, evasion and migration. These effects were mediated by a profound down-regulation of non-canonical β -catenin levels [188]. In addition, Myc, a downstream target of β -catenin, was markedly down-regulated by decorin, whereas phosphorylation of Myc at Thr58 was markedly induced [234]. The latter is known to destabilize Myc and target it for proteasomal degradation [235]. We also discovered that systemic delivery of decorin using three distinct tumour xenograft models caused down-regulation of Met and a concurrent suppression of β -catenin and Myc levels [234].

It is well established that there is a positive feedback loop between HGF/Met axis and β -catenin in sustaining the growth of several malignant tumours [236–238]. In addition, HGF induces Wnt-independent nuclear translocation of β -catenin following Met/ β -catenin dissociation in hepatocytes [239]. Under growing conditions, β -catenin and Myc are stabilized by activation of the Wnt signalling and various RTK pathways which are often hyperactive in cancer. Activation of Wnt and RTK pathways leads to phosphorylation of glycogen synthase kinase-3 β (GSK-3 β) which causes an inactivation of this important serine-threonine kinase and a concurrent release of active β -catenin and Myc (Fig. 3A). Both β -catenin and Myc translocate to the nucleus where they activate a large number of genes including Myc itself [240, 241], thereby establishing a positive feedback loop that favours growth and survival (Fig. 3A). Among the genes that are induced by Myc is activating enhancer-binding protein (AP)4, a transcriptional repressor of p21 [242], which also enhances the proliferative potential of transformed cells. Decorin, by neutralizing the activity of RTKs such as EGFR and Met, releases GSK-3 β from being phosphorylated and thus inactivated. This leads to

