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A current view of perlecan in physiology and pathology: A mosaic of functions

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Abstract

Perlecan, a large basement membrane heparan sulfate proteoglycan, is expressed in a wide array of tissues where it regulates diverse cellular processes including bone formation, inflammation, cardiac development, and angiogenesis. Here we provide a contemporary review germane to the biology of perlecan encompassing its genetic regulation as well as an analysis of its modular protein structure as it pertains to function. As perlecan signaling from the extracellular matrix converges on master regulators of autophagy, including AMPK and mTOR, via a specific interaction with vascular endothelial growth factor receptor 2, we specifically focus on the mechanism of action of perlecan in autophagy and angiogenesis and contrast the role of endorepellin, the C-terminal fragment of perlecan, in these cellular and morphogenic events.

Keywords

Angiogenesis; Autophagy; Endorepellin; Heparan sulfate; Proteoglycan

Introduction

Proteoglycans, ubiquitous residents of the extracellular matrix, participate in a range of both structural and signaling roles in order to maintain cellular homeostasis [1]. Proteoglycans participate in the genesis and maintenance of the extracellular milieu, the complex meshwork of proteins comprising multicellular organisms [2,3]. An example of proteoglycan versatility comes in the form of perlecan, an immense heparan sulfate proteoglycan primarily localized to basement membranes and pericellular spaces [4–9]. The modular structure of perlecan enables homeostatic regulation within a vast array of cellular processes including cell adhesion [10,11], endocytosis [12], bone and cartilage formation [13–16], lipid metabolism [17], peripheral node assembly [18], inflammation and wound healing [19,20], thrombosis [21], cancer angiogenesis [9,11,22–33], cardiovascular development [34], and autophagy [35,36]. Given the gargantuan structure and multitude of

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functions of perlecan, it is no surprise that its gene, *HSPG2*, is similarly large and transcriptionally controlled by complex promoter interactions.

In this review, we will provide a current analysis of the biology of perlecan focusing on its genetic regulation and protein structure as well as its functional role in physiological processes. In particular, we will examine the dichotomy between perlecan and its C-terminal fragment, endorepellin, in coordinating angiogenesis and autophagy as well as provide commentary on the emerging paradigm regarding the connection between these two vital cellular pathways.

Form follows function: Overview of the perlecan gene and protein structure

Perlecan is one of the largest proteoglycans discovered, possessing a protein core of approximately 500 kDa that can be modified by the addition of N-terminal heparan sulfate (HS) side chains, measuring ~65 kDa each [37]. The protein core is divided into several unique structural regions, each imparting distinct biofunctional diversity to perlecan [1,38]. The complexity and sheer size of the core protein is rivaled only by the modular nature [39] and tight regulation of the gene encoding this key proteoglycan. Here, we will discuss the genetics of perlecan as well as analyze the structure of each intra-protein domain and ascribed functions (Fig. 1). A more extensive description of the role of specific perlecan protein modules in angiogenesis and autophagy will be considered later.

Genomic organization and transcriptional regulation of perlecan

The gene encoding perlecan, *HSPG2*, spans over 120 Kb and encompasses 97 exons [39,40] and is highly conserved across species [41]. The genomic DNA is organized in a modular fashion, with specific exons corresponding to distinct protein domains conventionally found in other basement membrane constituents (Fig. 1). Further, this exonic organization is evolutionarily conserved for the corresponding regions seen in homologous *HSPG2* genes [40], suggesting a common ancestor arising from gene duplication and exon shuffling. Structurally, *HSPG2* is one of a subset of so-called TATA-less driven-genes and is seemingly regulated by a variety of housekeeping transcription factors (e.g. Sp1 and ETF) while also being responsive to growth factor-controlled gene induction. This response is evidenced by *HSPG2* transcriptional activation downstream of canonical TGF-β signaling via direct binding of nuclear factor-1 to its promoter [42] and transcriptional suppression mediated by IFN-γ [43]. Moreover, transcriptional activation by NFκB enhances *HSPG2* gene expression in the desmoplastic prostate cancer microenvironment [44]. Due to this regulatory circuit, perlecan is considered an early response gene. Additionally, consistent with the lack of a traditional TATA-box, the perlecan promoter harbors multiple transcriptional start sites scattered over an 80-bp region of genomic DNA [40].

Complementing the transcriptional complexity mentioned above, the perlecan pre-mRNA is subject to alternative splicing, as splice variants occur in the HMC-1 human mast cell line [19]. Interestingly, these shorter perlecan isoforms encode biologically active endorepellin [20], an effective anti-angiogenic and pro-autophagic proteolytic cleavage product.

