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Decoding the matrix: Instructive roles of proteoglycan receptors

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Abstract

The extracellular matrix is a dynamic repository harboring instructive cues that embody substantial regulatory dominance over many evolutionarily conserved intracellular activities including proliferation, apoptosis, migration, motility, and autophagy. The matrix also coordinates and parses hierarchical information, such as angiogenesis, tumorigenesis, and immunological responses, typically providing the critical determinants driving each outcome. We provide the first comprehensive review focused on proteoglycan receptors, that is, signaling transmembrane proteins that use secreted proteoglycans as ligands, in addition to their natural ligands. The majority of these receptors belong to an exclusive subset of receptor tyrosine kinases and assorted cell surface receptors that specifically bind, transduce, and modulate fundamental cellular processes following interactions with proteoglycans. The class of small leucine-rich proteoglycans is the most studied so far, and constitutes the best understood example of proteoglycan/receptor interactions. Decorin and biglycan evoke autophagy and immunological responses that deter, suppress, or exacerbate pathological conditions such as tumorigenesis, angiogenesis, and chronic inflammatory disease. Basement membrane-associated heparan sulfate proteoglycans - perlecan, agrin and collagen XVIII - represent a unique cohort and provide proteolytically-cleaved bioactive fragments for modulating cellular behavior. The receptors that bind the genuinely multifactorial and multivalent proteoglycans represent a nexus in understanding basic biological pathways and opens new avenues for therapeutic and pharmacological intervention.

Keywords

decorin; biglycan; perlecan; lumican; agrin; collagen XIV; small leucine rich proteoglycans

Introduction

Instructive cues fundamental for all aspects of multicellular life reside within the ubiquitous and evolutionarily conserved extracellular matrix (ECM). These functional signals range from fully embedded solid phase ligands to soluble mediators that function in a paracrine and/or autocrine fashion by engaging in high-affinity interactions with cell surface receptors^{1–6}. Integrating and parsing bidirectional inputs and outputs allow the ECM to reign

as key regulator for maintaining optimal cell and tissue homeostasis^{7,8}. Cells interpret and process this dynamic repository of information through various supramolecular signaling complexes including RTKs, innate immune receptors, and integrins^{6,9}.

The prominent subclasses of matrix constituents responsible for instructive signal transduction include the diverse and multifaceted small leucine-rich proteoglycan (SLRP) gene family and several members of the pericellular heparan sulfate proteoglycans that reside within basement membranes^{8,10}. The original discovery that soluble, monomeric decorin¹¹ is capable of binding EGFR and communicating with the intracellular signaling apparatus via cell surface signaling receptors¹² pioneered the paradigm-changing concept that matrix components embody a critical regulatory network^{5,13–17}. Following this initial discovery, decorin emerged as the leading candidate for a matrix-derived repressor of tumorigenic growth with inherent angiostatic and pro-autophagic activities^{18–20} that wholly manifests from RTK partial agonism. The repertoire of functions ascribed to decorin has since expanded exponentially and now participates in a plethora of diverse processes such as fibrillogenesis and wound healing^{21–23}, keratinocyte function²⁴, allergen-induced asthma²⁵, delayed hypersensitivity²⁶, diabetic nephropathies and renal diseases^{27,28}, skeletal muscle homeostasis²⁹, nurturing hematopoietic stem/progenitor cell niches³⁰, and ensuring proper convergent extension³¹.

Biglycan, a member of the class I SLRP family with highest homology to decorin, has also been involved in receptor engagement and coordination of intracellular signaling pathways. For example, both circulating biglycan and decorin have been implicated in regulating innate inflammatory responses downstream of TLR2/4 (see below)^{5,9,32–34}. Biomechanically, decorin and biglycan also share overlapping functions in the mechanobiology of tendon structure^{35–37}, and have distinct roles in fetal membrane signaling³⁸. Further, the control of cellular phenotype via ligation of distinct signaling receptors has also been attributed to molecules associated with basement membranes such as perlecan, agrin, and collagen XVIII³⁹. Moreover, some of the basement membrane HSPGs (perlecan, collagen XVIII) are proteolytically processed to liberate a soluble, bioactive fragment capable of engaging cognate receptors^{40,41}.

Although proteoglycans have been previously shown to play a role as endocytic receptors^{42–44}, in this Current Topics, we will evaluate a unique class of signaling receptors that engage and transduce proteoglycan-derived cues. These activities can profoundly impact and reprogram the central processing and integration networks responsible for cell behavior, phenotype, and the development of various pathologies. Therefore, we will critically evaluate the pathways downstream of proteoglycan receptor engagement relevant for tumorigenesis, angiogenesis, autophagy, and immunomodulation.

DECORIN ENGAGES A MULTITUDE OF RTKS FOR PROTRACTED TUMORIGENIC SUPPRESSION

Decorin has emerged as a soluble pan-RTK inhibitor. However, decorin was initially discovered and characterized as a putative collagen binding factor active in competent collagen synthesis, assembly, deposition and fibrillogenesis^{37,45–48}. Decorin also binds

multiple matrix components necessary for structural integrity¹⁴ and sequesters pleiotropic growth factors, with a predominant tendency to inactivate several TGF- β family members^{14,18}, which consequently suppress downstream TGF- β signaling in an indirect fashion^{14,18}. One receptor-dependent mechanism of TGF- β regulation involves decorin-mediated engagement of the endocytic receptor, lipoprotein receptor-related protein 1 (LRP-1)⁴⁹ resulting in PI3K activation and TGF- β modulation via trimeric Smad signaling. Famously and primarily, decorin is a soluble tumor repressor that neutralizes tumorigenic growth and unchecked neovascularization, vis-à-vis RTK-mediated pro-autophagic signaling pathways¹⁹. For this reason, decorin has been appropriately designated as “a guardian from the matrix”¹⁴. Importantly, the well-established anti-tumorigenic and anti-angiogenic properties borne from the interaction of decorin and receptor (as discussed below) are independent of the covalently attached chondroitin/dermatan sulfate glycosaminoglycan chain^{50–52}. Therefore, this review will focus exclusively on signaling events and cellular responses as mediated by the respective proteoglycan core protein.

EGFR AND MET: IT STARTED WITH A TALE OF TWO RECEPTORS

The epidermal growth factor receptor (EGFR) was the original RTK discovered that binds decorin with high affinity¹² (Figure 1). Following stimulation of A431 cells, decorin promotes EGFR receptor dimerization, rapid trans-autophosphorylation of the unstructured intracellular tails, increased cytosolic calcium levels⁵³, and evokes EGFR internalization in caveolin-1 positive endosomes¹⁴. Presumably, following receptor clearance from the tumor cell surface, the ternary complex of decorin/EGFR/caveolin-1 traffics and ultimately fuses with the lysosomal compartment for receptor complex degradation concomitant with a cessation of EGFR signaling⁵⁴. Intriguingly, the ligand-binding site of decorin on EGFR partially overlaps with that of EGF, as decorin significantly competes off bound EGF⁵⁵. Despite this narrow binding cleft shared by EGF and decorin, biologically distinct phenotypes occur insofar as receptor stability, signal intensity, and signal duration. Indeed, EGF sustains the maximal phosphorylation state and signaling capacity of post-internalized EGF:EGFR complexes and permits additional waves of signaling (e.g. bound MAPK, PI3K, PLC- γ 1 components) followed by sorting into an endosomal recycling pathway that repopulates the cell surface with active EGFR⁵⁶. In contrast, after evoking a brief burst of EGFR phosphorylation, declining EGFR cell surface levels (>50% of total), and transient activation of MAPK (ERK1/2), decorin exerts a protracted attenuation of downstream signaling effectors that paradoxically results in cell cycle arrest (induction of the cyclin-dependent kinase inhibitor, p21^{WAF1}) and caspase-3 mediated apoptosis (Figure 1)^{4,57}. Interestingly, depending on the phosphorylation signature decorin evokes, phosphorylated EGFR is required for association with caveolin-1 coated pits⁵⁸. Unsurprisingly, EGFR is not the only Erb family member by which decorin serves as a direct soluble repressor as ErbB4 is targeted by decorin during scar tissue repair in the central nervous system⁵⁹.

