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A Role for Proteoglycans in Vascular Disease

Thomas N. Wight

Matrix Biology Program, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101, United States

Abstract

The content of proteoglycans (PGs) is low in the extracellular matrix (ECM) of vascular tissue, but increases dramatically in all phases of vascular disease. Early studies demonstrated that glycosaminoglycans (GAGs) including chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS) and heparan sulfate (HS) accumulate in vascular lesions in both humans and in animal models in areas of the vasculature that are susceptible to disease initiation (such as at branch points) and are frequently coincident with lipid deposits. Later studies showed the GAGs were covalently attached to specific types of core proteins that accumulate in vascular lesions. These molecules include versican (CSPG), biglycan and decorin (DS/CSPGs), lumican and fibromodulin (KSPGs) and perlecan (HSPG), although other types of PGs are present, but in lesser quantities. While the overall molecular design of these macromolecules is similar, there is tremendous structural diversity among the different PG families creating multiple forms that have selective roles in critical events that form the basis of vascular disease. PGs interact with a variety of different molecules involved in disease pathogenesis. For example, PGs bind and trap serum components that accumulate in vascular lesions such as lipoproteins, amyloid, calcium, and clotting factors. PGs interact with other ECM components and regulate, in part, ECM assembly and turnover. PGs interact with cells within the lesion and alter the phenotypes of both resident cells and cells that invade the lesion from the circulation. A number of therapeutic strategies have been developed to target specific PGs involved in key pathways that promote vascular disease. This review will provide a historical perspective of this field of research and then highlight some of the evidence that defines the involvement of PGs and their roles in the pathogenesis of vascular disease.

Keywords

Vascular disease; Versican; Biglycan; Decorin; Fibromodulin; Lumican; Perlecan

Correspondence to: Thomas N. Wight, PhD, Director, Matrix Biology Program, Benaroya Research Institute, 1201 9th Avenue, Seattle, WA 98101, twight@benaroyaresearch.org, (206) 287-5666.

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Introduction

The extracellular matrix (ECM) of blood vessels is a mixture of collagens, elastic fibers, proteoglycans (PGs), hyaluronan and an assortment of glycoproteins that interact by entanglement and crosslinking to form higher-ordered polymers which, in part, regulate the biomechanical properties of blood vessels as well and the phenotype of the cells that reside in them. The relative contributions of the different ECM components in normal blood vessels are fixed so as to maintain optimal vascular structure and function throughout life. Any deviation from this balance changes blood vessel architecture and mechanics, altering function and resulting in disease [1, 2]. For example, the ECM of most normal and medium-sized arteries contains a large amount of elastic fiber proteins (as much as 50% of total ECM protein), lesser amounts of collagen, and small amounts of PGs and glycoproteins. The low amounts of PGs in normal vessels help stabilize vessel architecture and maintain appropriate tissue turgor. However, in vascular disease, these proportions change, with PGs predominating in the early lesion phase, with collagens and elastic fibers reduced and considerably less amounts of glycoproteins. As the lesions become more advanced, collagens tend to predominate with lesser amounts of elastic fibers, PGs, and glycoproteins (Fig. 1). These changes differentially impact several pathological events that promote lesion development and dictate to a large extent the severity of vascular disease (reviewed in [1]).

Our interest over the years has been in defining the involvement of PGs in the development of vascular disease and the role that these molecules play in regulating key events in vascular pathogenesis. PGs are complex macromolecules that have a common domain structure consisting of a core protein to which one or more glycosaminoglycans (GAGs) are covalently attached. GAGs are negatively charged polysaccharides of various lengths that consist of repeating disaccharide units where one of the two residues is an amino-sugar (n-acetyl glucosamine or n-acetylgalactosamine) and the other is a hexuronic acid (glucuronic or its C-5 epimerized homolog, iduronic acid or galactose). The GAGs are sulfated and carboxylated giving rise to strong polyanions forming four types of GAG : chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and keratan sulfate (KS), which are linked to different core proteins through O-glycosidic linkage to serine residues forming distinct PG families: CSPGs, DSPGs, KSPGs, and HSPGs [3, 4]. It is possible that more than one type of GAG can be attached to a single core protein, creating a number of PG subtypes. Such diversity, plus their splice variants, have identified more than 20 different PGs in blood vessels or synthesized by vascular cells [5]. Virtually every cell in normal and diseased blood vessels synthesizes PGs whether they are resident cells such as endothelial cells, smooth muscle cells (SMCs), and adventitial fibroblasts, or cells that gain access to the blood vessel wall from the circulation such as macrophages, mast cells, lymphocytes, and neutrophils. PGs within normal and diseased blood vessels can be found in the interstitial space between cells, associated with basement membranes and basal laminae, present on cell surfaces, and intracellularly. PGs are promiscuous in that they interact with multiple ligands within the interstitial space to aid in the regulation of vascular structure and function. PGs also interact with multiple receptors on the surface of vascular cells to, in part, regulate vascular cell phenotype.

The purpose of this review is to focus on the major PGs present in blood vessel disease and to highlight some of the work that we and others have done to advance our understanding of their importance in vascular disease. Some historical perspective on the development of this field has been included as a point of general interest and to highlight some of the work that has set the stage for our current thinking. This review will emphasize versican because of its importance in many different types of vascular diseases and since this PG has been our focus for the past several years, but this review will also cover other PGs that clearly play critical roles in vascular pathogenesis. Other excellent reviews covering different aspects of this topic are also available [6-10].