This complex regulatory scheme underscores the dynamic expression profile for perlecan in various tissues throughout development [15,45–48] and in several pathological processes [29]. These areas include vascularized and non-vascularized regions as well as the connective tissue stroma [38,49]. This expression pattern leads to several downstream functional consequences. For example, as it is found ubiquitously within a diverse population of basement membranes [8,50], perlecan aids in the formation and maintenance of polarized epithelial cells, chiefly at the apical cell surface [27]. In addition, perlecan takes part in a variety of other functions such as the modulation of vascular endothelial cell adhesion [10] and proliferation [51], modulation of growth factor signaling [11,52–56], maintenance of biomechanical properties and cartilage homeostasis [57–59], skeletal muscle and cardiovascular development [34], and skin and endochondral bone formation [60,61]. These actions occur, in part, by the ability of perlecan to regulate the bioavailability of growth factors resulting in the modification of the bioactivity of various signaling pathways. Moreover, perlecan is involved in regulating solute transport and mechanosensing in the osteocyte lacunar-canalicular system [62], and its protein core can withstand over 100 pN of tension, suggesting that perlecan could act as an elastic tether in the lacunar-canalicular system [63]. Collectively, these properties culminate in a multifaceted biological agent that has been implicated in diseases such as osteoarthritis [64], atherosclerosis [65,66], muscle hypertrophy [67] cancer progression [22,24,27,68,69], aging [70], and pulmonary hypertension [71]

Domain I: An N-terminal region exclusive to perlecan

The N-terminus of perlecan possesses the primary location for HS side-chain attachment. Indeed, this region of perlecan contains three areas comprising the signature Ser-Gly-Asp (SGD) sequence [37], an amino acid arrangement that resembles the putative glycosaminoglycan (GAG) consensus site (Fig. 1). Structurally, domain I contains no cysteine residues, consists of many acidic amino acids, and, unlike the other perlecan domains, does not include any known sequential repeats, such as EGF-like repeats [37]. Surprisingly, following in-depth analysis utilizing several known protein sequence databases, no homology occurs between this domain and any recognized motif in other proteins [37], conferring specificity for this region to perlecan. The notable caveat to this statement is the Sea urchin sperm protein, Enterokinase, and Agrin (SEA) module of domain I, which is shared by some other extracellular matrix proteins from which the name originates.

The HS side chains of domain I allow it to sequester and present growth factors to their appropriate receptors [52,72–74]. This role is quite important for establishing morphogen gradients required for developmental processes and regulating angiogenesis, which will be discussed in more detail in a subsequent section.

Domain II: A pro-atherosclerotic domain

Unlike domain I, which is unique to perlecan, domain II contains four cysteine-rich repeats resembling the sequence of the LDL-binding region of the LDL receptor [37,39,75] (Fig. 1). As such, it is not surprising that this domain of perlecan interacts with LDL in a glycosylation-dependent manner [76]. This finding denotes a role for perlecan in the

retention of LDL in the arterial sub-endothelium, where expression levels of perlecan correlate well with the progression of atherosclerosis [77]. Also worthy of mention is the single Ig-like repeat reminiscent of the repetitive structure of N-CAM that separates domain II from domain III. Notably, several of these repeats are prominently featured later in domain IV. However, there is currently no known function for this region in domain II.

Domain III: Homologue of the short arm of laminin A

Domain III is divided into several segments including three laminin globular domains interspersed among 11 laminin-like EGF repeats [37,39] (Fig. 1). Given this information, it is predictable that domain III shares homology with the short arm of laminin A [78–80] affording a high degree of probability that these two proteins descended from a shared ancestor over a common evolutionary period.

Functionally, domain III may modulate efflux of perlecan into the extracellular space as evidenced by a recent case study of a patient with Schwartz-Jampel syndrome [81], a disease known to be linked to altered perlecan expression and characterized by a short stature phenotype as well as myotonia and chondrodysplasia [82]. Expression studies utilizing perlecan harboring the reported domain III-specific missense mutation revealed impaired secretion of perlecan into cell culture media [81], suggesting the importance of this domain for proper localization of perlecan to enable its function in downstream signaling cascades. Additionally, in mice, perlecan domain III contains an RGD sequence, implicating it in cell attachment [39]. Indeed, recombinant murine domain III promotes adhesion of epithelial-like mouse mammary tumor cells, which can be inhibited upon introduction of a synthetic RGDS peptide [83].

Domain IV: N-CAM immunoglobulin stretch

Penultimate domain IV is the largest, encompassing over 2000 amino acid residues to form 21 Ig-like repeats in human perlecan that are homologous to those found in N-CAM [37,39,84] (Fig. 1). It is important to note that there is inter-species variation in the number of these Ig-like repeats [75]. Interestingly, though the most common GAG chain attachment sites are found in domain I, there exists a prospective GAG chain attachment sequence in the 14^{th} Ig-like repeat of human perlecan [75]. With regard to function, this sizable domain is hypothesized to act as a scaffold, where it could mediate a constellation of heterotypic interactions to sustain extracellular matrix assembly and architecture. Indeed, domain IV binds several structural matrix components including nidogen, fibronectin, and fibulin-2 [85]. Recently, it has been shown that fragments of domain IV are markedly elevated in the blood of patients with advanced prostate carcinomas, but absent in normal sera, suggesting that domain IV might be linked to advanced prostate cancer [86]. These elevated levels of domain IV correlate with increased levels of MMP-7 [86], an enzyme known to cleave perlecan [29].

Domain V: An entity known as endorepellin

The final domain of perlecan, christened endorepellin due to its ability to act as an independent entity to inhibit endothelial cell motility [25], contains three laminin globular domains separated by dual EGF-like repeats [37,39] (Fig. 1). Like domain III, these globular

domains share similarity with laminin A [78,79]. These sub-domains allow endorepellin to function in dual receptor antagonism (see below). These concurrent actions culminate in decreased endothelial cell migration and, consequently, angiogenesis. Intriguingly, recent studies also show soluble endorepellin as a key autophagic inducer in endothelial cells [35].