A recurring hallmark of decorin binding served as the utilitarian means for the identification of Met (known as hepatocyte growth factor or scatter factor receptor) as the primary RTK by which decorin transduces biological information^{7,60} (Figure 1). In analogy with EGFR, decorin evoked a rapid, but transient burst at a phosphotyrosine residue detectable via a targeted phosphotyrosine RTK array platform⁶⁰. Moreover, Met was characterized as the

central RTK for decorin mediated anti-tumorigenicity and angiostatic properties^{50,52,60} vis-à-vis higher affinity binding for Met⁶⁰ as well as via biological, pharmacological, and genetic methodologies^{52,60}. Binary decorin/Met complexes avidly colocalize with caveolin-1 positive endosomes (for proficient lysosomal degradation) whereas pro-tumorigenic HGF/Met complexes associate with clathrin for receptor recycling and additional cycles of signaling⁵⁰. Consequentially, two potent effector oncogenes downstream of Met signaling, β -catenin and Myc, are targeted for protracted degradation via the 26S proteasome^{14,50,61}. Mechanistically, degradation of Myc - following phosphorylation at Thr58, which is situated within an established degron - permits *CDKN1A* derepression via loss of the AP4 transcriptional repressor⁵⁰. Moreover, decorin utilizes Met as the nexus for angiogenic suppression⁵² in HeLa and MDA-MB-231 cells (Figure 1). Signaling positively through Met, decorin non-canonically suppresses *HIF1* expression via transcriptional repression. Compromising HIF-1 α expression subsequently decreases VEGFA and MMP2/9 with a concurrent increase of puissant anti-angiogenic effectors such as thrombospondin-1 (TSP-1) (see below) and TIMP-3^{14,18,52}. Collectively, decorin subverts the pro-angiogenic signaling network via prolonged attenuation of Met in neoplastic cells.

Similar binding mechanics notwithstanding, decorin may integrate signaling among multiple RTKs expressed by a single cell via the empirically determined binding constants of each receptor, prevailing density of the receptor, and the uniform inclusion of specific structural motifs (e.g. the IgG domain). Moreover, decorin may functionally titrate total receptor levels (via degradation) and thereby deprive signaling clusters of key receptors necessary for competent signal transduction for the development and progression of cancer. This biological process has already been observed via the physical sequestration of EGFR away from EGFR/ErbB1:Her2/Neu/ErbB2 heterodimers^{7,14}. A pertinent example of differential signal integration is the rapid release of TSP-1 from basal breast carcinoma cells⁶² in a RhoA/ROCK1 dependent manner. Although MDA-MB-231 cells constitutively express EGFR and Met, pharmacological inhibition and RNAi-mediated silencing of Met did not perturb decorin-induced TSP-1 secretion whereas blocking EGFR completely abrogated this effect⁶² (Figure 1). Functionally, differences may reside in the phosphorylation signatures of the flexible intracellular tails flanking the kinase domain or in the geometrically-constrained structural conformations the receptors adopt following decorin binding.

Recently, a novel mechanism has emerged wherein decorin, acting as a partial Met agonist, induces tumor cell mitophagy (mitochondrial autophagy) as the molecular basis for the observed angiostatic effects in basal breast carcinoma^{18,19,63}. Mitophagic induction may be a general phenomenon as it also occurs in prostate carcinomas transduced with decorin-expressing adenovirus⁶¹. Induction of tumor cell mitophagy is entirely dependent on the complex interaction between PGC-1 α and mitostatin^{19,63}. Loss of mitostatin, a putative tumor suppressor gene^{64,65} via RNAi-silencing prevents mitophagy and significantly compromises VEGFA suppression following decorin administration⁶³ (Figure 1). Thus, decorin can negatively regulate two RTKs, EGFR and Met, potent drivers of cancer and angiogenesis and this influence could be due to an endogenous, stromal-derived force to restrain cancer growth and infiltration.

INSULIN-LIKE GROWTH FACTOR RECEPTOR 1 (IGF-IR): THE DECORIN DUALITY

The role of environmental and context-specific signaling is further illustrated with the interaction between decorin and IGF-IR⁶⁶. An intriguing duality between normal, genomically-stable cells (e.g. endothelial cells)⁶⁷ and tumor cells (e.g. bladder carcinoma)⁶⁸ emerges following decorin engagement of IGF-IR (Figure 1). In endothelial cells, decorin triggers levels of IGF-IR phosphorylation comparable to that evoked by IGF-I coincident with downstream Akt signaling via the N-terminus of decorin^{66,68}. Further, decorin is capable of stimulating endothelial cell adhesion and migration of endothelial cells over fibrillar collagen networks in an IGF-IR/ α 2 β 1 integrin dependent manner upstream of Rac activation⁶⁹. Decorin also regulates renal fibrosis by directly engaging IGF-IR present on renal fibroblasts and indirectly by inhibiting the biological activity of CTGF (CCN2)^{70,71}. Moreover, decorin promotes the PI3K/Akt/mTOR pathway in renal cells, which indirectly promotes fibrillin-1 translation, thereby curbing TGF- β bioavailability⁷²⁻⁷⁴. Substantially antithetic to the aforementioned role of decorin as a pan-RTK inhibitor, decorin can be a full IGF-IR agonist, analogous to IGF-I, in genomically-stable cells. In this case, soluble decorin exerts positive IGF-IR phosphorylation consistent with improved receptor stabilization and robust downstream effector activity under physiologically-relevant conditions⁶⁶. In contrast, the net output of IGF-I/IGF-IR signaling in a neoplastic setting promotes urothelial tumor cell motility via Akt/MAPK-dependent paxillin activation⁷⁵, where decorin can bind IGF-I and associated IR-A ligands⁷⁶ and the IGF-IR in a region that does not overlap with the IGF-I-binding domain⁶⁸. Orthodox paradigms regarding the interaction of decorin with cognate receptors no longer apply when discussing the effect of decorin/IGF-IR complexes in a tumorigenic setting^{66,68}. Decorin does not compromise or enhance the IGF-IR phosphorylation state upon binding but allosterically competes and suppresses IGF-I-mediated activation of IGF-IR and downstream Akt/MAPK pathways⁶⁸. Moreover, prolonged stimulation by decorin neither perturbs receptor stability nor does trigger internalization of the receptor complex with caveolin-1, unlike EGFR and Met, but does trigger degradation of IRS-1⁶⁸. This decorin/IGF-IR interaction is the only known instance whereby decorin does not cause RTK internalization and association with caveosomes. Decorin-evoked negative regulation of IGF-IR culminates in decreased IRS-1 stability that will ultimately prove insufficient for sustainable Akt/MAPK (and paxillin) activity; thereby abrogating IGF-I induced tumor cell motility (Figure 1). It was recently discovered that IGF-I requires a novel kinase involved in urothelial cell motility known as Pyk2⁷⁷. It remains unknown whether Pyk2 is downstream of IGF-IR/IRS-1/Akt/MAPK signaling and whether decorin inactivates this kinase for motility termination. Thus, decorin can exert opposite effects on the IGF-IR system and these effects are dependent on the cell context.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (VEGFR2): A NEW ERA FOR SLRPS

