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Novel Interactions of Perlecan: Unraveling Perlecan's Role in Angiogenesis

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Abstract

Perlecan, a highly conserved and ubiquitous basement membrane heparan sulfate proteoglycan, is essential for life, inasmuch as its absence results in embryonic lethality in mice and *C. elegans*, and neonatal lethality in humans. Perlecan plays an essential role in vasculogenesis and chondrogenesis, as well as in pathological states where these processes are maladapted. Although a large body of evidence supports a pro-angiogenic role for perlecan, recent findings suggests that portions of the perlecan protein core can be antiangiogenic, requiring a further evaluation of the functioning of this complex molecule. This review is focused on the genetics of mammalian and nonmammalian perlecan, the elucidation of its novel interacting partners and its role in angiogenesis. By more fully understanding perlecan's functioning in angiogenesis, we may gain invaluable insight that could lead to therapeutic interventions in cancer and other pathologic states.

Keywords

endorepellin; heparan sulfate proteoglycan; extracellular matrix; $\alpha 2\beta 1$ integrin

INTRODUCTION

Perlecan, a highly conserved modular heparan sulfate proteoglycan (HSPG), is one of the most abundant HSPGs of the vertebrate extracellular matrix (ECM). Its eponym reflects a “beads on a string” domain structure (Noonan and Hassell, 1993) as visualized by rotary shadowing electron microscopy (Paulsson et al., 1987). It has a preferred localization to vascular basement membranes (BMs) (Iozzo et al., 1994; Timpl, 1993), thin sheets of ECM that separate endothelial cells from surrounding connective tissue (Fig. 1). However, perlecan is also located at epithelial BMs and cell surfaces (Iozzo, 1984), to which it is likely bound via specific integrin receptors (Battaglia et al., 1993; Hayashi et al., 1992), diffusely in ovarian stroma (Fig. 1), or in avascular cartilage (SundarRaj et al., 1995). In conjunction with its ubiquity in BMs, perlecan plays a significant role in key cellular processes from ontogeny to adulthood, including roles in cell growth, differentiation, development, and

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inflammation (Handler et al., 1997; Hassell et al., 2003; Murdoch and Iozzo, 1993; Peng et al., 1999; Rops et al., 2004). In particular, perlecan appears to play a prominent role in the development of blood vessels (vasculogenesis), the heart, cartilage (chondrogenesis), and the nervous system (Arikawa-Hirasawa et al., 1999; Costell et al., 1999). Perlecan has also been shown to bind various growth factors (Aviezer et al., 1994; Iozzo, 1998), and BM proteins including nidogen, laminin, and several types of collagen (Hopf et al., 1999; Knox and Whitelock, 2006; Whitelock et al., 1999).

Evidence from humans, mice, *Drosophila*, and *C. elegans* further establishes the significance of perlecan as a fundamental biological entity (Iozzo, 2005; Whitelock and Iozzo, 2005). More recently, perlecan has been implicated in angiogenesis, the process of new blood vessel formation from preexisting blood vessels (Iozzo and San Antonio, 2001). This review, after briefly describing the structure and basic genetics of perlecan, will focus on our expanding knowledge of perlecan functional interactions and how these interactions fit into a role for perlecan in angiogenesis.

PERLECAN PROTEIN CORE: A COLLAGE OF PROTEIN MODULES

A single gene encodes mammalian and nonmammalian perlecan, and it is located on the short arm of human chromosome 1 (1p36) (Dodge et al., 1991; Kallunki et al., 1991) and on a syntenic region of mouse chromosome 4 (Chakravarti et al., 1991). Over a decade ago, we cloned the human gene, named HSPG2, which spans ~120 kb of contiguous DNA resulting in a very large message of ~14.5 kb (Cohen et al., 1993). The human perlecan promoter has been partially characterized and shown to be induced by TGF- β (Iozzo et al., 1997) and suppressed by interferon- γ (Sharma and Iozzo, 1998), two factors intimately associated with tumor growth and angiogenesis. Originally, it was thought that HSPG2 comprised 94 exons; however, a recent working draft of human chromosome 1 revealed the presence of an additional three exons, bringing the total number of exons to 97 (GeneBank no. NT_004576). There is a remarkable conservation of the intron/exon organization when compared with that of homologous genes, that is, genes that encode one or more modules with features similar to those present in the perlecan gene. This suggests that the perlecan gene has evolved by utilizing exon shuffling and gene duplication of a common ancestor. Indeed, mammalian perlecan core protein is a “collage” of protein modules utilized by various classes of gene products involved in lipid uptake, growth control, and adhesion.

Perlecan is composed of five domains, each with sequence homology to other proteins (Kallunki and Tryggvason, 1992; Murdoch et al., 1992; Noonan et al., 1991). Perlecan homologues in *Drosophila* and *C. elegans* have also been identified. *Drosophila* perlecan is encoded by the *trol* (terribly reduced optical lobes) gene, whereas *C. elegans* perlecan is encoded by *unc-52* (uncoordinated phenotype) gene located on chromosome 2 (see below). Figure 2 schematically shows mammalian and nonmammalian perlecan core protein structures. The N-terminal domain I contains an SEA (Sperm protein, Enterokinase, Agrin) module and three, closely-spaced SGD (Ser-Gly-Asp) tripeptide sequences to which HS chains covalently attach (specifically to the Ser residue). The SEA module is unique to perlecan, has no invertebrate counterpart, and has no known function other than enhancing O-linked glycosylation and heparan sulfate attachment (Dolan et al., 1997). Domain II

possesses homology to the class A low-density lipoprotein (LDL) receptor (LA) containing four of these modules and one immunoglobulin (Ig)-like repeat. Rotary shadowing electron microscopy of mouse recombinant domain II reveals a globular structure connected to a short rod-like segment of various length (Costell et al., 1996). Chicken domain II is capable of binding LDL and very low-density lipoprotein (VLDL) (Hummel et al., 2004).

Domain III contains three laminin-like domain modules (L4) and eight epidermal growth factor-like repeats (LE). Of note, mouse, but not human domain III, has been demonstrated to have an RGDS (Arg-Gly-Asp-Ser) sequence, a conserved sequence that promotes cell binding via integrin receptors (Chakravarti et al., 1995). However, the functional integrin binding ability of this murine domain III RGD sequence remains somewhat controversial (Battaglia et al., 1993; Chakravarti et al., 1995; Schulze et al., 1996). Domain IV, the largest domain, contains N-CAM-like Ig repeats whose number is species-dependent. The C-terminal domain V contains three laminin globular (LG) domains separated by four EGF like repeats (EG). Domain V has been demonstrated to be the major cell-binding domain and also is potentially a very interactive protein module because of its laminin and agrin homologies (Brown et al., 1997; Friedrich et al., 1999). Additionally, murine domain V contains alternative glycosaminoglycan attachment sites (Tapanadechopone et al., 1999; Timpl, 1993). *Drosophila* perlecan lacks domain I, but harbors a large number of LA modules (22 units) and two Ig-like repeats in domain II (Fig. 2). The nematode perlecan also lacks a homologous domain I, but it contains instead an Asn-rich module. Overall, mammalian perlecan domains II–V are remarkably similar to their nonmammalian counterparts, whereas domain I has no counterpart (Fig. 2). In addition, the nematode perlecan (*unc-52*) undergoes extensive and complex alternative splicing giving rise to large number of isoforms, which can be classified into three distinct groups: (1) Long isoforms, which contain all five domains, (2) Medium isoforms, which contain the first four domains, and (3) Short isoforms that contain only the first three domains (Mullen et al., 1999).

MAMMALIAN PERLECAN GENETICS

Given the high conservation of perlecan across species, its ubiquitous distribution, and the fact that it is a single prototype family member gene, it is not surprising that “life without perlecan has its problems” (Olsen, 1999) (Table 1). In mice, perlecan is expressed in tissues undergoing vasculogenesis at embryonic day 10.5 (E10.5). By E11–E13, more expression can be seen in developing cartilage, and by E13–17.5 perlecan is seen in conjunction with the development of other internal organs (Handler et al., 1997). Absence of perlecan in mice frequently results in embryonic lethality at E10–12 with severe embryonic cephalic and cartilage abnormalities (Arikawa-Hirasawa et al., 1999; Costell et al., 1999), although BMs are still able to develop. The majority of *Hspg2*^{−/−} mice develop severe chondrodysplasia and die of respiratory failure just after birth. This is in agreement with a chondrogenic role for perlecan in vitro (French et al., 1999, 2002). The phenotype of perlecan null animals is quite complex insofar as other reports have shown that many of the embryos that survive to E17.5 and later stages possess malformations of the cardiac outflow tract with complete transposition of the great vessels in the vast majority (Costell et al., 2002), as well as abnormal coronary arteries (González-Iriarte et al., 2003). Interestingly, *Hspg2*^{−/−} mice do

not have acetylcholinesterase (AChE) in their neuromuscular junctions because of the lack of perlecan/AChE binding (Arikawa-Hirasawa et al., 2002b).