Endorepellin is found *in vivo* in the blood and in several body fluids where it is liberated from perlecan by partial proteolysis of the protein core [20] via matrix metalloproteinases, a family of enzymes involved in a multitude of biological processes [87–92]. In addition, the LG3 fragment is found in circulation as both Cathepsin L and BMP-1-Tolloid-like proteases catalyze proteolytic cleavage from the C-terminus of endorepellin [20,93]. LG3 is increased in the urine of women with premature rupture of fetal membranes [94], patients with end-stage renal failure and chronic allograft rejection [95,96], and in the amniotic fluid of women bearing fetuses with trisomy 21 [97]. Furthermore, LG3 may act as a useful biomarker for disease detection, as low levels of LG3 are associated with progression and increased invasion of breast cancer [98]. LG3 can also modulate homing of mesenchymal stem cells and neointima formation during vascular rejection [99], and can evoke auto-antibody production and allograft inflammation [100].

Defective perlecan is coupled with genetic conditions penetrant in multiple model organisms

As mentioned above, perlecan is present as a single, unique gene and is highly conserved across several organisms including *C. elegans* (*unc-52*, uncoordinated phenotype 52), *D. melanogaster* (*trol*, terribly reduced optical lobes), *D. rerio* (*Hspg2*), *M. musculus* (*Hspg2*), and *H. sapiens* (*HSPG2*) (Table 1). Predominantly, perlecan is expressed and functions, both structurally and as a signaling node, within developing and mature cartilages [48,73,101–103]. Cumulatively, these findings establish a pivotal role for perlecan in cartilage formation, making it indispensable for life. Given its importance in cartilage and other tissues, mutations in perlecan result in several distinct phenotypes observed across a spectrum of different organisms (Table 1).

In the most basic model organism, *C. elegans*, *unc-52* mutations lead to aberrant gonadal leader cell migration via disrupted growth factor signaling (e.g. FGF, TGF-β, Wnt) operative during gonadogenesis [104]. As mentioned previously, there is evidence for alternative splicing of perlecan in mast cells [19]. Likewise, in *C. elegans*, *unc-52* is heavily spliced and mediates spatiotemporal deposition and localization of perlecan [105]. Nonsense mutations occurring within these splice variants trigger progressive paralysis [106].

Moving to *D. melanogaster,* studies of *trol* reveal critical roles for perlecan in the proliferative activation of quiescent neuroblasts [107] and neural stem cell signaling [108] (Table 1). These functions seemingly depend on FGF and Shh pathways [109] inasmuch as reintroduction of FGF2 into *trol* mutants re-establishes the neural proliferative defect [109]. Therefore, *trol* may orchestrate key regulatory roles in neurogenesis and stem cell niches. This role is further reinforced by the requirement of *trol* in the proliferation of hematopoietic progenitor cells and with the prevention of premature hemocyte differentiation. Analogous

to the FGF2 rescue, this phenotype can be reversed with exogenously expressed Hedgehog [110].

The comparatively more sophisticated model organism, *D. rerio*, also sustains the effects of aberrant perlecan expression (Table 1). Zebrafish morphants, created via morpholino-mediated knockdown of perlecan, demonstrate disorganized and reduced amounts of actin filaments leading to abnormal sarcomeres [34]. These structural alterations in the absence of perlecan result in severe myopathy. Furthermore, these same morphants do not demonstrate proper extension of aortic vessel sprouts resulting in abnormal angiogenesis and decreased circulation [34]. Moreover, morpholino-mediated knockdown of the major receptor for perlecan/endorepellin, the α2β1 integrin, causes an even more dramatic impairment of the intersegmental vessels which are normally generated by angiogenic sprouting from the dorsal aorta [111]. Taken together, the absence of perlecan and/or its cell surface receptor α2β1 in zebrafish demonstrate the necessity for this proteoglycan signaling axis in muscle development and angiogenesis.

The perlecan null mice (*Hspg2−/−)* are embryonic lethal [112]. Indeed, an estimated 50% of *Hspg2−/−* mice succumb to pericardial hemorrhage between embryonic days 10–12 [112] (Table 1). Due to the biomechanical properties ascribed to perlecan, it is thought that regions of the pericardial cavity basement membrane are significantly compromised, leading to deterioration and ultimately, mechanical breakdown [112]. As the perlecan null embryos reach more progressive stages of development, stark morphological complications of the cardiovascular system arise [113] including transposition of the great vessels, malformed coronary arteries, and irregular cardiac outflow tracts [113]. The few mice that do survive exhibit severe cartilage malformations and perish shortly after birth from respiratory failure [112,114]. Notably, some of these cardiac defects are similar to mutant mice lacking NDST-1, a key enzyme involved in the biosynthesis of heparan sulfate [115].