A unique high-resolution transcriptomic platform capable of differentiating species-specific transcripts from engrafted *H. sapiens* orthotopic tumors (MDA-MB-231) from the recipient *M. musculus* microenvironment has changed our understanding of decorin (and thus SLRP)

biology⁵¹. These analyses revealed an exclusive subset of genes that are differentially regulated only within the tumor stroma. Among these genes, *Peg3* emerged as a highly favored candidate⁵¹. Utilizing endothelial cells (MDEC and HUVEC) as a proxy for the mouse tumor stroma, we found that *Peg3* was intimately involved in orchestrating decorin-evoked endothelial cell autophagy^{18–20,78} under nutrient enriched conditions. Further analysis revealed a strict dependence on the primary endothelial cell RTK, VEGFR2, as the mechanism for decorin-mediated autophagy and angiostasis⁷⁸ (Figure 1). In a similar mechanism, decorin bioactivity requires competent RTK signaling as inhibition of VEGFR2 with the small molecule inhibitor SU11274 or genetic depletion of the receptor, abrogates *Peg3* induction and subsequently prevents an increase in Beclin 1 and LC3⁷⁸. Therefore, decorin acts as a partial VEGFR2 agonist, whose predicted ligand-binding domain partially overlaps with that of VEGFA, the natural ligand of VEGFR2^{20,78}. Proficient autophagic stimulation relies on decorin/VEGFR2 interactions and subsequent downstream AMPK α activation (at Thr172)^{19,79,80}. Critically, AMPK α serves as the chief energy sensing kinase responsible for autophagic initiation by inhibiting the anti-autophagic mTOR pathway⁸¹. This represents the first report that AMPK α can be activated by an upstream RTK and that it is stimulated in a manner commensurate with canonical autophagic stimuli (e.g. amino acid withdrawal and nutrient deprivation, rapamycin, or Torin-1)⁷⁹. Moreover, the magnitude of autophagic induction attained with decorin is comparable to traditional methods of inducing autophagy. Therefore, decorin can be considered a soluble pro-autophagic effector that requires and binds two distinct pro-autophagic receptors, i.e., Met for tumor cell mitophagy and VEGFR2 for endothelial cell autophagy. In both instances, their tyrosine kinase activity is necessary for downstream biological function.

PLATELET DERIVED GROWTH FACTOR RECEPTOR (PDGFR): DECORIN IS A GENUINE PAN-RTK INHIBITOR

A prime example of the pervasive and widespread effect of decorin mediated RTK antagonism lies with the identification of platelet derived growth factor receptor α/β (PDGFR α/β) as a key target for combatting tumorigenesis⁸². Utilization of two different chemically-induced models of hepatocellular carcinoma (HCC) in either a wild-type or *Dcn*^{-/-} background⁸², has identified several RTK targets. The screen revealed that when decorin is globally deleted, many RTKs become hyperactive, i.e., an increase in the phospho-Tyr signal, even under basal conditions⁸². From this screen, PDGFR α/β has emerged as a high-affinity interacting partner for decorin and proved a critical avenue by which decorin suppresses HCC development and progression^{82,83} (Figure 1). Thus, a genetic background lacking an important signaling SLRP causes a constitutive activation of several RTKs, a function not attributable to the collagen-binding role of decorin during development and tissue homeostasis⁸⁴. The constitutive activation of RTKs in the absence of decorin, thus, provides a mechanistic explanation for a permissive role of decorin in tumorigenesis as shown in other genetic cancer models^{85–87}.

INCITING INFLAMMATION

A second, closely-related class I SLRP, biglycan, shares greater than 65% homology with decorin in both human and mouse genes^{6,8}. Biglycan contains two covalently-attached glycosaminoglycan chains, hence earning the eponym of biglycan⁶. Functionally, biglycan sequesters and modulates the activity of several TGF- β superfamily members including TGF β /Smad2^{88,89}, and BMP-4⁹⁰. In addition, biglycan modulates the Wnt/ β -catenin signaling axis⁹¹ (see below). Biglycan also binds and potentiates VEGFA signaling during fracture healing under normal physiological conditions⁹². Indeed, decorin may also be pro-angiogenic in certain physiological settings¹⁴. Non-overlapping functions do exist between biglycan and decorin insofar as biglycan is a critical regulator of skeletal bone growth^{6,16,93} and cardiac remodeling following myocardial infarction⁹⁴. However, biglycan and decorin have functional commonality in the realm of regulating innate immunological responses.

BIGLYCAN LINKS SOLUBLE MATRIX WITH INNATE IMMUNE RESPONSES VIA TLR2/4 AND P2X SIGNALING

A groundbreaking discovery has been leading a revolution in further understanding biglycan biology⁶. Biglycan acts as an endogenous agonist for the innate immune receptors, Toll-like receptor 2 and 4 (TLR2/4) expressed on the surface of macrophages^{95–97} (Figure 2), and can aggravate ischemic acute renal injury²⁸. As decorin is a soluble tumor repressor, biglycan has been identified as a soluble signaling molecule, a so-called “danger signal”^{6,98,99} that interfaces with the innate immune system^{33,74} following sepsis or ischemic injury. *De novo* synthesized and secreted by circulating macrophages^{34,97}, biglycan engages TLR2/4. This initiates a pro-inflammatory cascade that converges on NF- κ B and evokes the synthesis and development of mature IL-1 β ⁹⁶, and release of TNF- α and IL-6. Mechanistically, biglycan promotes receptor clustering and cooperativity of TLR2/4 with the purinergic P2X₇/P2X₄ receptors (Figure 2)^{5,9,96}. This affords rapid generation of reactive oxygen species that is directly involved in activating the NLRP3/ASC inflammasome⁹⁶. Biglycan-mediated activation of the NLRP3/ASC inflammasome induces caspase-1-dependent cleavage of pro-IL-1 β into mature IL-1 β with subsequent secretion. Two feed-forward loops become established whereby biglycan promotes expression of *NLRP3* and *IL1B* transcripts⁹⁶ and, in turn, IL-1 β and IL-6 can promote *BGN* expression and synthesis. Moreover, biglycan mediated signaling via the MyD88/TRIF³⁴ arm downstream of TLR2/4 in kidneys result in CCL2 and CCL5 synthesis for the recruitment of macrophages and T-lymphocytes, respectively^{34,99,100}. The CXCL class chemokines (e.g. CXCL1, CXCL2 and CXCL13) are subsequently released for macrophage^{34,95} and B-lymphocyte conscription in murine lupus nephritis¹⁰¹. Biglycan, as a newly-discovered DAMP, fundamentally connects soluble mediators derived from the matrix with regulating and inducing robust innate immune responses.

These observations have been elegantly confirmed *in vivo*^{6,8,9}. Biglycan-deficient mice respond significantly less vigorously (e.g. decreased levels of active caspase-1 and lower titers of mature IL-1 β) when challenged with inflammatory renal injury or lipopolysaccharide⁹⁶. Physiologically, less IL-1 β is found in the circulation, kidneys, and

lungs⁹⁶. Moreover, in a mouse model of ischemic acute kidney injury where biglycan was over-expressed, appreciably increased plasma and renal levels of TNF- α , CXCL1, CCL-2, and CCL-5 were found concomitant with increased frequency of infiltrating macrophages, neutrophils, and T-lymphocytes⁹⁷. Overall, this resulted in considerably worse renal function and paints biglycan as a key mediator of inflammatory renal disease²⁸. Conversely, the impairment of renal function is markedly ameliorated in the compound knockout mouse lacking TLR2/4 indicating a crucial pathological role for the biglycan/TLR2/4 interaction *in vivo* (Figure 2)⁹⁷.

Biglycan also promotes a pro-inflammatory milieu within the lungs⁷⁴. Under septic conditions, biglycan levels are substantially increased within cells infiltrating the pulmonary parenchyma⁹⁵. In parallel with renal injury, biglycan deficiency dampens the immune cell population from breaching the lung tissue and results in less pulmonary damage⁹⁵. Functionally, diminutive amounts of active caspase-1 and lower amounts of mature IL-1 β were found within the lungs⁹⁶.

LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 6 (LRP6) IS A TUMOR RECEPTOR FOR BIGLYCAN

Not unforeseeable, and consistent with the abovementioned biological interactions (such as modulating Wnt signaling), biglycan is poised as a potent regulator of tumorigenesis and angiogenesis. Several solid malignancies are known to over-express biglycan which could contribute to tumor motility and even drug resistance¹⁰². The sources of biglycan in cancer currently remain elusive. However, it is known that TGF- β can induce biglycan from stromal fibroblasts¹⁰². Moreover, infiltrating immune cells (macrophages, neutrophils) constitutively secrete IL-1 β and IL-6 under malignant conditions, and this will mechanistically support the feed-forward loop via TLR2/4 signaling.

Biglycan represents a functional dyad for the documented tumorigenic effects. Anti-proliferative effects of biglycan stem from a study in which cells transformed with the Her2/Neu oncogene secrete decreased levels of biglycan in a PKC/CREB-dependent manner¹⁰³. RNAi-mediated silencing of biglycan in the Her2/Neu transformed fibroblasts augments growth rate and migration¹⁰³, suggesting that biglycan can stymie the malignant phenotype conveyed by the Her2/Neu oncogene. However, biglycan can also compromise tissue architecture and integrity by promoting tumorigenesis via enhanced Wnt/ β -catenin signaling⁹¹. Biglycan participates in a tripartite complex involving Wnt3a and the LRP6 (low-density lipoprotein receptor-related protein 6) co-receptor⁹¹ (Figure 2). Biglycan enhances LRP6 phosphorylation as cells deficient in biglycan have reduced cell surface retention of Wnt3a and decreased LRP6 phosphorylation⁹¹. Collectively, this trimeric complex is sufficient for the canonical induction of β -catenin/TCF target genes and also transactivates the RUNX2 transcriptional complex in osteoprogenitor cells⁹¹. Finally, biglycan may also promote angiogenesis via enhanced VEGFA signal potentiation⁹² and/or via the formation of reactive oxygen species downstream from TLR2/4-dependent signaling as a consequence of the NLRP3/ASC activation pathway (Figure 2).

DECORIN FOSTERS A PRO-INFLAMMATORY SIGNATURE VIA TLR2/4

Several lines of evidence have converged on the concept that decorin may also be involved in regulating immunological responses in a manner congruent with biglycan^{5,104}. Decorin suppresses TGF- β , thereby repressing the macrophage phenotype via p27^{Kip1} and p21^{WAF1} induction¹⁰⁵, promotes synthesis of the potent chemoattractant MCP-1¹⁰⁶, and potentiates IFN- γ for allergen-induced inflammation¹⁰⁷. In a mechanistic equivalence with biglycan function, LPS-induced sepsis greatly induces decorin expression in the plasma as well as in the perivascular regions and in bronchial epithelial cells³², whereas decorin deficiency attenuates the pro-inflammatory state induced by LPS³².

In a manner analogous to its relative, decorin binds TLR2/4 with high affinity on the surface of macrophages (Figure 2). Receptor binding initiates a pro-inflammatory cascade transduced by the MAPKs, ERK1/2 and the SAPK, and by p38 for the synthesis and secretion of TNF- α and IL-12p70³². Decorin also induces PDCD4 (programmed cell death 4), a translational repressor that post-transcriptionally suppresses the anti-inflammatory modulator, IL-10³². During active TGF- β signaling, microRNA-21 (miR-21) is in abundance and decreases levels of PDCD4, thereby permitting high levels of IL-10. Therefore, during LPS-induced sepsis, decorin levels increase and signal via TLR2/4 for TNF- α and IL-12p70 production, while concurrently blocking TGF- β from accessing the TGF β R. The sequestration of TGF- β by decorin circumvents miR-21-mediated repression of PDCD4, thereby enabling PDCD4 to translationally repress IL-10³². The total outcome of this intricate regulatory system advocates for the differential synthesis of pro-inflammatory immunomodulators while concurrently suppressing anti-inflammatory molecules and licenses decorin as a proinflammatory proteoglycan (Figure 2). This mechanism also operates within the tumor microenvironment and creates an inflammatory milieu that combats tumorigenic growth of established tumors via the differential regulation of PDCD4 and miR-21 and the secretion of TNF- α and IL-12p70³². Over-expressing decorin via adenovirus *in vivo* has provided robust evidence concerning the link among increased decorin abundance, the release of pro-inflammatory regulators, and the significant decrease in tumorigenicity³².

Collectively, these studies reveal entirely new roles for the circulating, soluble forms of biglycan and decorin in regulating and evoking protracted innate immune responses. Therefore, the importance of matrix-derived factors that would otherwise not be considered in the realm of immunology is furthered underscored by the range of homeostatic, physiologically-relevant, and pathobiological processes these dynamic proteoglycans often contribute, coordinate, and stop.

BASEMENT MEMBRANE HEPARAN SULFATE PROTEOGLYCANS INTEGRATE SIGNALING OVER MULTIPLE RECEPTORS

The three best characterized basement membrane HSPGs (perlecan, agrin, and collagen XVIII) are functionally and structurally diverse participants that contribute nanostructural architecture for tissue stability, homeostasis, and tether the pericellular matrix to the cell surfaces^{6,40}. Primarily harboring heparan sulfate (HS) glycosaminoglycan chains, these

HSPGs serve as sinks for multiple cytokines and growth factors⁶. Further, these elongated and multimodal molecules can act as co-receptors via HS-mediated presentation of growth factors in the proper orientation within three-dimensional space for optimal interactions with the cognate signaling receptor, e.g. FGF2/FGFR2¹⁰⁸. Despite maintaining structural integrity as a network in basement membranes and presenting growth factors, perlecan, agrin, and collagen XVIII also signal independently of bound cytokines^{2,109}. Indeed, each member can bind distinct and multiple cell surface receptors in a variety of tissues and microenvironments (Figure 3). Moreover, aberrant expression contributes significantly during pathobiological processes.

PERLECAN RECEPTORS: A DUAL RECEPTOR ANTAGONISM

Perlecan is a gigantic (470 kDa protein core), multimodular HSPG, comprising five distinct protein modules, that exemplifies angiogenic bivalency by concealing pro- and anti-angiogenic properties within the same molecule^{2,110,111}. Perlecan contributes positively by binding HS-interacting angiokines such as FGF2/7/18¹¹², VEGFA, PDGF, and progranulin¹¹³, and operates at multiple levels throughout developmental angiogenesis by modulating the VEGFA/VEGFR2 axis and $\alpha 2\beta 1$ integrin function^{114,115}. Perlecan can also exert biomechanical anti-thrombotic properties^{116–119}. Perlecan-null mice embryos are embryonic lethal and exhibit pericardial hemorrhage and deficits of the major cardiac vessels^{120,121}. Seemingly, morpholino-mediated knockdown of perlecan in zebrafish shows lack of angiogenesis and skeletal muscle defects¹²². In solid tumors, perlecan also contributes positively as a pro-angiogenic store of potent angiokines for rampant neovascularization via potentiated FGF2 and VEGFA signaling resulting in unchecked tumorigenic growth and spreading².

As a genuine diametric opposite, the most C-terminal domain of perlecan, known as endorepellin, confers potent inhibitory properties upon endothelial cells by specifically repelling (hence the eponym) migration and blunting capillary morphogenesis¹²³ (Figure 3). Exposure of recombinant endorepellin to stationary endothelial cells, both micro- and macro-vascular, disrupts the actin cytoskeleton and decreases β -actin levels downstream of the $\alpha 2\beta 1$ integrin^{124,125} thereby providing a mechanism for its ability to block endothelial cell migration. Structurally, endorepellin recapitulates the modular architecture of the parent molecule by containing three laminin-like globular domains (LG1, LG2, LG3) each interspersed by tandem EGF-like motifs (Figure 3). Proteolytic cleavage of endorepellin from the parent perlecan molecule occurs following release of active cathepsin L from dying endothelial cells N-terminal to LG1^{19,126}. However, in mast cells, evidence exists for a co-transcriptional mechanism involving alternative splicing of the primary perlecan mRNA transcript that ultimately yields functional endorepellin for regulating angiogenesis and wound healing¹²⁷.