In humans, two rare skeletal disorders, the severe neonatal lethal dyssegmental dysplasia, Silverman-Handmaker type (DDSH), and the relatively mild Schwartz-Jampel syndrome (SJS), are both due to mutations in HSPG2 gene encoding perlecan (Arikawa-Hirasawa et al., 2001a,b; 2002a; Nicole et al., 2000; Stum et al., 2006). In DDSH patients, truncated perlecan molecules are not properly secreted into the extracellular space, indicating that functional null mutations of HSPG2 gene cause DDSH (Arikawa-Hirasawa et al., 2001a). DDSH is a lethal autosomal recessive disorder that causes skeletal dysplasia characterized by ankylospondyly and micromelia, among other malformations. In contrast, patients with SJS, a nonlethal, autosomal recessive disorder, secrete truncated forms of perlecan lacking domain V or parts of domain V (35- or 64-amino acid deletions from the C-terminus of domain V), and these levels are relatively lower than normal patients (Arikawa-Hirasawa et al., 2002a; Nicole et al., 2000; Stum et al., 2006). The SJS phenotype is complex and includes varying degrees of myotonia, conceivably caused by AChE absence from the neuromuscular junctions, and chondrodysplasia. The mutations of the perlecan gene are quite numerous in SJS patients and all the aberrant splicing products predict the generation of premature termination codons, thereby generating truncated perlecan protein cores (Arikawa-Hirasawa et al., 2002a). However, there is a common theme: perlecan molecules are secreted and partially functional. Thus, phenotypic differences between DDSH and SJS patients are due to “quantitative” changes, that is, the phenotype severity depends on the amount of functional perlecan in the matrix.

NONMAMMALIAN PERLECAN GENETICS

The *Drosophila* perlecan (*trol*) is developmentally expressed at high levels around the visceral mesoderm, the hindgut, and the central nervous system, and via evidence from *trol*^{sd} mutants (a lethal mutation), is thought to play a role in neuroblast proliferation (Caldwell and Datta, 1998; Datta, 1995; Voigt et al., 2002). Recently, *Drosophila* perlecan has been shown to activate neuroblast division by modulating fibroblast growth factor (FGF) and Hedgehog signals (Park et al., 2003). Addition of mammalian *FGF2* to *trol* mutant brains in culture rescues the *trol* proliferation phenotype. Moreover, biochemical studies suggest a potential interaction between perlecan and Hedgehog proteins in both flies and mammals, a binding that is not affected by exogenous heparin (Park et al., 2003). Thus, it is possible that some of the phenotypic changes that occur in mammalian *Hspg2*^{−/−} animals might also be due to abnormal signaling through FGF and Hedgehog pathways.

Contrasting with mammalian perlecan which, as aforementioned, is quite ubiquitous, *C. elegans* perlecan (*unc-52*) has a limited distribution; that is, it is specifically localized to muscle tissues (Mullen et al., 1999) which express *unc-52* in an autonomous way (Moerman et al., 1996). Mutations in *unc-52* suggest that it plays an important role in myofilament assembly in the body wall muscle (Mullen et al., 1999; Rogalski et al., 1993, 1995). Null mutations result in the lethal Pat (Paralyzed, arrested elongation at twofold) phenotype, whereas various other mutations that affect some, but not all, isoforms of *unc-52* result in normally moving larvae and paralyzed adults due to progressive disruption of the body wall

at third-fourth larval stage (Gilchrist and Moerman, 1992). In the severely affected *unc-52* muscles, myosin forms large aggregates instead of orderly arrays of myofilaments. Furthermore, *unc-52* mutations have recently been demonstrated to affect the migration of distal tip cells that ultimately form gonad arms in the hermaphrodite *C. elegans* by misregulation of growth factor signaling (Merz et al., 2003). Thus, in *C. elegans*, perlecan has a dual role during larval development in maintaining proper myofilament lattice assembly and in regulating growth factor signaling pathways.

PERLECAN AND TUMOR ANGIOGENESIS

It is well established that HSPGs are implicated in cancer development, invasion, and metastasis (Bix and Iozzo, 2005; Esko et al., 1988; Iozzo, 2001; Sanderson, 2001; Timár et al., 2002; Whitelock and Iozzo, 2005). In addition to the evidence for a major role of perlecan in vasculogenesis derived from the analysis of the perlecan null mice, a growing body of literature suggests that perlecan plays a direct role in tumor progression and angiogenesis (Iozzo and San Antonio, 2001; Jiang and Couchman, 2003; Jiang et al., 2004; Tapanadechopone et al., 2001). For example, perlecan has been demonstrated to stimulate angiogenesis in a rabbit ear model (Aviezer et al., 1994), while the antiangiogenic antithrombin has been demonstrated to down-regulate endothelial cell perlecan expression (Zhang et al., 2004). Inasmuch as angiogenesis allows tumors to expand beyond a few millimeters by providing oxygen, nutrients, and waste disposal at distances greater than allowed by diffusion (Carmeliet, 2003; Folkman, 2002), highly expressed bioactive compounds found at tumor peripheries, such as perlecan, could conceivably play an important role in tumor cell-mediated angiogenesis (Jiang and Couchman, 2003). Perlecan protein core is found in abundance in blood vessel walls of many human primary tumors (Fig. 1) (Iozzo, 1994). Furthermore, down-regulation of tumor cell perlecan results in inhibited tumor growth and angiogenesis (Sharma et al., 1998), and tumor-derived perlecan has been recently suggested to exert greater control over tumor growth and angiogenesis than animal host-derived perlecan (Jiang and Couchman, 2003). Perlecan knockdown in metastatic prostate cancer cells reduces the responses to FGF2 and VEGF and in vivo tumor growth (Savore et al., 2005). Seemingly, perlecan and sonic hedgehog (Shh) proteins colocalize in aggressive prostate carcinomas and knockdown of perlecan in these cells leads to an attenuation of cell proliferation and Shh signaling (Datta et al., 2006a,b). Finally, increased perlecan expression in breast and colon carcinomas (Iozzo et al., 1994), and metastatic melanomas (Cohen et al., 1994), correlates with enhanced metastatic potential. Thus, perlecan possesses all the attributes for a molecule involved in angiogenesis, including its ability to modulate growth and pro-angiogenic factors (such as FGF and VEGF family members) and its strategic location at the interface between proliferating tumor and migrating endothelial cells.

PERLECAN INTERACTIONS WITH GROWTH FACTORS AND OTHER MEDIATORS OF ANGIOGENESIS

A way by which perlecan might modulate angiogenesis is via its interactions, through its protein core or HS chains, with growth factors (Table 2). The concept that endothelial cell

HS chains bind FGF2 and protect it from proteolytic degradation was put forward by Rifkin and collaborators in the late eighties (Saksela and Rifkin, 1990; Saksela et al., 1988). Perlecan interacts with various forms of FGF, powerful promoters of growth and angiogenesis found largely in areas of active tissue remodeling and tumor invasion (Faham et al., 1998; Nugent and Iozzo, 2000). Indeed, perlecan is a major candidate for the FGF2 low-affinity receptor (Aviezer et al., 1994) and is involved in the binding of FGF2 within the BM (Vigny et al., 1988). Moreover, exogenous perlecan restores FGF2 high-affinity binding to HS-deficient cells (Aviezer et al., 1994). In human melanoma cells, stable suppression of endogenous perlecan causes a concurrent suppression of autocrine and paracrine functions of FGF2 (Aviezer et al., 1997) as well as melanoma cell proliferation and invasion (Adatia et al., 1998). In addition to binding via HS side chains, the N-terminal portion of perlecan domain III protein core, and to a lesser extent domain V protein core, bind FGF7 (Mongiat et al., 2000), a product of stromal cells that affects, in a paracrine fashion, adjacent epithelial cells in vivo (Aaronson et al., 1991). Additionally, domain V protein core can interact with progranulin (Gonzalez et al., 2003), a bioactive precursor protein involved in wound healing and angiogenesis (He and Bateman, 1999). We have also recently demonstrated that domain V can modulate platelet responses, which play an increasingly recognized role in angiogenesis, to collagen (Bix et al., 2007). Perlecan has also been demonstrated to bind to platelet-derived growth factor (PDGF) (Göhring et al., 1998), a potent mitogen and chemo-attractant for connective tissue cells (Westermarck and Heldin, 1993). Collectively, by binding and protecting these various growth factors from degradation and misfolding (Knox et al., 2002; Whitelock et al., 1996), perlecan could modulate angiogenesis and tumor growth (Iozzo, 2001).