However, despite the embryonic lethality that follows from global perlecan deletion, there exist some viable mouse models that provide invaluable insight into the function of perlecan. The first transgenic model was generated by genomic deletion of exon 3 (designated as *Hspg2* Δ3/Δ3), which encodes two out of the three SGD consensus sites for HS attachment in domain I [116]. These mice are fertile, despite having reduced amounts of HS, and show lens degeneration three weeks post birth [116], indicating a key role for the HS chains in lens capsule homeostasis [117]. Further, the HS-deficient mice have narrowing of an experimentally occluded carotid artery due to augmented intimal hyperplasia concomitant with smooth muscle cell proliferation [118], as well as impaired tumor angiogenesis, tumorigenesis, and delayed wound healing [119]. These latter deficits are consistent with the co-receptor roles perlecan plays within the tumor and stroma microenvironment. The second transgenic model features reconstituted *Hspg2* expression specifically within the cartilage [120], obviating the lethality associated with cartilage failure in the *Hspg2−/−* mouse. The phenotype of these mice will be discussed in a later section due to its relevance to autophagy.

In humans, *HSPG2* has been linked to two well-characterized autosomal recessive genetic diseases: lethal dyssegmental dysplasia, Silver-Handmaker type (DSSH) [121] and

Schwartz-Jampel syndrome (SJS) [82,122] (Table 1). Clinically, DSSH patients exhibit anisospondyly, a marked difference in size and shape of the vertebral bodies, and micromelia, disproportionally short limbs. Genetically, truncated forms of perlecan, resulting from skipped exons caused by duplications in exon 34, frameshifts, and point mutations, are responsible for the clinical manifestations of this disease [123]. Intriguingly, these truncations generate a situation where perlecan is functionally null, as the shorter perlecan species are labile and quickly degraded. The end result is phenotypically akin to the *Hspg2−/−* mice [123] where loss of perlecan results in dysregulated FGF signaling, impaired cartilage architecture, and decreased chondrocyte proliferation [114,121,123].

In SJS, the prevalence of missense and alternative splicing mutations gives rise to a non-lethal muscle disorder [82] resulting in myotonia. These mutations either completely or partially (up to 64 amino acids) ablate domain V/endorepellin from the C-terminus of perlecan thereby rendering the secreted product non-functional or only partially functional [122]. Mechanistically, these deletions may affect the clustering and anchoring of multiprotein receptor complexes at neuromuscular junctions (i.e. acetylcholinesterase, acetylcholine receptor, MuSK [124]) and/or perturb association with adjacent proteoglycan scaffolds, such as agrin [125,126]. Loss of synaptic responsiveness, via defects in acetylcholine signaling or organization, could cause myotonia. Indeed, *Hspg2−/−* mice do not retain acetylcholinesterase at the neuromuscular junction [127].

Antithetic biological functions of perlecan and endorepellin

As perlecan is expressed in both vascular and avascular tissues, it follows that this proteoglycan is directly involved in a variety of cellular events including cell adhesion [10,11], thrombosis [21], and vascular development [34]. Though these examples emphasize cell signaling versatility, we will focus below on two hallmark processes in which the terminal domain of perlecan is strongly implicated: angiogenesis and autophagy (Fig. 2).

Perlecan is an archetypical HSPG required for bivalent angiogenic regulation

Perlecan intrinsically coordinates angiogenesis by harboring pro- and anti-angiogenic properties within the same molecule [128]. Most prominent are the intricate regulatory roles perlecan exerts over the VEGFA/VEGFR2 and α2β1 integrin signaling axes [69,129]. As a whole molecule, perlecan acts predominantly in a pro-angiogenic fashion (Fig. 2). The N-terminal HS side chains of domain I act as a reservoir for growth factors, which can then be presented in the proper arrangement to their respective cell surface receptors [52,72–74].

For example, perlecan induces neovascularization in a rabbit ear model of angiogenesis by promoting FGF2 binding to its receptor [130]. Furthermore, perlecan enhances VEGFA-mediated phosphorylation of VEGFR2 [131]. Additionally, mice lacking the HS attachment site of perlecan demonstrate compromised FGF2-mediated corneal angiogenesis [119], underscoring the importance of the N-terminal GAG chains for the regulation of this process. As mentioned above, morpholino-mediated knockdown of perlecan in zebrafish causes reduced extension of angiogenic sprouts from the dorsal aorta [34]. These perlecan morphants show aberrant distribution of pro-angiogenic VEGFA, which can be rescued by the addition of exogenous growth factor [131]. This information, combined with the

knowledge that VEGF itself can stimulate the synthesis of perlecan [132], supports the view that there is an evolutionarily-conserved positive feedback loop for this proteoglycan in the angiogenic signaling cascade.

Given the aforementioned role of perlecan in developmental angiogenesis, it is not surprising that, in the setting of tumorigenesis, perlecan functions as a cornucopia of pro-angiogenic factors involved in the promotion of unrestrained neovascularization and tumorigenic growth [27]. Indeed, perlecan favors pathologic angiogenesis via direct interactions with a multitude of HS-binding angiokines such as progranulin, VEGFA, and PDGF, as well as several FGF superfamily members, including FGF2, FGF7, and FGF18 [55,133]. This mechanism allows perlecan to assume the role of co-receptor for presentation of the appropriate ligand to the cognate receptor for efficient and robust signaling responses, resulting in tumor progression [27].

In contrast to its N-terminus, the C-terminal domain V of perlecan, endorepellin, takes on an opposing role to the parent molecule: it inhibits angiogenesis, capillary morphogenesis, and endothelial cell migration [25,134,135] (Fig. 2). Specifically, soluble endorepellin is a dual receptor antagonist by acting as a molecular bridge for simultaneous ligation of the α2β1 integrin and VEGFR2 [136]. The discovery of “dual receptor antagonism” could be fundamental for understanding the mechanism through which endorepellin, and perhaps other angiostatic factors such as endostatin, would halt neovascularization.