Upon liberation, soluble endorepellin engages in a novel form of suppression known as “dual receptor antagonism” and functions as a molecular bridge by ligating the $\alpha 2\beta 1$ integrin and VEGFR2¹²⁸ for angiostasis. Simultaneous interaction of both receptors conveys high sensitivity, cell type specificity, and wholly nullifies angiogenic responses *in vitro*¹²⁸ and *in vivo* by specific targeting of the tumor vasculature via the $\alpha 2\beta 1$ integrin^{129,130}, the only cell

type that expresses both receptors. Moreover, the BMP-1/Tolloid protease can release the bioactive fragment, LG3¹³¹, whose three-dimensional crystal structure has been solved¹³². The concept of “dual receptor antagonism” is also exhibited by other proteolytically processed matrix components that yield soluble effectors (e.g. endostatin from collagen XVIII, see below) for transducing and activating signaling programs fundamental for cellular behavior.

Physically, endorepellin interacts with VEGFR2 (between IgG domains 3–5 with an empirically derived K_d of 2.8 nM and in a region that overlaps with VEGFA binding) via the N-terminal LG1/2 domains while recruiting and binding the $\alpha2\beta1$ integrin with the C-terminal LG3 module via the $\alpha2\beta1$ domain¹³³. Mechanistically, this tethering brings the SHP-1 phosphatase, which is putatively bound to the cytoplasmic tail of the $\alpha2$ integrin subunit, into functional proximity with the intracellular tails of VEGFR2 and executes rapid VEGFR2 dephosphorylation, inactivation, and ternary complex internalization^{128,134,135} consisting of endorepellin, VEGFR2 and the $\alpha2\beta1$ integrin. Importantly, SHP-1 catalyzes dephosphorylation of Tyr1175 of VEGFR2¹³⁶, a critical docking site for Shb and PLC- γ (see below)¹³⁵. Biologically, dual receptor antagonism permits actin dissolution via the $\alpha2\beta1$ integrin, and angiogenic suppression via the down-regulation of VEGFR2¹¹⁰. Downstream of simultaneous VEGFR2/ $\alpha2\beta1$ binding and attenuation, multiple pro-angiogenic signaling pathways chiefly emanating from VEGFR2 are significantly compromised¹³⁵. As discussed, endorepellin promotes active dephosphorylation, via SHP-1, of Tyr1175 of VEGFR2 thereby disrupting the recruitment, coupling, and activation of the angiogenic PI3K/Akt signaling axis. Impaired Akt (Ser473) signaling downstream of VEGFR2 manifests as blunted activation of PDK (Ser241), eNOS (Ser1177), and mTOR (Ser2448) despite the overabundance of exogenous VEGFA, suggesting that endorepellin competently and allosterically abrogates pro-VEGFA signaling¹³⁵. The attenuation of mTOR signaling negatively impacted HIF-1 α expression (a major downstream effector of mTOR) and activity in an oxygen-independent manner and, unbeknownst at the time, would be involved in a much more sublime manner (see below). Endorepellin also prevents PLC- γ /VEGFR2 docking (via diminished P-Tyr1175), resulting in the protracted attenuation of the PKC/JNK/AP1 signaling arm with concomitant attenuation of calcineurin activity and subsequent cytosolic retention of NFAT¹³⁵. This dual receptor antagonism suppresses key pathways for VEGFA expression and secretion.

Recently, a novel finding has expanded our understanding of the angiostatic function of endorepellin¹⁹. Endorepellin evokes endothelial cell autophagy in a VEGFR2- and Peg3-dependent manner under nutrient-enriched conditions¹³⁷ characterized by dually positive (e.g. Beclin 1/LC3) autophagosomes and the dynamic regulation of p62/SQSTM1. Perhaps the previous discovery of mTOR suppression (mTOR is staunchly anti-autophagic¹³⁸) was prescient for endorepellin-evoked autophagy. Partial agonism is a recurring theme with soluble matrix constituents as endorepellin requires VEGFR2 signaling in much the same manner that decorin requires VEGFR2 kinase activity for Peg3-dependent autophagy¹³⁷. Intriguingly, truncated endorepellin consisting of only LG1/2 was sufficient for Peg3, Beclin 1, and LC3 induction and autophagosome formation that subsequently incorporates these pro-autophagic mediators^{19,137}. Endorepellin solely requires an interaction with VEGFR2 for endothelial cell autophagy; the LG3/ $\alpha2\beta1$ interaction appears dispensable and perhaps

even inhibitive¹³⁷ (Figure 3). Functionally, distinct signaling pathways are either activated or attenuated following dual receptor antagonism and thereby holds physiological relevance. A molecular dissection of endorepellin revealed a profound dependence on LG1/2 for autophagic induction and angiogenic suppression; however, inhibiting cell motility relies heavily on the $\alpha2\beta1$ -mediated arm. Therefore, we postulate that endorepellin-mediated prolonged autophagic induction may underlie its ability to suppress angiogenesis.

Clinically, endorepellin is released under physiological conditions¹³⁹, whereas LG3 is a candidate serological biomarker that has been amply identified under many pathological conditions, including breast cancer and allograft rejection^{140–145}. Moreover, LG3 possesses intrinsic angiostatic abilities via calcium regulation¹³¹ through the $\alpha2\beta1$ integrin. Additional binding partners of endorepellin include the α/β -dystroglycan complex whereby perlecan¹⁴⁶ and agrin¹⁴⁷ (see below) are interacting partners¹⁴⁸ (Figure 3). Specifically, the proximal LG1/2 modules of endorepellin contain high-affinity binding sites for α/β -dystroglycan binding in maintaining skeletal muscle integrity² and may have a role in the development of ameloblastoma¹⁴⁹. Disrupting perlecan: α/β -dystroglycan interactions compromises basement membrane stability¹⁵⁰. Recently, it was discovered that perlecan is recruited to the nodes of Ranvier and participates in rapid neural conduction¹⁵¹. Dystroglycan selectively recruits perlecan as a novel component of the nodal matrix and is involved in nodogenesis via gliomedin clustering¹⁵¹ (Figure 3). Overall, these findings propose a novel role for protein core fragments of basement membrane HSPGs in concurrently affecting cell adhesion and pro-angiogenic signaling receptors. The convergence of signaling towards a pro-autophagic pathway could exacerbate the growth of vascular cells and thus contributes to the angiostatic properties of endorepellin and perhaps other bioactive protein modules such as endostatin (see below).

AGRIN: A SYNAPTIC PROTEOGLYCAN THAT ENGAGES A DIVERSE ARRAY OF RECEPTORS

Structurally, agrin appears similar to the aforementioned proteoglycan, perlecan. Agrin is a multimodular HSPG with up to three HS that are apparently dispensable for function¹⁵². Agrin protein core has an additional complexity conveyed by alternative splicing of the *AGRN* mRNA¹⁵³. Substitution of the N-terminal domain of agrin via an alternatively-spliced mRNA converts agrin into a type-II transmembrane proteoglycan¹⁵⁴ and can modulate Fyn and MAPK signaling pathways¹⁵⁵. Chiefly involved in forming and maintaining homeostasis of neural and neuromuscular synapses¹⁵⁶, agrin can also modulate neurite and motor neuron outgrowth via FGF2 binding^{157,158}, and retinal development¹⁵⁹. In combination with perlecan, agrin promotes oral squamous cell carcinoma¹⁶⁰, and also accumulates in HCC¹⁶¹ with dynamic expression in cholangiocarcinoma¹⁶².