In addition to binding growth factors, perlecan has varying degrees of affinity for several ECM components, growth promoting proteins, and transmembrane receptors (Fuki et al., 2000; Iozzo, 1998; Peng et al., 1999; Timpl, 1993; Whitelock et al., 1999). Domain I of perlecan interacts with thrombospondin (Vischer et al., 1997), a matricellular protein involved in the regulation of cell adhesion, migration, and proliferation in many different cells (Armstrong and Bornstein, 2003). Domain I and Domain II interact with the fibrillin-1 component of ECM microfibrils at the BM, which may serve to anchor microfibrils to the BM (Tiedemann et al., 2005). Domain III of human perlecan interacts with FGF-BP, a secreted protein that modulates FGF activity (Mongiat et al., 2001). Domain V of perlecan binds to secreted ECM protein-1, a glycoprotein involved in bone formation and angiogenesis (Mongiat et al., 2003a). Moreover, perlecan protein core in the ECM of cultured lung fibroblasts interacts with the matrix macromolecule fibronectin (Heremans et al., 1990). The HS-containing domains of murine perlecan have also been demonstrated to interact with the N-terminal domain of the laminin $\alpha 1$ chain (Ettner et al., 1998). Perlecan also binds to collagens type I, III, IV, and V (Villar et al., 1999; Whitelock et al., 1999) as well as to the nonfibrillar collagen type XIII (Tu et al., 2002), a transmembrane collagen involved in cell adhesion events. The HS chains of murine perlecan domain I and domain V bind to PRELP, a leucine-rich repeat ECM protein thought to function as a BM anchor (Bengtsson et al., 2002). Domain V of murine perlecan also interacts with fibulin-2, an ECM protein, nidogen, a central BM scaffolding component, the $\beta 1$ integrin, a transmembrane cell adhesion protein (Brown et al., 1997), and α -dystroglycan (the LG1 and LG2 domains

account for most of the high affinity binding), a transmembrane protein with important ECM and cytoskeletal interactions (Peng et al., 1999).

PERLECAN FRAGMENTS AS INHIBITORS OF ANGIOGENESIS

Because of perlecan's interactions with growth factors and various growth promoting and adhesive structures (Dunlevy and Hassell, 2000; Klein et al., 1995; Whitelock et al., 1999), as well as a demonstrated growth-related presence and function in many tumors, perlecan is typically described as a pro-angiogenic and tumor-growth promoting HSPG (Iozzo and San Antonio, 2001; Jiang and Couchman, 2003). However, some studies have suggested that the specific cellular context may play a key role in perlecan's biological role in angiogenesis. For example, in fibrosarcoma cells, perlecan acts as a negative regulator of growth and invasion (Mathiak et al., 1997). In human Kaposi's sarcoma cells, reduction of perlecan expression has the paradoxical effect of reducing tumor cell proliferation in vitro, whereas stimulating tumor growth in vivo (Marchisone et al., 2000). Furthermore, perlecan is required to inhibit thrombosis after deep vascular injury and contributes to endothelial cell-mediated inhibition of intimal hyperplasia (Nugent et al., 2000). It has been known for a long time that exogenous heparin and heparan sulfate from endothelial cells prevent the proliferation of vascular smooth muscle cells (Benitz et al., 1990; Clowes and Karnovsky, 1977; Nugent et al., 1993). Only recently it has been demonstrated that perlecan itself inhibits the proliferation of smooth muscle cells (Garl et al., 2004; Walker et al., 2003) and that altered perlecan gene expression plays a direct role in the development of late atherosclerotic lesions (Kinsella et al., 2003). Moreover, transgenic animals that express a mutated perlecan lacking the N-terminal HS chains [these animals lack exon 3 in the *Hspg2* gene and thus lack the three SGD sequences to which HS chains are covalently attached (Rossi et al., 2003)] exhibit an enhanced intimal hyperplasia and smooth muscle cell proliferation following carotid artery injury (Tran et al., 2004). Thus, altered expression of perlecan could significantly contribute to blood vessel remodeling in various pathological conditions, including cancer growth and metastasis. It should be pointed out, however, that one report showed perlecan-dependent growth stimulation of vascular smooth muscle cells (Whitelock et al., 1997), and another report demonstrated that cultured apoptotic endothelial cells release a C-terminal fragment of perlecan domain V that inhibits vascular smooth muscle cell apoptosis (Raymond et al., 2004) suggesting that the in vivo regulation of perlecan expression and its proteolytic fragments might have diverse effects depending on the cellular context and the specific pathological process.

In our search for novel binding partners of human perlecan, we discovered that recombinant domain V, at low nanomolar concentrations, potently inhibited angiogenesis (Fig. 3). Accordingly, we renamed it "endorepellin" to denote its antiendothelial effects (Mongiat et al., 2003b). Endorepellin was active in various models of angiogenesis including: endothelial cell migration through a collagenous matrix, collagen-induced endothelial capillary morphogenesis, and blood vessel growth in the chorioallantoic membrane, and Matrigel plug assays (Mongiat et al., 2003b). Recently, we demonstrated that endorepellin is capable of inhibiting tumor growth when administered in vivo (Bix et al., 2006). In these studies, endorepellin inhibited solid tumor angiogenesis and was surprisingly found to localize around the remaining tumor vasculature. This raises the intriguing possibility that

endorepellin possesses tumor-homing properties. Interestingly, the LG2 domain of endorepellin interacted with and counteracted the antiangiogenic effects of endostatin, an antiangiogenic portion of collagen XVIII (O'Reilly et al., 1997). Furthermore, endorepellin interfered with the adhesion of endothelial cells and tumor cells derived from colon, neuroectoderm, or mesenchyme to fibrillar collagen and fibronectin. On further examination of the mechanism of action of endorepellin, we discovered that endorepellin and its LG3 component, but not the LG1 or LG2 components, reversibly altered the endothelial cell actin cytoskeleton and focal adhesions (Bix et al., 2004). This calcium-dependent mechanism of action involves an initial stimulation of cAMP and protein kinase A, followed by stimulation of focal adhesion kinase (FAK), transient activation of p38 MAPK and Hsp27, which ultimately culminates in dissolution of actin stress fibers and focal adhesions. This mechanism of action is unique since, unlike endostatin, which disrupts the endothelial cell actin cytoskeleton through a HS-dependent $\alpha 5\beta 1$ integrin interaction (Wickström et al., 2003), endorepellin directly interacts with the $\alpha 2\beta 1$ integrin, independently of cell surface HS (Bix et al., 2004). This novel signaling cascade for an angiogenesis inhibitor is cell specific and limited to human umbilical vein and dermal microvascular endothelial cells. Endorepellin appears to inhibit angiogenesis in a dominant negative fashion since endothelial cells secrete perlecan. Furthermore, LG1 and LG2 do not by themselves possess antiangiogenic activity; thus, LG3 harbors most of antiangiogenic activity of endorepellin. Because LG1 and LG2, but not LG3, possess high affinity binding to other components (i.e., α -dystroglycan and endostatin), LG3 is relatively free of high affinity interactions within BMs, possibly allowing proteolytic cleavage to occur during the early stages of angiogenesis. Indeed, such an endogenous processing mechanism is common to most LG domains of laminin (Smirnov et al., 2002; Timpl et al., 2000) and there is evidence that LG3 is proteolytically cleaved from perlecan both in vitro (Mongiat et al., 2003b) and in vivo (Oda et al., 1996; Vuadens et al., 2003). We recently identified specific enzymes of the bone morphogenetic protein-1 (BMP-1)/Tolloid family of metalloproteases that cleave LG3 (at a physiologically relevant site) from recombinant endorepellin and from endogenous perlecan in cultured mouse and human cells (Gonzalez et al., 2005). Furthermore, other potent inhibitors of angiogenesis result from the proteolytic processing of BM components (Kalluri, 2003; Marnaros and Olsen, 2001; Sage, 1997). It is tempting to speculate that, in vivo, endorepellin/LG3 could be proteolytically liberated during angiogenesis that was "initially" induced and supported by perlecan and other factors. Liberated endorepellin/LG3, in conjunction with various other endogenous angiogenesis inhibitors, might then serve to reign in angiogenesis (Bix and Iozzo, 2005). In pathological states such as cancer, these anti-angiogenesis signals could be overwhelmed by an over-abundance of pro-angiogenic factors. Recently, two independent studies using $\alpha 2\beta 1$ null mice have shown an increased angiogenesis in the granulation tissues of wounded mutant mice (Grenache et al., 2006; Zweers et al., 2007). A potential explanation, among others, is that endogenous endorepellin would not be able to exert its function in the absence of $\alpha 2\beta 1$ integrin.