At the receptor level, the N-terminal LG1/2 domains of endorepellin primarily interact with the VEGFR2 ectodomain in a region encompassing $Ig_{3–5}$ domains. Meanwhile, the most C-terminal LG module, LG3, binds the α2 I-domain of the α2β1 integrin [129,137–139]. The formation of the heterotrimeric endorepellin-VEGFR2-α2β1 integrin complex brings the SHP-1 phosphatase, constitutively bound to the intracellular tail of α2 integrin subunit, in close functional juxtaposition with the cytoplasmic tails of VEGFR2 [140]. SHP-1-mediated dephosphorylation of VEGFR2 has dramatic consequences for the ternary complex including inactivation of downstream signaling effectors [141] and caveolin-mediated internalization [141]. Interestingly, the bioactivities of endorepellin can be physically separated insofar as the VEGFR2-binding domains (i.e. LG1/2) are sufficient and embody the properties necessary for attenuating downstream VEGFA signaling [138]. Likewise, purified LG3, the α2β1-binding region, is competent for dissolution of the actin cytoskeleton [128,138].

Mechanistically, dephosphorylation of VEGFR2, particularly at Tyr^{1175} [142], in a SHP-1-dependent manner removes a crucial recruitment site for Shb and PLC-γ nucleation [141], ultimately resulting in protracted angiogenic suppression. Downstream of VEGFR2 effector attenuation, pro-angiogenic HIF-1α/VEGFA signaling is potently abrogated in a non-canonical, oxygen-independent manner [141]. Further, NFAT nuclear translocation is significantly inhibited via PLC-γ attenuation, resulting in diminished calcineurin and PKC/JNK/AP1 activity, culminating in decreased VEGFA expression and secretion [136,141].

Cumulatively, these studies acknowledge contrasting roles for perlecan, whereby its N-terminus sequesters and presents growth factors to specific receptors to promote angiogenesis, while its C-terminus possesses a starkly anti-angiogenic function through its intrinsic property of dual receptor antagonism.

Divergent roles of perlecan and endorepellin in autophagy

In analogy with their roles in angiogenesis, whole perlecan and endorepellin once again find themselves in opposition in the regulation of autophagy, an essential cellular process encompassing the lysosomal breakdown of cellular components following isolation in double-membraned autophagosomes [143]. Studies utilizing transgenic mice that harbor expression of *Hspg2* only in cartilage to ensure viability [112,114] depict perlecan as an autophagic inhibitor [36]. Indeed, perlecan-null soleus muscle demonstrates increased numbers of autophagosomes when compared to wild-type muscle [36]. Mechanistically, absence of perlecan results in inhibition of the canonical mTOR pathway, causing increased autophagic flux *vis-a-vis* wild-type mice [36]. Physiologically, perlecan expression is necessary to maintain muscle mass following tenotomy as its absence results in atrophy due to improper autophagic regulation [36].

As in the case of angiogenesis, endorepellin takes on a contrasting role to perlecan as an autophagic inducer. Exposure to nanomolar concentrations of endorepellin causes the formation of prominent autophagosomes in endothelial cells (Fig. 3B), similar to those evoked by canonical autophagic stimuli such as rapamycin, which inhibits the mTOR pathway (Fig. 3C), or nutrient deprivation (Fig. 3D). Operating as a partial agonist to VEGFR2 under nutrient-rich conditions, endorepellin evokes a pro-autophagic cascade composed of Peg3, Beclin 1, p62, and LC3 [35] (Fig. 2). This action inhibits mTOR signaling in a manner similar to that of the gold standard, rapamycin [143]. Curiously, though endorepellin typically serves to inhibit VEGFR2 signaling, its ability to induce autophagy in endothelial cells relies on the phosphorylation of the VEGFR2 residue Tyr^{1175} as the tyrosine kinase inhibitor, SU5416, abrogates this autophagic effect [35]. The autophagic induction by endorepellin is completely independent of its interaction with $\alpha 2\beta 1$ integrin [136] as endothelial cells treated with LG3 do not undergo autophagy [35]. In fact, LG3 quells autophagic gene expression, suggesting that, like the different domains of the parent proteoglycan, individual regions of the same domain may also partake in disparate activities [35].

Future work will likely deconstruct the link between angiogenesis and autophagy. It appears that a pattern is emerging in that pro-angiogenic perlecan is anti-autophagic whereas the converse holds true for endorepellin. Intriguingly, recent work in our laboratory [144], has demonstrated that endorepellin and Torin 1, a potent autophagic inducer [145], both inhibit vessel sprouting in an *ex vivo* model of angiogenesis (Fig. 3F,G), suggesting that autophagy may be one mechanism by which endorepellin inhibits neovascularization.