Agrin can be proteolytically cleaved by MMPs¹⁶³ and by the serine protease neurotrypsin immediately upstream of the SEA domain and distally between LG2 and LG3^{164,165} (Figure 3). Processing generates 110-, 90-, and 22-kDa fragments of agrin². Intriguingly, the processed fragments flanked by the neurotrypsin sites bear striking resemblance to the C-terminal perlecan fragment, endorepellin (Figure 3, see above section), which also harbor

the majority of the interaction sites (similar to perlecan and collagen XVIII, see below). Intact or proteolytically-processed agrin interacts with several receptors with the caveat that specific splice variants of agrin bind specific cell surface receptors^{2,166,167}. Agrin is an avid binding partner of the critical cell adhesion glycoprotein α/β -dystroglycan in muscle and non-muscle tissues alike¹⁴⁷. Mutations that disrupt or result in inappropriate glycosylation of the membrane-localized α/β -dystroglycan complex have been implicated in a broad spectrum (ranging from mild to severe) of muscular dystrophies. The formation of the α/β -dystroglycan heterodimer represents the fundamental component responsible for linking extracellular matrix constituents (e.g. perlecan, agrin, laminin) with dystrophin. These interactions primarily occur via carbohydrate moieties appended to the α/β -dystroglycan core complex by LARGE, which is necessary for proper α/β -dystroglycan function¹⁶⁸. This interaction is obligatory for agrin-mediated clustering of acetylcholinesterase at neuromuscular junctions¹⁶⁹ in conjunction with perlecan. Recently, agrin-binding dystroglycan has been implicated in promoting synaptic plasticity and specialized GABAergic synapses¹⁷⁰. Further, α/β -dystroglycan exhibits strong affinity with a large stretch of the C-terminal portion of agrin that has been alternatively spliced in a manner that excludes the Y and Z inserts¹⁷¹. These inserts, if present, negatively regulate association of agrin with α/β -dystroglycan¹⁷¹. Agrin also interacts with the α/β -dystroglycan receptor in the formation of immunological synapses with lymphocytes and aids in activation¹⁷² as well as maintaining monocyte cell survival downstream in an α -dystroglycan dependent manner¹⁷³.

A second receptor for agrin has been identified as the $\alpha 3\text{Na}^+/\text{K}^+$ ATPase, which functions primarily as a neuronal ion pump for maintaining proper membrane potential¹⁷⁴ (Figure 3). Agrin binding $\alpha 3\text{Na}^+/\text{K}^+$ ATPase (interestingly, resting neuronal synapses harbor a small percentage of agrin/ $\alpha 3\text{Na}^+/\text{K}^+$ ATPase complexes) on pre- and post-synaptic neurons inhibits ion pump activity and results in a net loss of membrane polarization and a corresponding increase in neuronal action potential¹⁷⁴. Since cardiac tissue expresses both agrin and the $\alpha 3\text{Na}^+/\text{K}^+$ ATPase, it is more probable that agrin has a role in cardiac pathology (e.g. congestive heart failure) via the pro-inotropic effects of agrin-mediated $\alpha 3\text{Na}^+/\text{K}^+$ ATPase modulation¹⁷⁴. A third receptor, the $\alpha V\beta 1$ integrin, binds the LG2 domain of agrin and aids in proper synaptic localization of agrin (Figure 3)¹⁷⁵. The $\alpha V\beta 1$ integrin was discovered in a screen for interacting agrin partners, which revealed that $\alpha V\beta 1$ binds the second of three LG (LG2) domains. Further, agrin also contains a distinct $\alpha 1$ interaction motif in the last EGF (EGF4) repeat¹⁷⁵. The interaction of agrin with neurons is seemingly integrin and divalent cation (Mg^{2+}) dependent as EDTA and monoclonal blocking antibodies directed against these sites substantially abrogated neuronal adhesion to agrin¹⁷⁵. Moreover, $\alpha V\beta 1$ modulates the ability of agrin to appropriately cluster AChR on the surface of myotubes at neuromuscular junctions, orchestrates cation-coordination for proper cell-matrix adhesion, and may also fine-tune agrin-directed neurite outgrowth¹⁷⁶.

A fourth receptor, LRP4 (low-density lipoprotein receptor-related protein 4) is the agrin receptor responsible for MuSK (muscle-specific receptor tyrosine kinase) phosphorylation that permits appropriate AChR clustering at the neuromuscular junction¹⁷⁷ (Figure 3). Unlike binding α/β -dystroglycan, the Z-insert and the C-most terminal LG domain (LG3) are sufficient for LRP4 binding and downstream activation of MuSK¹⁷⁷. Using SILAC

quantitative proteomics, a role of the agrin/LRP4/MuSK signaling axis has been determined as a driving oncogenic force in the development of hepatocellular carcinoma¹⁷⁸.

Unfortunately, the downstream signaling effectors for the agrin receptors remain elusive and very poorly understood for this versatile proteoglycan. Deciphering the downstream apparatus will open novel therapeutic avenues for mitigating agrin pathologies (e.g. muscular dystrophies). However, despite this lack of basic signal transduction knowledge, the agrin LG3 domain has been used as a biomarker, akin with the LG3 domain of perlecan as a serological marker, as a detection method of prematurely ruptured fetal membranes¹⁷⁹.

COLLAGEN XVIII: A UNIQUE HSPG THAT HARBORS ENDOSTATIN

Collagen XVIII is a unique HSPG¹⁸⁰ that contains ten interrupted collagenous domains flanked by non-collagenous regions localized at the N- and C-termini². Collagen XVIII, member of the multiplexin gene family¹⁸¹, is a homotrimer comprising three identical $\alpha 1$ chains, encoded by the *COL18A1* gene. It also harbors consensus sites for the attachment of HS chains¹⁸². Despite the multifactorial nature of this particular proteoglycan and the nearly global expression pattern of *Col18a1* within vascular basement membranes, mice lacking collagen XVIII are not embryonic lethal and are fertile¹⁸³ suggesting it does not play a role in developmental vasculogenesis or angiogenesis. However, genetic ablation of *Col18a1* disrupts the structural integrity of the choroid plexus basement membrane and results in hydrocephaly¹⁸⁴. Lack of *Col18a1* also causes hypertriglyceridemia in mice and humans¹⁸⁵ given modulation of LDL complexes within the subendothelial matrix via endostatin (see below)¹⁸⁶. Collagen XVIII is required for proper eye development^{2,183}, vision, and retinal pigment function¹⁸⁷. Moreover, collagen XVIII may have very specific and context-dependent roles for regulating angiogenesis¹⁸⁸ and tumor growth¹⁸³. However, during HCC development, collagen XVIII expression is decreased¹⁸⁹.

Despite not having a large role in developmental angiogenesis and seemingly not observing any enhanced tumorigenic growth upon genetic inactivation, collagen XVIII harbors a potent anti-angiogenic inhibitor, endostatin^{190–192} that completely neutralizes tumorigenic growth in various models^{191,193}. As mentioned above, collagen XVIII is composed of three identical $\alpha 1$ chains. Each chain has an NC1 (non-collagenous 1) region that includes the trimerization domain, various sites sensitive to MMPs¹⁹⁴, cathepsin L¹⁹⁵, and elastase^{2,196}, a hinge region, and the most C-terminal endostatin domain (Figure 3)^{197–199}. Endostatin orchestrates anti-angiogenic activities in a zinc-dependent manner^{200,201}.

Mechanistically, endostatin binds multiple endothelial cell-specific integrins (e.g. $\alpha 5\beta 1$, $\alpha V\beta 3$, $\alpha V\beta 5$)^{202,203}, and exhibits low affinity interactions for glypicans²⁰⁴. In analogy to the bioactivity of endorepellin, endostatin also binds VEGFR2²⁰⁵ (Figure 3) and can suppress *VEGFA* and *Myc* expression^{199,206}. Ligation of endostatin with the cognate receptor results in a total hijacking of the malignant gene expression programs and counteracts tumorigenicity by reprogramming the responsive cells that ultimately interfere with endothelial cell migration and survival. As an additional mechanism of endothelial cell regulation, endostatin is one of a newly emergent class of proteoglycans that regulates autophagy^{19,207} in an $\alpha 5\beta 1$ -dependent manner²⁰⁷, resulting in apoptosis²⁰⁸. Indeed, loss of

Beclin 1 exacerbates hypoxia-driven angiogenesis²⁰⁹ while the induction of autophagy, in turn, inhibits tumorigenesis in a Beclin 1-dependent manner²¹⁰. Therefore, endostatin mediated autophagy, via Beclin 1 function, may ameliorate hypoxia-driven angiogenesis in an autophagy-dependent manner.