Whatever the ultimate in vivo role of endorepellin is determined to be, future studies of perlecan's interactions and role(s) in angiogenesis should provide important insights into this complex process that may lead to novel therapeutic interventions.

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REFERENCES

- Aaronson SA, Bottaro DP, Miki T, Ron D, Finch PW, Fleming TP, Ahn J, Taylor WG, Rubin JS. 1991 Keratinocyte growth factor. A fibroblast growth factor family member with unusual target cell specificity. *Ann NY Acad Sci* 638:62–77. [PubMed: 1664700]
- Adatia R, Albin A, Carlone S, Giunciuglio D, Benelli R, Santi L, Noonan DM. 1998 Suppression of invasive behavior of melanoma cells by stable expression of anti-sense perlecan cDNA. *Ann Oncol* 8:1257–1261.
- Arikawa-Hirasawa E, Watanabe E, Takami H, Hassell JR, Yamada Y. 1999 Perlecan is essential for cartilage and cephalic development. *Nat Genet* 23:354–358. [PubMed: 10545953]
- Arikawa-Hirasawa E, Wilcox WR, Le AH, Silverman N, Govindraj P, Hassell JR, Yamada Y. 2001a Dyssegmental dysplasia. Silverman-Handmaker type, is caused by functional null mutations of the perlecan gene. *Nat Genet* 27:431–434. [PubMed: 11279527]
- Arikawa-Hirasawa E, Rossi SG, Rotundo RL, Yamada Y. 2002b Absence of acetylcholinesterase at the neuromuscular junctions of perlecan-null mice. *Nat Neurosci* 5:119–123. [PubMed: 11802174]
- Arikawa-Hirasawa E, Le AH, Nishino I, Nonaka I, Ho NC, Franco- mano CA, Govindraj P, Hassell JR, Devaney JM, Spranger J, Stevenson RE, Iannaccone S, Dalakas MC, Yamada Y. 2002a Structural and functional mutations of the perlecan gene cause Schwartz-Jampel syndrome, with myotonic myopathy and chondrodysplasia. *Am J Hum Genet* 70:1368–1375. [PubMed: 11941538]
- Arikawa-Hirasawa E, Wilcox WR, Yamada Y. 2001b Dyssegmental dysplasia. Silverman-Handmaker type: Unexpected role of perlecan in cartilage development. *Am J Med Genet* 106:254–257. [PubMed: 11891676]
- Armstrong LC, Bornstein P. 2003 Thrombospondins 1 and 2 function as inhibitors of angiogenesis. *Matrix Biol* 22:63–71. [PubMed: 12714043]
- Aviezer D, Hecht D, Safran M, Eisinger M, David G, Yayon A. 1994 Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth factor-receptor binding, mitogenesis, and angiogenesis. *Cell* 79:1005–1013. [PubMed: 7528102]
- Aviezer D, Iozzo RV, Noonan DM, Yayon A. 1997 Suppression of autocrine and paracrine functions of basic fibroblast growth factor by stable expression of perlecan antisense cDNA. *Mol Cell Biol* 17:1938–1946. [PubMed: 9121441]
- Battaglia C, Aumailley M, Mann K, Mayer U, Timpl R. 1993 Structural basis of $\beta 1$ integrin-mediated cell adhesion to a large heparan sulfate proteoglycan from basement membranes. *Eur J Cell Biol* 61:92–99. [PubMed: 7693469]
- Bengtsson E, Mörgelin M, Sasaki T, Timpl R, Heinegård D, Aspberg A. 2002 The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor. *J Biol Chem* 277:15061–15068. [PubMed: 11847210]
- Benitz WE, Kelley RT, Anderson CM, Lorant DE, Bernfield M. 1990 Endothelial heparan sulfate proteoglycan. I. Inhibitory effects on smooth muscle cell proliferation. *Am J Resp Cell Mol Biol* 2:13–24.
- Bix G, Iozzo RV. 2005 Matrix revolutions: “Tails” of basement-membrane components with angiostatic functions. *Trends Cell Biol* 15: 52–60. [PubMed: 15653078]
- Bix G, Fu J, Gonzalez E, Macro L, Barker A, Campbell S, Zutter MM, Santoro SA, Kim JK, Höök M, Reed CC, Iozzo RV. 2004 Endorepellin causes endothelial cell disassembly of actin cytoskeleton and focal adhesions through the $\alpha 2\beta 1$ integrin. *J Cell Biol* 166:97–109. [PubMed: 15240572]
- Bix G, Castello R, Burrows M, Zoeller JJ, Weech M, Iozzo RA, Cardi C, Thakur MT, Barker CA, Camphausen KC, Iozzo RV. 2006 Endorepellin in vivo: Targeting the tumor vasculature and retarding cancer growth and metabolism. *J Natl Cancer Inst* 98:1634–1646. [PubMed: 17105986]