In our study, we discovered that protracted endothelial cell autophagy evoked by endorepellin can curtail neovascularization, as both endorepellin and Torin 1 reduce sprouting in *ex vivo* aortic ring assays [144]. Notably, the angiostatic activity of endorepellin could be blocked using Compound C, an inhibitor of AMPKα (Fig. 2). Interestingly, the

synthesis of hyaluronan, a major constituent of the provisional angiogenic matrix whose biosynthetic pathway is under complex metabolic control [146–148] is seemingly inhibited by AMPK [149]. Mechanistically, AMPK directly phosphorylates hyaluronan synthase 2 at Thr^{110}, a post-translational modification that blocks its enzymatic activity [149]. Thus, together with autophagic induction, endorepellin could also modify the tumor microenvironment to favor angiostasis by reducing the expression of this pro-angiogenic glycosaminoglycan. These new findings, together with previous *in vivo* data depicting endorepellin as a powerful means to curtail tumor growth and angiogenesis [150], could potentially yield unique therapeutic targets for novel drug design.

Interestingly, other matrix components, most soluble, follow this same paradigm. For example, endostatin, the C-terminal fragment of the HSPG collagen XVIII and an established inhibitor of angiogenesis, induces autophagy in endothelial cells via α5β1 integrin signaling to increase Beclin 1 expression [151]. Additionally, decorin, an anti-angiogenic small, leucine-rich proteoglycan (SLRP) [152], correspondingly induces autophagy in HUVEC using a mechanism almost identical to that of endorepellin [153–155]. Decorin also promotes mitochondrial autophagy within the tumor proper [156]. Notably, decorin is an autophagy-inducible proteoglycan and its absence results in diminished cardiac autophagy [157]. Therefore, as perlecan deficiency results in enhanced autophagic activity, an intricate *in vivo* signaling network could take place whereby proteoglycans and other matrix residents would ensure appropriate flux through this pathway under different cellular conditions [158].

It is also worthy to mention a recent study which demonstrates that biglycan, an extracellular matrix SLRP and close relative to decorin, can counteract the anti-angiogenic effects of endostatin [159]. As endostatin is pro-autophagic, it is conceivable that future studies may show that biglycan is yet another matrix component that follows the aforementioned model where it could potentially inhibit autophagy to promote angiogenesis.

Finally, in this section we have focused primarily on the action of the N- and C-termini of perlecan in angiogenesis and autophagy. It is possible that the three internal domains of perlecan may also contribute to one or both of these processes. For example, laminin-α2 knockout mice, which exhibit a congenital muscular dystrophy phenotype, display increased muscle autophagy *vis-à-vis* wild-type mice [160]. Moreover, as in the case of the perlecan knockout, there is increased atrophy in the *Lama2*$^{-/-}$ model, which can be rescued with autophagic abatement [160]. Thus, domain III, which shares homology with laminin, may possess the anti-autophagic properties conferred to perlecan and could possibly act independently as an inhibitor of this catabolic process.

Final considerations

The long-standing dogma of large, HS-substituted matrix constituents acting strictly as structural components is rapidly evolving. The emergent paradigm involves proteoglycans as central signaling hubs, coordinating and integrating a multitude of signals for maintaining proper cellular homeostasis. Perlecan exemplifies this concept via strategic localization in various basement membranes and subsequent homo- and heterotypic interactions with

adjacent matrix molecules and vectorial juxtaposition with diverse subsets of cell surface receptors. The continued study of perlecan genetics, particularly of mutations of the gene that lead to defective or absent protein, is fundamental for advances in disease management. Furthermore, though the functions of perlecan are broad in scope, its key roles in angiogenesis and autophagy are most interesting, especially as they pertain to tissue homeostasis and dysregulation of normal cellular signaling, and thus should be the paramount focus of future work in order to understand better the mechanisms of disease progression.

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Abbreviations used

Akt/PKB	protein kinase B
AP1	activator protein 1
BMP	bone morphogenetic protein
DSSH	dyssegmental dysplasia Silver-Handmaker type
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
ETF	Tef-1 and abaA domain family member 2
FGF	fibroblast growth factor
GAG	glycosaminoglycan
HIF-1α	hypoxia-inducible factor-1α
HMC-1	human mast cell line 1
HS	heparan sulfate
HSPG	heparan sulfate proteoglycan
HUVEC	human umbilical vein endothelial cell
IFN-γ	interferon gamma
Ig	immunoglobulin
JNK	Jun N-terminal kinase
LC3	microtubule-associated protein light chain 3
LDL	low-density lipoprotein

LG	laminin globular domain
MEK	mitogen-activated protein kinase kinase
mTOR	mammalian target of rapamycin
MuSK	muscle-specific kinase
N-CAM	neural cell adhesion molecule
NFAT	nuclear factor of activated T-cells
p62	sequestosome 1
PDGF	platelet-derived growth factor
Peg3	paternally expressed gene 3
PI3K	phosphoinositide 3-kinase
PKC	protein kinase C
PLC-γ	phospholipase C gamma
Raf	rapidly accelerated fibrosarcoma
Ras	rat sarcoma
SEA	Sea urchin sperm protein, enterokinase, and agrin domain
Shh	Sonic hedgehog
SHP-1	Src homology phosphatase-1
SJS	Schwartz-Jampel Syndrome
SLRP	small, leucine-rich proteoglycan
Sp1	specificity protein 1
TGF-β	transforming growth factor β
Unc	uncoordinated phenotype
VEGFA	vascular endothelial growth factor A
VEGFR2	vascular endothelial growth factor receptor 2
Wnt	wingless-type