Collectively, these studies stress an important concept, that is, fragments of basement membrane HSPGs, which are located close to the plasma membrane, are in constant dialogue with signaling receptors and their liberation from a matrix-bound state during tissue remodeling would have a strong effect on cell behavior.

CONCLUSIONS

Our aggregate knowledge concerning the underlying fundamental molecular and cellular mechanisms governed by interactions between soluble matrix constituents and cell surface receptors is growing at an exponential rate. Since the initial pioneering discovery that a considerable fraction of decorin is soluble and engages EGFR via high-affinity interactions and compromises oncogenic intracellular signaling, numerous similar paradigms have emerged as functional explanations for a variety of biological phenotypes⁶. The most recent example is lumican, a class II SLRP implicated in cancer and angiogenesis²¹¹, which promotes wound healing via direct ALK5 (TGF β receptor 1) binding and activation²¹². By integrating signaling among multiple receptors, where each receptor is differentially expressed, cognizant of the cell- and tissue-specific resident microenvironment, and the empirically computed differential binding constants, licenses decorin (and related SLRPs) with an ability to affect a wide variety of processes. The perceived promiscuity of decorin is an inherent and critical facet of decorin (and related SLRPs) biology. We should point out that Myc is capable of regulating 1,500 genes and this ability is widely accepted in the literature. We propose that some matrix proteoglycans, because of their structure endowed with leucine-rich repeats, are designed to interact with other proteins and several RTKs, many of which contain Ig-like repeats (VEGFR2, etc.) or leucine-rich repeats (TLR2/4). Canonically, decorin has been viewed as an obligate antagonistic ligand for the EGFR and Met for tumorigenic and angiogenic suppression^{16,50}. With the new dawn that decorin evokes endothelial cell autophagy and tumor cell mitophagy by interacting with VEGFR2 and Met, respectively, the effector landscape has shifted and decorin seemingly acts as a partial agonist for autophagic-mediated tumorigenic and angiogenic abrogation^{14,18,80}. Mounting evidence suggests the requirement of the tyrosine kinase domain for proficient signal transduction and autophagic and mitophagic induction and ensuing angiostasis. Moreover, the case of IGF-IR epitomizes the concept of context- and environmental-dependent signaling in normal, genomically stable cells when compared to transformed cells.

Decorin has long been heralded as a pan-RTK inhibitor that potently opposes tumorigenesis and angiogenesis in a multitude of solid tumors. However, in analogy with biglycan, decorin engages the innate immune receptors TLR2/4 and evokes a proinflammatory phenotype. Indeed, biglycan and decorin have been implicated in regulating immune responses during ischemic acute renal diseases, fibrosis, and tumorigenesis⁹. Indeed, generating a pro-inflammatory milieu within the tumor stroma appears as an additional layer of activity for

the tumoricidal functions of decorin. Interestingly, transcriptome wide analysis of basal breast carcinoma tumor xenografts following systemic administration of decorin protein core has shown a suppression of multiple immunoregulatory genes within the tumor stroma⁵¹. Conversely, soluble biglycan signaling via LRP6 potentiates canonical Wnt signaling and may contribute to enhance cancer growth and progression via β -catenin-driven tumorigenesis.

Lastly, the pericellular matrix, responsible for organizing and maintaining the basement membrane structure and reliability in a multitude of tissues, directly participates in regulating centrally conserved cellular processes such as synaptogenesis, nodogenesis, autophagy⁸⁰, angiogenesis, and tumorigenesis. Perlecan, agrin, and collagen XVIII are unique insofar as being multifunctional effectors that are proteolytically processed and yield modular effector units². These soluble fragments can bind multiple receptors as diverse as integrins, glypicans, and RTKs for exercising their inherent biological functions across an array of tissues and maladies. Commanding a comprehensive understanding of the intricacies of proteoglycan signaling via this specialized, but ever-expanding, community of proteoglycan receptors will permit more personalized and targeted therapeutic options that will effectively combat our most insidious and devastating diseases.

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ABBREVIATIONS

SLRP	small leucine-rich proteoglycan
RTK	receptor tyrosine kinase
HUVEC	human umbilical vein endothelial cells
ECM	extracellular matrix
HS	heparan sulfate
HSPG	HS proteoglycan
EGF	epidermal growth factor
EGFR	EGF receptor
IGF-I	insulin like growth factor I
IGF-IR	IGF-I receptor
VEGFA	vascular endothelial cell growth factor A
VEGFR2	VEGF receptor 2
PDGFR	platelet-derived growth factor receptor

TLR	toll-like receptor
LPS	lipopolysaccharide
PDCD4	programmed cell death 4
MAPK	mitogen activated protein kinase
LRP6	Low-density lipoprotein receptor-related protein 6
ERK1/2	extracellular regulated kinase 1/2
SAPK	stress activated protein kinase
mTOR	mammalian target of rapamycin
PI3K	phosphoinositidyl-3-kinase
HIF-1α	hypoxia inducible factor-1 α
NFAT	nuclear factor of activated T-cell
NFκB	nuclear factor kappa B
MMP	matrix metalloproteinase
TSP-1	Thrombospondin-1
TIMP-3	tissue inhibitor of metalloproteinase-3
AMPKα	AMP-activated protein kinase α subunit
DAMP	damage associated molecular patterns
MCP-1	monocyte chemoattractant protein-1
SHP-1	Src homology region 2 domain-containing phosphatase-1
LRP4	Low-density lipoprotein receptor-related protein 4
MuSK	muscle-specific receptor tyrosine kinase
AChR	acetylcholinesterase receptor
HCC	hepato-cellular carcinoma
LRP-1	lipoprotein receptor-related protein 1

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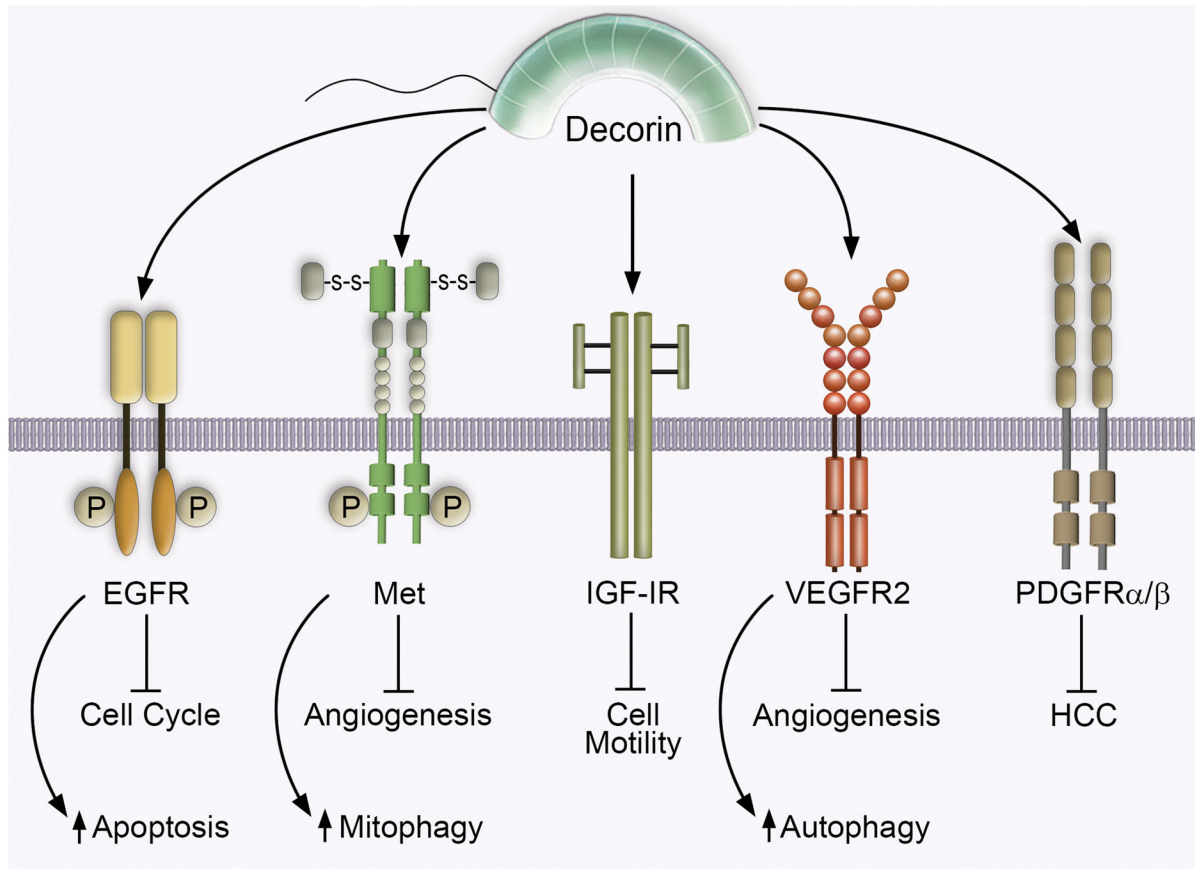