- Bix G, Iozzo RA, Woodall B, Burrows M, McQuillan A, Campbell S, Fields GB, Iozzo RV. 2007 Endorepellin, the C-terminal angiostatic module of perlecan, enhances collagen-platelet responses via the $\alpha 2\beta 1$ integrin receptor. *Blood* 109:3745–3748. [PubMed: 17197432]
- Brown JC, Sasaki T, Göhring W, Yamada E, Timpl R. 1997 The C-terminal domain V of perlecan promotes $\beta 1$ integrin-mediated cell adhesion, binds heparin, nidogen and fibulin-2 and can be modified by glycosaminoglycans. *Eur J Biochem* 250:39–46. [PubMed: 9431988]
- Caldwell MC, Datta S. 1998 Expression of cyclin E or DP/E2F rescues the G1 arrest of trol mutant neuroblasts in the Drosophila larval central nervous system. *Mech Dev* 79:121–130. [PubMed: 10349625]
- Carmeliet P. 2003 Angiogenesis in health and disease. *Nat Med* 9: 653–660. [PubMed: 12778163]
- Chakravarti S, Phillips SL, Hassell JR. 1991 Assignment of the perlecan (heparan sulfate proteoglycan) gene to mouse chromosome 4. *Mamm Genome* 1:270–272.
- Chakravarti S, Horchar T, Jefferson B, Laurie GW, Hassell JR. 1995 Recombinant domain III of perlecan promotes cell attachment through its RGDS sequence. *J Biol Chem* 270:404–409. [PubMed: 7814401]
- Clowes AW, Karnovsky MJ. 1977 Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature* 265:625–626. [PubMed: 859561]
- Cohen IR, Grässel S, Murdoch AD, Iozzo RV. 1993 Structural characterization of the complete human perlecan gene and its promoter. *Proc Natl Acad Sci USA* 90:10404–10408. [PubMed: 8234307]
- Cohen IR, Murdoch AD, Naso MF, Marchetti D, Berd D, Iozzo RV. 1994 Abnormal expression of perlecan proteoglycan in metastatic melanomas. *Cancer Res* 54:5771–5774. [PubMed: 7954396]
- Costell M, Sasaki T, Mann K, Yamada Y, Timpl R. 1996 Structural characterization of recombinant domain II of the basement membrane proteoglycan perlecan. *FEBS Lett* 396:127–131. [PubMed: 8914972]
- Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R. 1999 Perlecan maintains the integrity of cartilage and some basement membranes. *J Cell Biol* 147:1109–1122. [PubMed: 10579729]
- Costell M, Carmona R, Gustafsson E, Gonzalez-Iriarte M, Fässler R, Munoz-Chápuli R. 2002 Hyperplastic conotruncal endocardial cushions and transposition of great arteries in perlecan-null mice. *Circ Res* 91:158–164. [PubMed: 12142349]
- Datta MW, Hernandez AM, Schlicht MJ, Kahler AJ, DeGueme AM, Dhir R, Shah RB, Farach-Carson C, Barrett A, Datta S. 2006a Perlecan, a candidate gene for the CAPB locus, regulates prostate cancer cell growth via the Sonic Hedgehog pathway. *Mol Cancer* 5:9. [PubMed: 16507112]
- Datta S. 1995 Control of proliferation activation in quiescent neuroblasts of the Drosophila central nervous system. *Development* 121:1173–1182. [PubMed: 7743929]
- Datta S, Kankel DR. 1992 *l(1)trol* and *l(1)devl*, loci affecting the development of the adult central nervous system in Drosophila melanogaster. *Genetics* 130:523–537. [PubMed: 1551575]
- Datta S, Pierce M, Datta MW. 2006b Perlecan signaling: Helping hedgehog stimulate prostate cancer growth. *Int J Biochem Cell Biol* 38:1855–1861. [PubMed: 16750652]
- Dodge GR, Kovalszky I, Chu M-L, Hassell JR, McBride OW, Yi HF, Iozzo RV. 1991 Heparan sulfate proteoglycan of human colon: Partial molecular cloning, cellular expression, and mapping of the gene (HSPG2) to the short arm of human chromosome 1. *Genomics* 10:673–680. [PubMed: 1679749]
- Dolan M, Horchar T, Rigatti B, Hassell JR. 1997 Identification of sites in domain I of perlecan that regulate heparan sulfate synthesis. *J Biol Chem* 272:4316–4322. [PubMed: 9020150]
- Dunlevy JR, Hassell JR. 2000 Heparan sulfate proteoglycans in basement membranes: Perlecan, agrin and collagen XVIII In: Iozzo RV, editor. *Proteoglycans: Structure, biology and molecular interactions*. New York: Marcel Dekker pp. 275–326.
- Esko JD, Rostand KS, Weinke JL. 1988 Tumor formation dependent on proteoglycan biosynthesis. *Science* 241:1092–1096. [PubMed: 3137658]
- Ettner N, Göhring W, Sasaki T, Mann K, Timpl R. 1998 The N-terminal globular domain of the laminin $\alpha 1$ chain binds to $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins and to the heparan sulfate-containing domains of perlecan. *FEBS Lett* 430:217–221. [PubMed: 9688542]

- Faham S, Linhardt RJ, Rees DC. 1998 Diversity does make a difference: Fibroblast growth factor-heparin interactions. *Curr Opin Struct Biol* 8:578–586. [PubMed: 9818261]
- Folkman J. 2002 Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 29:15–18.
- French MM, Smith SE, Akanbi K, Sanford T, Hecht J, Farach-Carson MC, Carson DD. 1999 Expression of the heparan sulfate proteoglycan, perlecan, during mouse embryogenesis and perlecan chondrogenic activity in vitro. *J Cell Biol* 145:1103–1115. [PubMed: 10352025]
- French MM, Gomes RR Jr, Timpl R, Höök M, Czymmek K, Farach-Carson MC, Carson DD. 2002 Chondrogenic activity of the human heparan sulfate proteoglycan perlecan maps to the N-terminal domain I. *J Bone Miner Res* 17:48–55. [PubMed: 11771669]
- Friedrich MVK, Göhring W, Mörgelin M, Brancaccio A, David G, Timpl R. 1999 Structural basis of glycosaminoglycan modification and of heterotypic interactions of perlecan domain V. *J Mol Biol* 294:259–270. [PubMed: 10556044]
- Fuki I, Iozzo RV, Williams KJ. 2000 Perlecan heparan sulfate proteoglycan. A novel receptor that mediates a distinct pathway for ligand catabolism. *J Biol Chem* 275:25742–25750. [PubMed: 10818109]
- Garl PJ, Wenzlau JM, Walker HA, Whitelock JM, Costell M, Weiser- Evans MCM. 2004 Perlecan-induced suppression of smooth muscle cell proliferation is mediated through increased activity of the tumor suppressor PTEN. *Circ Res* 94:175–183. [PubMed: 14656929]
- Gilchrist EJ, Moerman DG. 1992 Mutations in the sup-38 gene of *Caenorhabditis elegans* suppress muscle-attachment defects in unc-52 mutants. *Genetics* 132:431–442. [PubMed: 1427037]
- Göhring W, Sasaki T, Heldin CH, Timpl R. 1998 Mapping of the binding of platelet-derived growth factor to distinct domains of the basement membrane proteins BM-40 and perlecan and distinction from the BM-40 collagen-binding epitope. *Eur J Biochem* 255:60–66. [PubMed: 9692901]
- Gonzalez EM, Mongiat M, Slater SJ, Baffa R, Iozzo RV. 2003 A novel interaction between perlecan protein core and progranulin: Potential effects on tumor growth. *J Biol Chem* 278:38113–38116. [PubMed: 12900424]
- Gonzalez EM, Reed CC, Bix G, Fu J, Zhang Y, Gopalakrishnan B, Greenspan DS, Iozzo RV. 2005 BMP-1/Tolloid-like metalloproteases process endorepellin, the angiostatic C-terminal fragment of perlecan. *J Biol Chem* 280:7080–7087. [PubMed: 15591058]
- González-Iriarte M, Carmona R, Pérez-Pomares JM, Maicas D, Costell M, Munoz-Chaápulí R. 2003 Development of the coronary arteries in a murine model of transposition of great arteries. *J Mol Cell Cardiol* 35:795–802. [PubMed: 12818570]
- Grenache DG, Zhang Z, Wells LE, Santoro SA, Davidson JM, Zutter MM. 2006 Wound healing in the $\alpha 2\beta 1$ integrin-deficient mouse: Altered keratinocyte biology and dysregulated matrix metalloproteinase expression. *J Invest Dermatol* 127:455–466. [PubMed: 17068473]
- Handler M, Yurchenco PD, Iozzo RV. 1997 Developmental expression of perlecan during murine embryogenesis. *Dev Dyn* 210:130–145. [PubMed: 9337134]
- Hassell JR, Yamada Y, Arikawa-Hirasawa E. 2003 Role of perlecan in skeletal development and diseases. *Glycoconj J* 19:263–267.
- Hayashi K, Madri JA, Yurchenco PD. 1992 Endothelial cells interact with the core protein of basement membrane perlecan through $\beta 1$ and $\beta 3$ integrins: An adhesion modulated by glycosaminoglycans. *J Cell Biol* 119:945–959. [PubMed: 1385448]
- He Z, Bateman A. 1999 Progranulin gene expression regulates epithelial cell growth and promotes tumor growth in vivo. *Cancer Res* 59:3222–3229. [PubMed: 10397269]
- Heremans A, De Cock B, Cassiman JJ, Van Den Berghe H, David G. 1990 The core protein of the matrix-associated heparan sulfate proteoglycan binds to fibronectin. *J Biol Chem* 265:8716–8724. [PubMed: 2140362]
- Hopf M, Göhring W, Kohfeldt E, Yamada Y, Timpl R. 1999 Recombinant domain IV of perlecan binds to nidogens, laminin-nidogen complex, fibronectin, fibulin-2 and heparin. *Eur J Biochem* 259:917–925. [PubMed: 10092882]
- Hummel S, Osanger A, Bajari TM, Balasubramani M, Halfter W, Nimpf J, Schneider WJ. 2004 Extracellular matrices of the avian ovarian follicle. Molecular characterization of chicken perlecan. *J Biol Chem* 279:23486–23494. [PubMed: 15016830]