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Highlights

- A modern view of the biological properties of perlecan, an archetypal basement membrane proteoglycan
- The complex genomic organization of perlecan translates into a large, modular proteoglycan capable of regulating numerous cellular pathways
- Mutations in the perlecan gene across multiple organisms result in diverse phenotypes
- Perlecan and its C-terminal fragment, endorepellin, display antagonistic roles in angiogenesis and autophagy

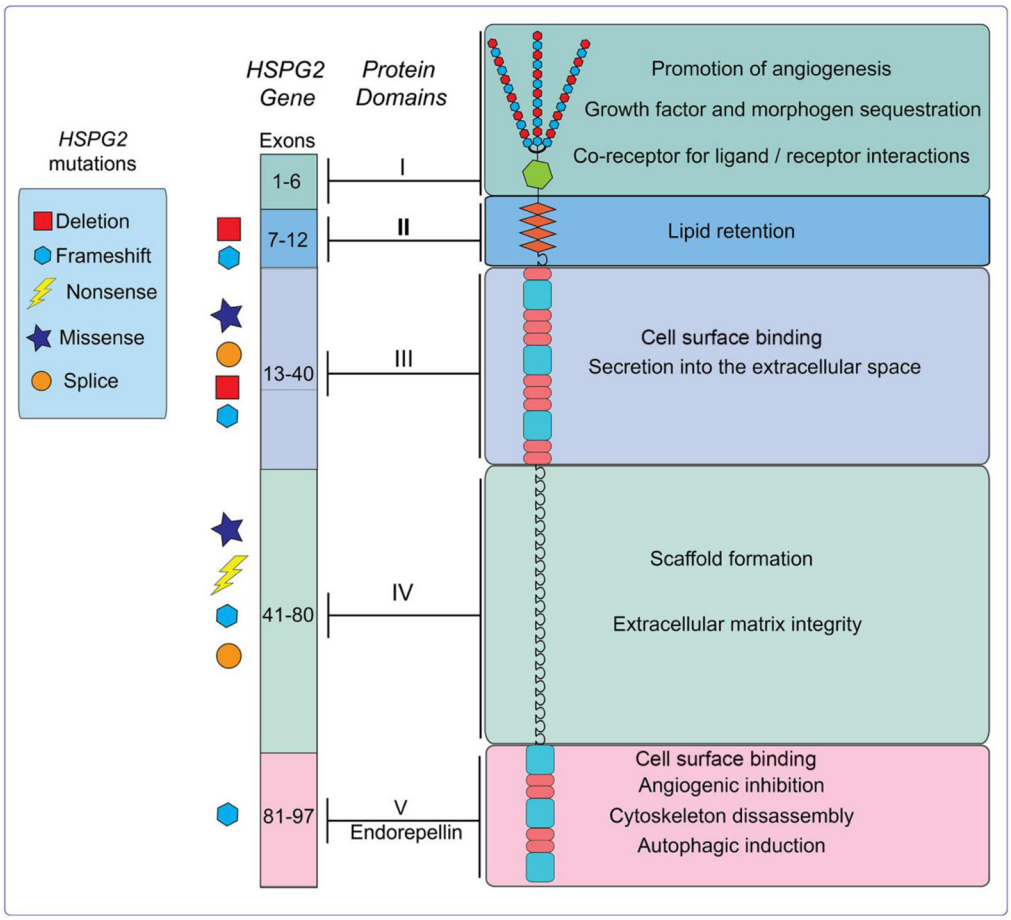

Fig. 1.

Graphical representation of the human perlecan gene, *HSPG2*, and protein. Reported disease-causing mutations in the gene are symbolically depicted to the left of the exons. Individual domains of the protein are highlighted and grouped with their corresponding functions.

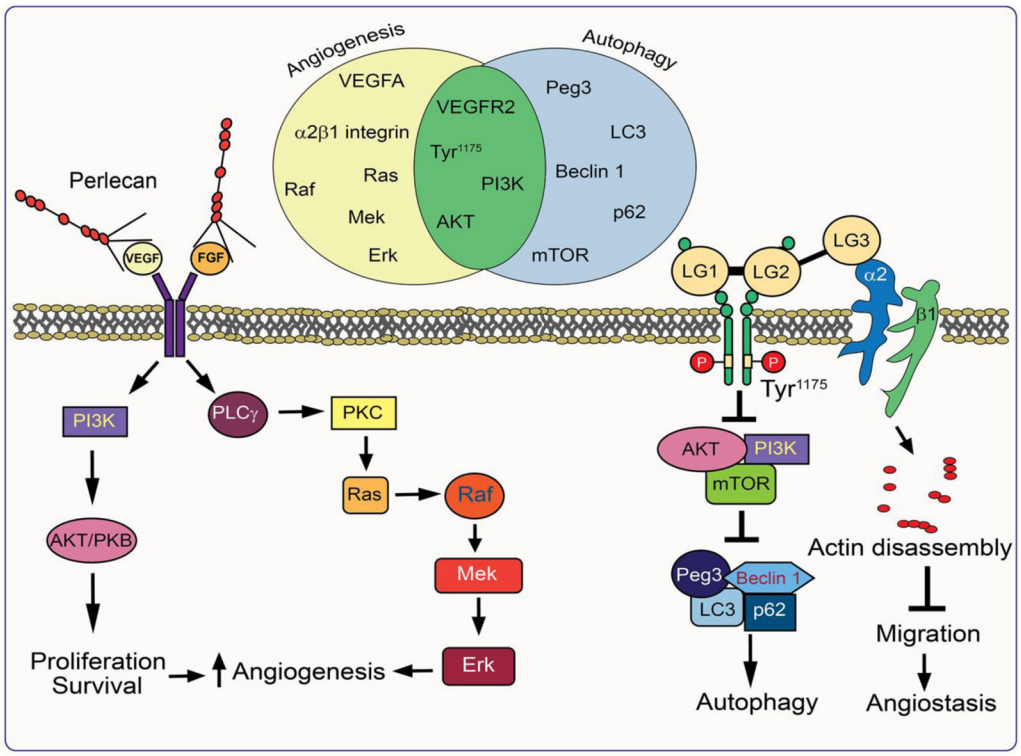

Fig. 2.