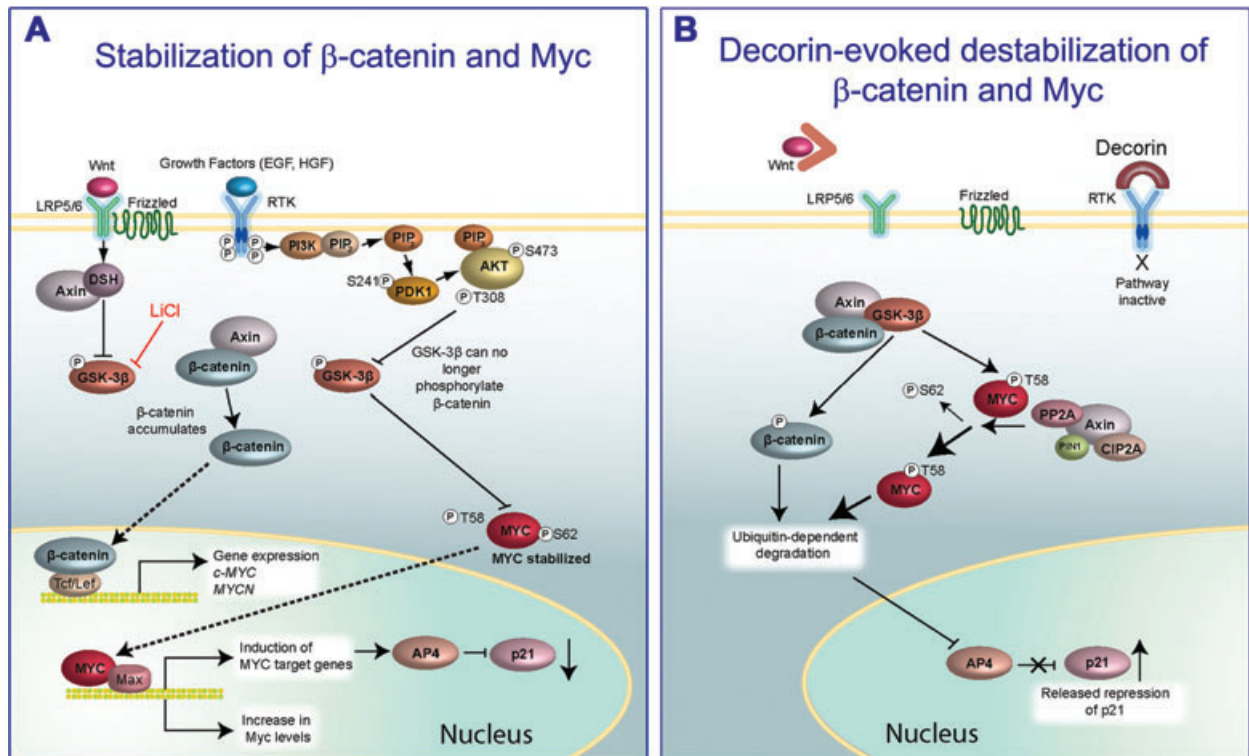


Fig. 3 Schematic representation of decorin's effects on β -catenin and Myc stabilization by directly down-regulating EGFR and Met. **(A)** Activation of both the Wnt and RTK pathways leads to stabilization of β -catenin and Myc proteins and their subsequent translocation into the nuclei where downstream transcription is activated. **(B)** When the Wnt pathway is blocked by absence or block of Wnt factors, or when RTK activity is attenuated by exogenous decorin, there is phosphorylation of β -catenin and Myc at specific residues. Especially important is phosphorylation at Threonine 58 (T58) of Myc, because this modification acts as priming for subsequent ubiquitination and 26S proteasomal degradation. For additional details see the text. Adapted in part from Albihn *et al.*

GSK-3 β evoked phosphorylation of both β -catenin and Myc at specific sites that lead to recognition by ubiquitin ligases and degradation within the 26S proteasome (Fig. 3B). Notably, in the case of Myc, degradation through this pathway is initiated by phosphorylation at Ser62, which functions as a priming site for binding of GSK-3 β which then phosphorylates Thr58, an activity facilitated by axin 1 [243]. Additional steps in this pathway include the removal of the phosphate from Ser62 by protein phosphatase 2A and the subsequent degradation of Myc. Thus, the synthesis of AP4, among other genes, would be stopped and consequently p21 would be released from Myc/AP4 repression. Collectively, the decorin-evoked suppression of RTK activity can provide a plausible mechanistic explanation for the decorin-evoked induction of endogenous p21 levels found in a variety of experimental conditions as discussed above. Overall the marked suppression of β -catenin by decorin appears to occur *via* a non-canonical pathway, *i.e.* insensitive to lithium chloride, an inhibitor of GSK-3 β [233]. The concurrent induction of phosphorylated Thr58 in Myc and its down-regulation at the protein and transcriptional levels [234] indicate that this is also a consequence of decorin-evoked suppression of RTK activity.

Lumican in cancer biology

Lumican is a keratan sulphate SLRP that participates in the acquisition of corneal transparency, hence its eponym, by regulating collagen fibril diameter and interfibrillar spacing [244, 245]. However, various forms of lumican often carrying polyglucosamine chains, variants of undersulphated keratan sulphate, have been reported in various tissues outside of the cornea. Notably, lumican expression is increased in breast cancer, especially in the tumour stroma, and this correlates with higher tumour grade and lower oestrogen receptor levels [246]. More recently, lumican expression has been reported to be altered in various types of human malignancies, including melanomas [247, 248], osteosarcomas [249], pulmonary [250], pancreatic [251, 252] and colorectal carcinomas [253]. However, there are contrasting reports regarding the role of lumican in terms of potential biological function, *i.e.* pro- or anti-oncogenic role, and prognostic indicator. For example, in some studies lumican overexpression correlates with poor prognosis in advanced colorectal [253] and pancreatic [251] cancers. Similarly, in lung adenocarcinomas and squamous cell carcinomas lumican

expression correlates with poor outcome [250]. It is not clear, however, whether the elevated expression of lumican in the tumour stroma would correlate with poor prognosis because of more invasive tumours or because lumican is indeed a biomarker for poor outcome. One possibility is that the poor prognosis might be related to the abundance of tumour stroma which is indicative of more advanced neoplasms. In contrast, lumican might play an opposite role in osteosarcomas where its expression correlates positively with differentiation and negatively with tumour progression [249]. In melanomas, stroma lumican levels decrease markedly with increasing malignancy as measured by vertical growth of the melanomas [247]. Moreover, B16F1 melanoma cells engineered to express lumican showed a marked reduction in the number and sizes of the pulmonary tumour nodules as compared to the wild-type cells [254, 255], suggesting that lumican might indeed be an anti-oncogenic agent. This concept is supported by a mechanistic study which has shown that *de novo* expression of lumican inhibits focus formation in cells transformed with the potent *H-ras* oncogene [256]. Moreover, colony formation in soft agar, a hallmark of malignant transformation, induced by the two viral oncogenes *v-K-ras* and *v-src* is suppressed in cells stably expressing lumican, without affecting cell proliferation [256]. These findings suggest that lumican might be able to selectively inhibit transformation by *v-K-ras* and *v-src* viral oncogenes. In support of these studies, cleavage of lumican by membrane type 1 metalloprotease (MT1-MMP) abrogates lumican-ability to suppress colony formation in soft agar [257]. Analogous to decorin, lumican-mediated cytostatic effects on cancer cells are associated with an induction of the cyclin-dependent kinase (CDK) inhibitor p21^{WAF1} [257]. In murine embryonic fibroblasts induction of endogenous p21^{WAF1} evoked by lumican follows a p53-dependent pathway and leads to suppression of cyclins A, D1 and E [258].