- Iozzo RV. 1984 Biosynthesis of heparan sulfate proteoglycan by human colon carcinoma cells and its localization at the cell surface. *J Cell Biol* 99:403–417. [PubMed: 6235235]
- Iozzo RV. 1994 Perlecan: A gem of a proteoglycan. *Matrix Biol* 14: 203–208. [PubMed: 7921536]
- Iozzo RV. 1998 Matrix proteoglycans: From molecular design to cellular function. *Annu Rev Biochem* 67:609–652. [PubMed: 9759499]
- Iozzo RV. 2001 Heparan sulfate proteoglycans: Intricate molecules with intriguing functions. *J Clin Invest* 108:165–167. [PubMed: 11457866]
- Iozzo RV. 2005 Basement membrane proteoglycans: From cellar to ceiling. *Nature Rev Mol Cell Biol* 6:646–656. [PubMed: 16064139]
- Iozzo RV, San Antonio JD. 2001 Heparan sulfate proteoglycans: Heavy hitters in the angiogenesis arena. *J Clin Invest* 108:349–355. [PubMed: 11489925]
- Iozzo RV, Cohen IR, Grässel S, Murdoch AD. 1994 The biology of perlecan: The multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices. *Biochem J* 302:625–639. [PubMed: 7945186]
- Iozzo RV, Pillarisetti J, Sharma B, Murdoch AD, Danielson KG, Uitto J, Mauviel A. 1997 Structural and functional characterization of the human perlecan gene promoter. Transcriptional activation by transforming factor- β via a nuclear factor 1-binding element. *J Biol Chem* 272:5219–5228. [PubMed: 9030592]
- Jiang J, Multhaupt H, Chan E, Schaefer L, Schaefer RM, Couchman JR. 2004 Essential contribution of tumor-derived perlecan to epidermal tumor growth and angiogenesis. *J Histochem Cytochem* 52:1575–1590. [PubMed: 15557212]
- Jiang X, Couchman JR. 2003 Perlecan and tumor angiogenesis. *J Histochem Cytochem* 51:1393–1410. [PubMed: 14566013]
- Kallunki P, Tryggvason K. 1992 Human basement membrane heparan sulfate proteoglycan core protein: A 467-kD protein containing multiple domains resembling elements of low density lipoprotein receptor, laminin, neural cell adhesion molecules and epidermal growth factor. *J Cell Biol* 116:559–571. [PubMed: 1730768]
- Kallunki P, Eddy RL, Byers MG, Kestilä M, Shows TB, Tryggvason K. 1991 Cloning of human heparan sulfate proteoglycan core protein, assignment of the gene (HSPG2) to 1p36.1-p35 and identification of a BamHI restriction fragment length polymorphism. *Genomics* 11:389–396. [PubMed: 1685141]
- Kalluri R. 2003 Basement membranes: Structure, assembly and role in tumor angiogenesis. *Nat Rev Cancer* 3:422–433. [PubMed: 12778132]
- Kinsella MG, Tran P-K, Weiser-Evens MCM, Reidy M, Majack RA, Wight TN. 2003 Changes in perlecan expression during vascular injury. Role in the inhibition of smooth muscle cell proliferation in the late lesion. *Arterioscler Thromb Vasc Biol* 23:608–614. [PubMed: 12615671]
- Klein G, Conzelmann S, Beck S, Timpl R, Müller CA. 1995 Perlecan in human bone marrow: A growth-factor presenting, but anti-adhesive, extracellular matrix component for hematopoietic cells. *Matrix Biol* 14:457–465. [PubMed: 7795884]
- Knox S, Merry C, Stringer S, Melrose J, Whitelock J. 2002 Not all perlecans are created equal. Interactions with fibroblast growth factor (FGF) 2 and FGF receptors. *J Biol Chem* 277:14657–14665. [PubMed: 11847221]
- Knox SM, Whitelock JM. 2006 Perlecan: How does one molecule do so many things? *Cell Mol Life Sci* 63:2435–2445. [PubMed: 16952056]
- Marchisone C, Del Grosso F, Masiello L, Prat M, Santi L, Noonan DM. 2000 Phenotypic alterations in Kaposi's sarcoma cells by antisense reduction of perlecan. *Pathol Oncol Res* 6:10–17. [PubMed: 10749582]
- Marneros AG, Olsen BR. 2001 The role of collagen-derived proteolytic fragments in angiogenesis. *Matrix Biol* 20:337–345. [PubMed: 11566268]
- Mathiak M, Yenisey C, Grant DS, Sharma B, Iozzo RV. 1997 A role for perlecan in the suppression of growth and invasion in fibrosarcoma cells. *Cancer Res* 57:2130–2136. [PubMed: 9187109]
- Merz DC, Alves G, Kawano T, Zheng H, Culotti JG. 2003 UNC-52/perlecan affects gonadal leader cell migrations in *C.elegans* hermaphrodites through alterations in growth factor signaling. *Dev Biol* 256:173–186. [PubMed: 12654300]

- Moerman DG, Hutter H, Mullen GP, Schnabel R. 1996 Cell autonomous expression of perlecan and plasticity of cell shape in embryonic muscle of *Caenorhabditis elegans*. *Dev Biol* 173:228–242. [PubMed: 8575624]
- Mongiat M, Taylor K, Otto J, Aho S, Uitto J, Whitelock J, Iozzo RV. 2000 The protein core of the proteoglycan perlecan binds specifically to fibroblast growth factor-7. *J Biol Chem* 275:7095–7100. [PubMed: 10702276]
- Mongiat M, Otto J, Oldershaw R, Ferrer F, Sato JD, Iozzo RV. 2001 Fibroblast growth factor-binding protein is a novel partner for perlecan protein core. *J Biol Chem* 276:10263–10271. [PubMed: 11148217]
- Mongiat M, Fu J, Oldershaw R, Greenhalgh R, Gown A, Iozzo RV. 2003a Perlecan protein core interacts with extracellular matrix protein 1 (ECM1), a glycoprotein involved in bone formation and angiogenesis. *J Biol Chem* 278:17491–17499. [PubMed: 12604605]
- Mongiat M, Sweeney S, San Antonio JD, Fu J, Iozzo RV. 2003b Endorepellin, a novel inhibitor of angiogenesis derived from the C terminus of perlecan. *J Biol Chem* 278:4238–4249. [PubMed: 12435733]
- Mullen GP, Rogalski TM, Bush JA, Gorji PR, Moerman DG. 1999 Complex patterns of alternative splicing mediate the spatial and temporal distribution of perlecan/UNC-52 in *Caenorhabditis elegans*. *Mol Biol Cell* 10:3205–3221. [PubMed: 10512861]
- Murdoch AD, Iozzo RV. 1993 Perlecan: The multidomain heparan sulphate proteoglycan of basement membrane and extracellular matrix. *Virchows Archiv A Pathol Anat Histopathol* 423:237–242.
- Murdoch AD, Dodge GR, Cohen I, Tuan RS, Iozzo RV. 1992 Primary structure of the human heparan sulfate proteoglycan from basement membrane (HSPG2/perlecan). A chimeric molecule with multiple domains homologous to the low density lipoprotein receptor, laminin, neural cell adhesion molecules, and epidermal growth factor. *J Biol Chem* 267:8544–8557. [PubMed: 1569102]
- Nicole S, Davoine C-S, Topaloglu H, Cattolico L, Barral D, Beighton P, Hamida CB, Hammouda H, Cruaud C, White PS, Samson D, Urtiz-berea JA, Lehmann-Horn F, Weissenbach J, Hentati F, Fontaine B. 2000 Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondro-dystrophic myotonia). *Nat Genet* 26:480–483. [PubMed: 11101850]
- Noonan DM, Hassell JR. 1993 Perlecan, the large low-density proteoglycan of basement membranes: Structure and variant forms. *Kidney Int* 43:53–60. [PubMed: 8433569]
- Noonan DM, Fulle A, Valente P, Cai S, Horigan E, Sasaki M, Yamada Y, Hassell JR. 1991 The complete sequence of perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein-receptor, and the neural cell adhesion molecule. *J Biol Chem* 266:22939–22947. [PubMed: 1744087]
- Nugent MA, Iozzo RV. 2000 Fibroblast growth factor-2. *Int J Biochem Cell Biol* 32:115–120. [PubMed: 10687947]
- Nugent MA, Karnovsky MJ, Edelman ER. 1993 Vascular cell-derived heparan sulfate shows coupled inhibition of basic fibroblast growth factor binding and mitogenesis in vascular smooth muscle cells. *Circ Res* 73:1051–1060. [PubMed: 8222077]
- Nugent MA, Nugent HM, Iozzo RV, Sanchack K, Edelman ER. 2000 Perlecan is required to inhibit thrombosis after deep vascular injury and contributes to endothelial cell-mediated inhibition of intimal hyperplasia. *Proc Natl Acad Sci USA* 97:6722–6727. [PubMed: 10841569]
- Oda O, Shinzato T, Ohbayashi K, Takai I, Kunitatsu M, Maeda K, Yamanaka N. 1996 Purification and characterization of perlecan fragment in urine of end-stage renal failure patients. *Clin Chim Acta* 255:119–132. [PubMed: 8937755]
- Olsen BR. 1999 Life without perlecan has its problems. *J Cell Biol* 147:909–911. [PubMed: 10579711]
- O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. 1997 Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88:277–285. [PubMed: 9008168]