Figure 1.

Decorin interacts with several receptor tyrosine kinases for the control of fundamental cellular behaviors in normal and malignant circumstances. Schematic representation of cell surface receptor tyrosine kinases occupied by decorin. Biological consequences of binding and resultant signal transduction are outlined below the appropriate receptor. Please refer to the text for additional information.

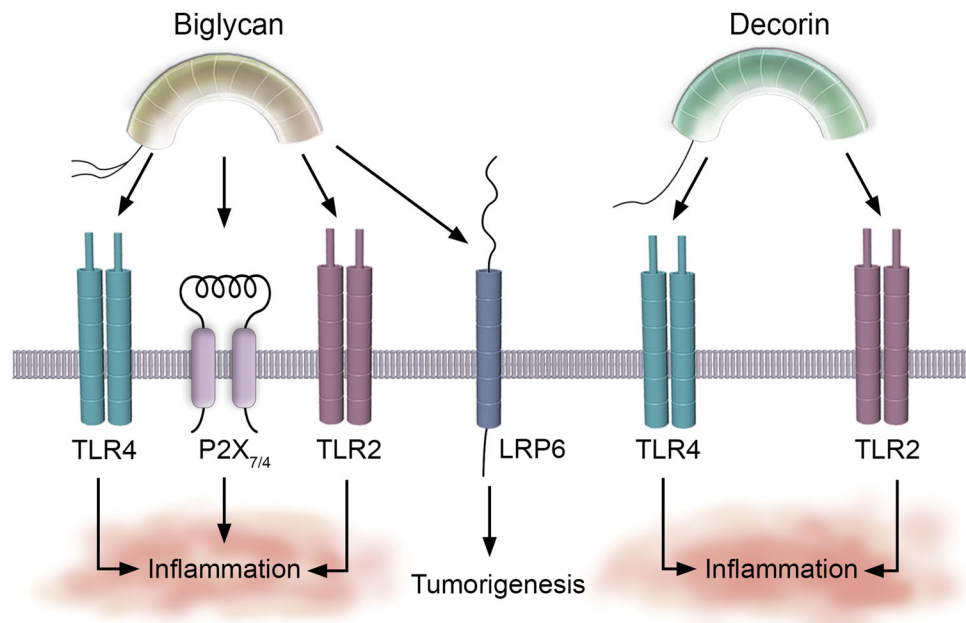


Figure 2. Biglycan and decorin bind innate immune system receptors for immunoregulation and tumorigenesis. The innate immune receptors TLR2, TLR4 and the purinergic P2X_{7/4} provide novel signaling circuits through which biglycan and decorin bind for regulating the innate immune system and cultivating a pro-inflammatory environment in sepsis and tumorigenesis. Biglycan also binds LRP6 for tumor promotion. Please refer to the text for more information.

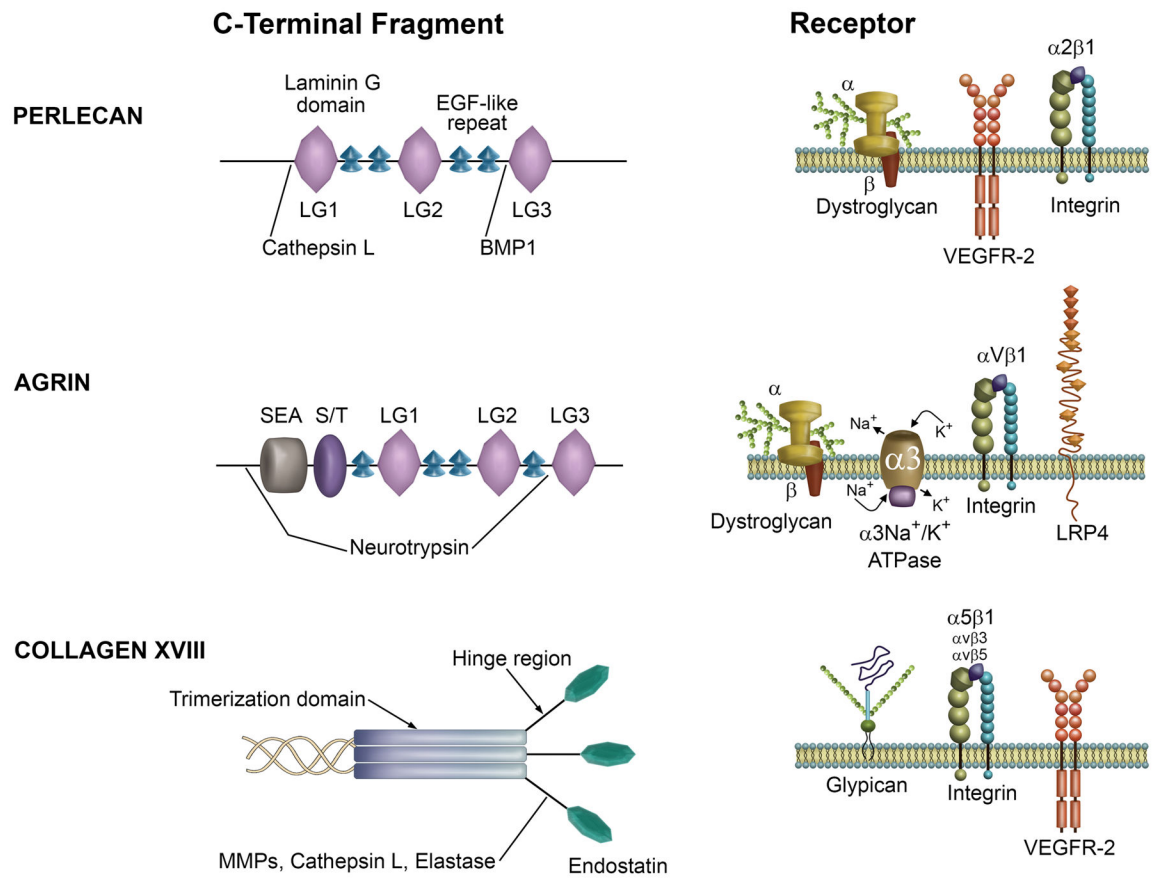


Figure 3. Basement membrane heparan sulfate proteoglycans ligate multiple receptors for effector function in a variety of cells and tissues. Graphical description of the C-terminal domains of the major basement membrane HSPGs demonstrating modular organization and architecture (left) and a summary of the primary receptors engaged (right). Figure adapted from Iozzo *et al*².