The precise molecular mechanisms of lumican's mode of action, including receptors and signalling pathways, have not been completely delineated. However there are several emerging candidates for receptors and signalling pathways that could mediate lumican's biological activity and most of these pathways are tissue specific. For example, lumican inhibits cell proliferation and aids apoptosis *via* Fas receptor (CD95)-Fas ligand [258], major regulators of programmed cell death. Moreover lumican regulates the induction of inflammatory cytokines and consequently wound healing by interacting with Fas-Fas ligand [259], it regulates neutrophil infiltration in experimental keratitis [260] and is required for neutrophil extravasation following corneal damage and inflammation [261]. Lumican adherent to the cell surface of neutrophils enhances their migration by interacting with β_2 -containing integrins [262]. In contrast, in melanoma cells lumican binds to β_1 -containing integrin receptors [263] and inhibits their migration by disrupting focal adhesion complexes [264]. The active site within the lumican protein core has been mapped to LRR₉ and named *lumcorin* [265]. Several components of focal adhesion complexes, including vinculin and focal adhesion kinase, are modulated by lumican which leads to a disruption of the relationship between actin filaments and β_1 integrin. Indeed, lumican protein core can directly bind the $\alpha_2\beta_1$ domain of the $\alpha_2\beta_1$ integrin receptor on melanoma cells with

relatively high affinity [266]. Thus, the reported anti-adhesive properties of lumican could be mediated by a specific interaction with the $\alpha_2\beta_1$ integrin receptor, thereby providing a plausible explanation for the anti-invasive properties of this SLRP [264]. Finally, a recent study has shown that lumican can block not only melanoma invasion and metastasis, but it also can also induce tumour cell apoptosis and inhibit angiogenesis [255]. Collectively, these *in vivo* and *in vitro* studies suggest that lumican, in analogy to decorin, is an antagonist of cancer growth and possibly angiogenesis. These effects likely involve several receptors including integrins containing β_1 and β_2 subunits and other receptor complexes such as the Fas-Fas ligand complex involved in apoptosis.

Conclusions and perspectives

As a prototype basement membrane and cell-associated HSPG, perlecan, can exert pro- and anti-angiogenic activity. The concept of cryptic fragments of large protein cores with biological activity that differ from those of the parent molecule is now being accepted, insofar as other HSPGs such as collagen XVIII also contain similar bioactivities embedded at their C-terminus. Syndecans and glypicans can mediate an array of functions within tumours and have the ability to promote or inhibit the initiation and progression of cancer dependent on the structure, function and localization of the proteoglycan and on the tumour type and stage. The diversity inherent in these proteoglycans thus provides the potential for multiple layers of regulation of tumour behaviour. Although not discussed in this review, cell surface proteoglycans may represent viable targets for cancer therapy. Based on their functional attributes, therapeutic targets may include specific domains of proteoglycan core proteins and/or heparan sulphate chains. Additionally, the proteoglycan modifying enzymes, particularly the endosulfatases and heparanase, represent attractive therapeutic targets. Reports that heparan sulphate present within the nucleus can regulate gene transcriptional activity [267, 268] has led to speculation that proteoglycans may play direct roles in regulating transcription of genes that control tumour behaviour. This exciting possibility remains to be explored.

The SLRPs have emerged as powerful signalling molecules that control a wide variety of processes and could represent novel therapeutic modalities against cancer [188] as well as being targets themselves [269]. Deciphering the molecular mechanisms through which these stromal-derived proteoglycans affect cell cycle, survival and cancer growth could provide novel clues for a better understanding of how tumour cells modulate their environments.

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Conflicts of interest

The authors state that there are no conflicts of interest.

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