- Park Y, Rangel C, Reynolds MM, Caldwell MC, Johns M, Nayak M, Welsh CJR, McDermott S, Datta S. 2003 *Drosophila* perlecan modulates FGF and hedgehog signals to activate neural stem cell division. *Dev Biol* 253:247–257. [PubMed: 12645928]
- Paulsson M, Yurchenco PD, Ruben GC, Engel J, Timpl R. 1987 Structure of low density heparan sulfate proteoglycan isolated from a mouse tumor basement membrane. *J Mol Biol* 197:297–313. [PubMed: 2960821]
- Peng HB, Xie H, Rossi SG, Rotundo RL. 1999 Acetylcholinesterase clustering at the neuromuscular junction involves perlecan and dystroglycan. *J Cell Biol* 145:911–921. [PubMed: 10330416]
- Raymond M-A, Dásormeaux A, Laplante P, Vigneault N, Filep JG, Landry K, Pshezhetsky AV, Hébert M-J. 2004 Apoptosis of endothelial cells triggers a caspase-dependent anti-apoptotic paracrine loop active on vascular smooth muscle cells. *FASEB J* 18:705–707. [PubMed: 14977881]
- Rogalski TM, Williams BD, Mullen GP, Moerman DG. 1993 Products of the unc-52 gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev* 7:1471–1484. [PubMed: 8393416]
- Rogalski TM, Gilchrist EJ, Mullen GP, Moerman DG. 1995 Mutations in the unc-52 gene responsible for body wall muscle defects in adult *Caenorhabditis elegans* are located in alternatively spliced exons. *Genetics* 139:159–169. [PubMed: 7535716]
- Rops AL, van der Vlag J, Lensen JF, Wijnhoven TJ, van den Heuvel LP, van Kuppevelt TH, Berden JH. 2004 Heparan sulfate proteoglycans in glomerular inflammation. *Kidney Int* 65:768–785. [PubMed: 14871397]
- Rossi M, Morita H, Sormunen R, Airenne S, Kreivi M, Wang L, Fukai N, Olsen BR, Tryggvason K, Soininen R. 2003 Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. *EMBO J* 22:236–245. [PubMed: 12514129]
- Sage EH. 1997 Pieces of eight-bioactive fragments of extracellular proteins as regulators of angiogenesis. *Trends Cell Biol* 7:182–186. [PubMed: 17708942]
- Saksela O, Rifkin DB. 1990 Release of basic fibroblast growth factor-heparan sulfate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. *J Cell Biol* 110:767–775. [PubMed: 2137829]
- Saksela O, Moscatelli D, Sommer A, Rifkin DB. 1988 Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. *J Cell Biol* 107:743–751. [PubMed: 2971068]
- Sanderson RD. 2001 Heparan sulfate proteoglycans in invasion and metastasis. *Semin Cell Dev Biol* 12:89–98. [PubMed: 11292374]
- Savore C, Zhang C, Muir C, Liu R, Wyrwa J, Shu J, Zhau HE, Chung LW, Carson DD, Farach-Carson MC. 2005 Perlecan knockdown in metastatic prostate cancer cells reduces heparin-binding growth factor responses in vitro and tumor growth in vivo. *Clin Exp Metastasis* 22:377–390. [PubMed: 16283481]
- Schulze B, Sasaki T, Costell M, Mann K, Timpl R. 1996 Structural and cell-adhesive properties of three recombinant fragments derived from perlecan domain III. *Matrix Biol* 15:349–357. [PubMed: 8981331]
- Sharma B, Iozzo RV. 1998 Transcriptional silencing of perlecan gene expression by interferon- γ . *J Biol Chem* 273:4642–4646. [PubMed: 9468523]
- Sharma B, Handler M, Eichstetter I, Whitelock J, Nugent MA, Iozzo RV. 1998 Antisense targeting of perlecan blocks tumor growth and angiogenesis in vivo. *J Clin Invest* 102:1599–1608. [PubMed: 9788974]
- Smirnov SP, McDearmon EL, Li S, Ervasti JM, Tryggvason K, Yurchenco PD. 2002 Contribution of the LG modules and furin processing to laminin-2 functions. *J Biol Chem* 277:18928–18937. [PubMed: 11886875]
- Stum M, Davoine C-S, Vicart S, Guillot-Noël L, Topaloglu H, Carod-Artal FJ, Kayserili H, Hentati F, Merlini L, Urtizberea JA, Hammouda E-H, Quan PC, Fontaine B, Nicole S. 2006 Spectrum of HSPG2 (perlecan) mutations in patients with Schwartz-Jampel syndrome. *Hum Mutat* 27:1082–1091.

- SundarRaj N, Fite D, Ledbetter S, Chakravarti L, Hassell JR. 1995 Perlecan is a component of cartilage matrix and promotes chondro- cyte attachment. *J Cell Sci* 108:2663–2672. [PubMed: 7593307]
- Tapanadechopone P, Hassell JR, Rigatti B, Couchman JR. 1999 Localization of glycosaminoglycan substitution sites on domain V of mouse perlecan. *Biochem Biophys Res Commun* 265:680–690. [PubMed: 10600481]
- Tapanadechopone P, Tumova S, Jiang X, Couchman JR. 2001 Epidermal transformation leads to increased perlecan synthesis with heparin-binding-growth-factor affinity. *Biochem J* 355:517–527. [PubMed: 11284741]
- Tiedemann K, Sasaki T, Gustafsson E, Göhring W, Bätge B, Notbohm H, Timpl R, Wedel T, Schlötzer-Schrehardt U, Reinhardt D. 2005 Microfibrils at basement membrane zones interact with perlecan via fibrillin-1. *J Biol Chem* 280:11404–11412. [PubMed: 15657057]
- Timár, Lapis KDJ, Sebestyén A, Kopper L, Kovalszky I. 2002 Proteoglycans and tumor progression: Janus-faced molecules with contradictory functions in cancer. *Semin Cancer Biol* 12:173–186. [PubMed: 12083848]
- Timpl R. 1993 Proteoglycans of basement membranes. *Experientia* 49:417–428. [PubMed: 8500597]
- Timpl R, Tisi D, Talts JF, Andac Z, Sasaki T, Hohenester E. 2000 Structure and function of laminin LG modules. *Matrix Biol* 19:309–317. [PubMed: 10963991]
- Tran P-K, Tran-Lundmark K, Soininen R, Tryggvason K, Thyberg J, Hedin U. 2004 Increased intimal hyperplasia and smooth muscle cell proliferation in transgenic mice with heparan sulfate-deficient perlecan. *Circ Res* 94:550–558. [PubMed: 14739157]
- Tu H, Sasaki T, Snellman A, Göhring W, Pirlä P, Timpl R, Pihlajaniemi T. 2002 The type XIII collagen ectodomain is a 150-nm rod and capable of binding to fibronectin, nidogen-2, perlecan, and heparin. *J Biol Chem* 277:23092–23099. [PubMed: 11956183]
- Vigny M, Ollier-Hartmann MP, Lavigne M, Fayein N, Jeanny JC, Laurent M, Courtois Y. 1988 Specific binding of basic fibroblast growth factor to basement membrane-like structures and to purified heparan sulfate proteoglycan of the EHS tumor. *J Cell Physiol* 137:321–328. [PubMed: 2973466]
- Villar MJ, Hassell JR, Brandan E. 1999 Interaction of skeletal muscle cells with collagen type IV is mediated by perlecan associated with the cell surface. *J Cell Biochem* 75:665–674. [PubMed: 10572249]
- Vischer P, Feitsma K, Schon P, Volker W. 1997 Perlecan is responsible for thrombospondin 1 binding on the cell surface of cultured porcine endothelial cells. *Eur J Cell Biol* 73:332–343. [PubMed: 9270876]
- Voigt A, Pflanz R, Schafer U, Jackle H. 2002 Perlecan participates in proliferation activation of quiescent *Drosophila* neuroblasts. *Dev Dyn* 224:403–412. [PubMed: 12203732]
- Vuadens F, Benay C, Crettaz D, Gallot D, Sapin V, Schneider P, Binevenut W-V, Lémery D, Quadroni M, Dastugue B, Tissot J-D. 2003 Identification of biologic markers of the premature rupture of fetal membranes: Proteomic approach. *Proteomics* 3:1521–1525. [PubMed: 12923777]
- Walker HA, Whitelock JM, Garl PJ, Nemenoff RA, Stenmark KR, Weiser-Evans WCM. 2003 Perlecan up-regulation of FRNK suppresses smooth muscle cell proliferation via inhibition of FAK signaling. *Mol Biol Cell* 14:1941–1952. [PubMed: 12802066]
- Westermarck B, Heldin CH. 1993 Platelet-derived growth factor. Structure, function and implications in normal and malignant cell growth. *Acta Oncol* 32:101–105. [PubMed: 8323751]
- Whitelock JM, Iozzo RV. 2005 Heparan sulfate: A complex polymer charged with biological activity. *Chem Rev* 105:2745–2764. [PubMed: 16011323]
- Whitelock JM, Murdoch AD, Iozzo RV, Underwood PA. 1996 The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin and heparanases. *J Biol Chem* 271:10079–10086. [PubMed: 8626565]
- Whitelock J, Mitchell S, Underwood PA. 1997 The effect of human endothelial cell-derived proteoglycans on human smooth muscle cell growth. *Cell Biol Int* 21:181–189. [PubMed: 9151995]