Schematic diagram demonstrating the mechanism of action of perlecan and endorepellin in angiogenesis and autophagy. Note the overlap between some of the signaling pathway intermediates as illustrated by the Venn diagram.

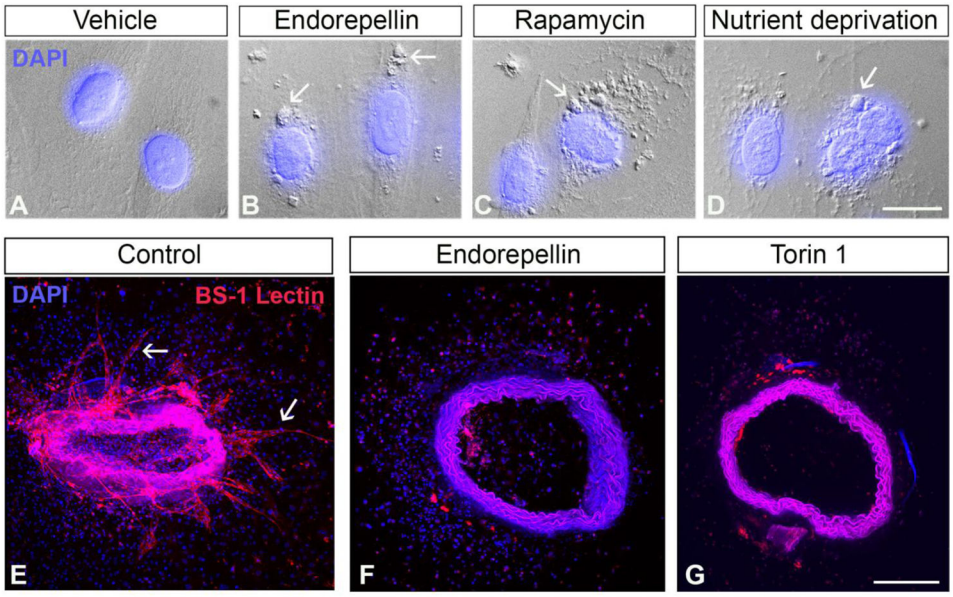

Fig. 3.

Autophagy may be a mechanism by which endorepellin curtails angiogenesis. (**A–D**) Differential interference contrast microscopy images of HUVEC under specified conditions. Note the presence of autophagosome-like structures (white arrows) in the endorepellin, rapamycin, and nutrient-deprived cells Bar ~ 15 μm. (**E–G**) Representative images of murine aortic rings treated with vehicle, endorepellin, or Torin 1. Note the sprouts in the control ring (white arrows) and the absence of sprouts following endorepellin and Torin 1 treatment. Bar ~ 100 μm.

Table 1

Tabulated snapshot of mutations of perlecan in various model organisms and resultant phenotypes and clinical manifestations

Model organism	Gene symbol	Mutation	Phenotype	Reference
C. elegans	*unc-52*	Chemical mutagenesis	Abnormal gonadal leader cell migration due to disruptions in the UNC-6/netrin system	[104]
		Non-sense mutations in diverse splice variants	Systemic paralysis and alterations in muscle morphology	[106]
D. melanogaster	*trol*	P-element insertion	Neural proliferative failure and deficient neural stem cells deriving from disrupted FGF2 and Shh signaling pathways	[107,108]
D. rerio	*Hspg2*	Morpholino-mediated knockdown	Myopathy, disorganized sarcomeres, reduced extension of vessel sprouts; mis-localized VEGFA	[34]
M. musculus	*Hspg2*	Global knockout	Embryonic lethality due to respiratory failure, pericardial hemorrhage, transposition of great vessels, malformation of coronary arteries, chondrodysplasia	[112]
	Hspg2 Δ3/Δ3	Exon 3 deletion	Loss of proper corneal structure, arterial narrowing, delayed wound healing; impaired angiogenesis and tumorigenesis; reduced heparan sulfate content	[116–119]
	Hspg2−/−;Tg	Global knockout with re- introduction in cartilage to prevent lethality	Impaired vascular relaxation, enhanced unloading-induced atrophy, increased autophagy	[36,120]
H. sapiens	*HSPG2*	Duplicated exon 34 and frameshifts; functionally null	Dyssegmental dysplasia, Silver-Handmaker type due to altered FGF2 signaling leading to aberrant cartilage architecture	[121,123]
		Missense and errors in alternative splicing resulting in truncated or ablated domain V	Schwartz-Jampel syndrome originating from disrupted neuromuscular junction arrangement and function	[82,122]