- Whitelock JM, Graham LD, Melrose J, Murdoch AD, Iozzo RV, Underwood PA. 1999 Human perlecan immunopurified from different endothelial cell sources has different adhesive properties for vascular cells. *Matrix Biol* 18:163–178. [PubMed: 10372557]
- Wickström SA, Alitalo K, Keski-Oja J. 2003 Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. *J Biol Chem* 278:37895–37901. [PubMed: 12851410]
- Zhang W, Chuang Y-J, Swanson R, Li J, Seo K, Leung L, Lau LF, Olson ST. 2004 Antiangiogenic antithrombin down-regulates the expression of the proangiogenic heparan sulfate proteoglycan, perlecan, in endothelial cells. *Blood* 103:1185–1191. [PubMed: 14563633]
- Zweers MC, Davidson JM, Pozzi A, Hallinger R, Janz K, Quondamatteo F, Leutgeb B, Krieg T, Eckes B. 2007 Integrin $\alpha 2 \beta 1$ is required for regulation of murine wound angiogenesis but is dispensable for reepithelialization. *J Invest Dermatol* 127:467–478. [PubMed: 16977325]

Fig. 1. Immunolocalization of perlecan protein core in the blood vessels of normal human breast (A), renal arteriole (B), renal tubules (C), astrocytoma (D), renal cell carcinoma (E), and in the stroma of an ovarian cancer (F). The dark brown pigment is indicative of strong perlecan expression and deposition. Immunohistochemistry utilized an antidiomain V specific antibody at 1:1000 dilutions. Bar 5 100 μ m.

Fig. 2.

Schematic model of perlecan from human, mouse, *Drosophila*, and *C. elegans*. In *Drosophila*, for schematic purposes, 19 additional LA modules in domain II (+19) are not shown. Also, alternate GAG binding sites on mouse domain V are not shown. Wavy lines represent no corresponding sequence. Note the significant differences in domain I between mammalian and nonmammalian perlecan [completely absent in *Drosophila* and substituted with an Arg-rich domain in *C. elegans*], and the relative conservation of structure in domains II–V.

Fig. 3.

Effects of endorepellin on capillary morphogenesis (tubulogenesis) induced by Matrigel (A, B) and endothelial actin stress fibers (C, D). In panels **A** and **B**, human umbilical vein endothelial cells were seeded on Matrigel and incubated for 24 h in the absence or presence of endorepellin (200 nM). In panels **C** and **D**, human dermal microvascular endothelial cells were grown to confluence on collagen I, washed briefly and then incubated with or without endorepellin (150 nM) for 10 min. Notice the endorepellin-induced disassembly of actin stress fibers as visualized by rhodamine-phalloidin staining (red). Nuclei were stained with DAPI (blue) Bars = 20 μ m.

TABLE 1.

Comparative perlecan genetics

Perlecan genetics	Genotype	Phenotype	Reference
Human			
Dyssegmental, dysplasia, Silverman-Handmaker type (DDSH)	Autosomal recessive disorder, 89-bp deletion from perlecan exon 36, or point mutations in perlecan exon 54 or 73; synthesis of truncated, nonsecreted/nonfunctional perlecan	Skeletal dysplasia characterized by ankyospondyly and micromelia; lethal	(Arikawa-Hirasawa et al., 2001a)
Schwartz-Jampel syndrome (SJS)	Homozygous missense and splicing mutations; partially functional/secreted truncated perlecan, missing part of domain IV and/or all of domain V, defective domain III, or reduced wild-type perlecan	Myotonia, chondrodysplasia; nonlethal	(Arikawa-Hirasawa et al., 2002; Nicole et al., 2001; Stum et al., 2006)
Mouse			
Perlecan knockout	Null mutation	Lethal in many embryos at E 10.5 with severe cephalic and cartilage abnormalities; surviving embryos exhibit malformations of the cardiac outflow tract with complete transposition of great vessels.	(Arikawa-Hirasawa et al., 1999; Costell et al., 2002)
<i>Drosophila</i>			
<i>Tro^{pd}</i>	Mutation of <i>tro</i> (terribly reduced optical lobe) gene involved in the control of neuroblast proliferation in optic lobe and thorax, and in modulation of FGF and Hedgehog signaling	Abnormal larval brain lobes and small imaginal discs; lethal	(Datta and Kankel, 1992; Park et al., 2003)
<i>C. elegans</i>	Null mutation	Pat (paralyzed, arrested elongation at twofold); lethal	(Rogalski et al., 1995)
<i>Unc-52</i>	Mutations in exons 16, 17, 18, affecting some, but not all <i>unc-52</i> isoforms	Larvae move normally, adults paralyzed due to progressive disruption of body wall at third-fourth larval stage	(Gilchrist and Moerman, 1992)

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TABLE 2.

Interactions of mammalian perlecan protein core with various partners, including: growth factors, growth promoting ECM components, and adhesion molecules

Perlecan domain	Interacting partner	Reference
I. N-terminal domain harboring an SEA module and three SGD tripeptides, to which heparan sulfate chains are covalently attached	Laminin-111 Collagen type IV Fibronectin FGF2 PDGF Thrombospondin PRELP Fibrillin-1 LDL, VLDL Fibrillin-1	(Eitner et al., 1998) (Whitelock et al., 1999) (Heremans et al., 1990) (Aviezer et al., 1994) (Göhring et al., 1998) (Vischer et al., 1997) (Bengtsson et al., 2002) (Tiedemann et al., 2005) (Hummel et al., 2004) (Tiedemann et al., 2005)
II. Four LDL receptor-like (LA) modules and one Ig repeat		
III. Three laminin-like (L4) modules and eight EGF (LE) repeats. Mouse, but not human, harbors an RGDS sequence	FGF7 FGF-BP PDGF	(Mongiat et al., 2000) (Mongiat et al., 2001) (Göhring et al., 1998)
IV. N-CAM-like domain with longest array (17–21) of Ig-like repeats	Nidogen-1 Nidogen-2 Fibulin-2 Fibronectin, Collagen type IV PDGF	(Hopf et al., 1999) (Hopf et al., 1999) (Hopf et al., 1999) (Hopf et al., 1999) (Whitelock et al., 1999) (Göhring et al., 1998)
V. C-terminal domain with three LG modules and four EGF repeats	Nidogen-1 Fibulin-2 β1 integrin FGF-7 Collagen XVIII/endostatin ECM1 Progranulin α2 integrin PRELP α-dystroglycan	(Brown et al., 1997) (Brown et al., 1997) (Brown et al., 1997) (Mongiat et al., 2000) (Mongiat et al., 2003b) (Mongiat et al., 2003a) (Gonzalez et al., 2003) (Bix et al., 2004) (Bengtsson et al., 2002) (Peng et al., 1999)