Vascular disease

Vascular disease involves diseases of blood vessels that can occur in both arteries and veins. The most prevalent and clinically significant vascular disease is atherosclerosis, which is responsible annually for the most deaths worldwide. Atherosclerosis is a disease of large and medium sized arteries. Blood vessels are composed of three layers: intima, media, and adventitia. Although any artery can be affected, the aorta, coronary, and cerebral arteries are the prime targets of this disease. Human atherosclerosis develops in different stages and usually involves intimal hyperplasia, lipid accumulation and formation of fatty streaks, early and late stage fibrous plaques and eventual plaque rupture with thrombosis and vessel occlusion leading to myocardial infarction or stroke [11-15]. Each of these stages is characterized by a unique cellular and ECM composition including an enrichment in different types of PGs [1, 6, 14-21]. Intimal hyperplasia is the progressive thickening of the intimal layer of the vessel wall due to increases in the number of SMCs and the accumulation of a PG-rich ECM. These events are then followed by the additional accumulation of cholesterol- and/or amyloid-carrying lipoproteins which enter the vascular wall from the circulation along with immune cells, ultimately giving rise to large atherosclerotic lesions with necrotic cores, cell debris, lipids, and inflammation. These plaques are likely to rupture, causing clot formation and thrombosis leading to vessel occlusion. Atherosclerosis, by definition, involves lipid accumulation and the overwhelming evidence suggests that vascular PGs are responsible for lipid retention in this disease [22-26] (also see below). In addition, atherosclerosis is an inflammatory disease with intense involvement of immune and inflammatory cells [27-29].

Clinically, atherosclerotic plaques can be removed by percutaneous angioplasty with a placement of a stent often coated with an anti-atherosclerotic agent to reinforce artery structure and to re-establish blood flow, but frequently lesions reform at these sites of injury, leading to restenosis and the need for further surgery. Restenotic lesions form primarily by an intimal hyperplastic response and are enriched in PGs [30-36]. Restenotic lesions can also develop into atherosclerosis, involving lipid accumulation at an accelerated rate leading to late-stage stent failure and the need for additional surgery [34, 37, 38].

Dissecting the sequence of events in the pathogenesis of any human disease is difficult because the early stages of the disease often go undetected until clinical symptoms appear. Thus, much of the early information on the involvement of PGs in vascular lesion development comes from animal studies in which initiating events can be controlled and

subsequent events clearly monitored both temporally and spatially. The two most common ways of creating atherosclerosis in animals are increasing plasma lipids by either fat feeding or the use of molecular genetics to create high levels of plasma lipids [39] and/or by vascular injury using mechanical devices such as balloon catheters or wires to injure the endothelium [40, 41]. Frequently, the best results are obtained by a combination of both approaches. While such approaches in experimental animals do create vascular lesions with some histopathological resemblance to human lesions, factors such as species differences in the native composition of blood vessels and the different mechanistic injury responses among different species highlight a need for caution when transferring information from animal studies to humans. Atherosclerosis in the mouse is not atherosclerosis in the human.

While different forms of atherosclerosis involve a significant lipid component coupled to PG changes, other human vascular diseases exist where lipids are a minor component, but where the accumulation of a PG-enriched ECM occurs contributing to plaque burden. Such vascular conditions in the human include early-stage restenosis [30, 32, 33, 36], aneurysms [42-45], temporal arteritis [46], pulmonary artery hypertension [47], and vascular graft failure [48-51].

Early studies

Interest in the importance of PGs in vascular disease goes back many years, with Virchow (1846) describing an increase in “mucosubstances” in atheromatous lesions in human arteries at autopsy. The early studies focused only on the GAGs and not the intact PGs, since it was common to purify the GAGs by treating the tissue with proteases to release the GAGs for analyses (reviewed in [52]). I remember well that one of the reviewers on my first grant application commented that by digesting the protein to purify the GAGs I was “throwing away” the most important part of the molecule! We now know, of course, that GAGs are not synthesized and deposited as free GAGs, with the exception of hyaluronan, but rather are covalently attached to core proteins and presented as PG.

Pioneering early work from a number of labs such as those of Berenson, Buddecke, Camejo, Hollander, Moore, Murata, and Schmid [22, 53-58] demonstrated that the principle types of GAGs found in vascular tissue and vascular lesions were chondroitin-4 and -6-sulfate (C-4-S, C-6-S), DS, HS, and KS (reviewed in [52]). Furthermore, early studies demonstrated that GAGs had a high propensity to interact with lipoproteins through ionic interactions between the positively-charged amino acids in the apoprotein of the lipoprotein and the negative charges of the GAG chains of the PGs, causing lipid build up in the vascular wall. In addition, the early studies showed that the GAG composition of the vasculature differed between different vascular beds within a given species and within the same vascular bed between species. For example, species susceptible to diet-induced atherosclerosis (chicken, pigeon, and rabbit) contained greater amounts of aortic GAGs enriched in CS than the aortae of diet-induced atherosclerosis-resistant species (rat, mouse, and hamster) which were enriched in HS [59, 60]. Interestingly, in a later study, HS was negatively associated with hyperglycemia and atherosclerosis in a diabetic monkey model [61]. Such findings led to some speculation that vascular GAG composition could predict susceptibility to atherosclerosis. Other studies showed that GAGs increased in the early phases of

atherosclerosis in both the human and in experimental animals and that areas prone to develop atherosclerotic lesions, such as at branch sites, often contained altered amounts and types of GAGs (reviewed in [52] and [62]). Lipids frequently accumulate in regions of high GAG content. For example, Minick and colleagues found that balloon-injured carotid arteries of rabbits fed a high-fat diet accumulated lipids in regions of neointima covered by regenerated endothelium, while regions devoid of endothelium showed minimal to no lipid deposits [41]. Analyses of these two regions revealed elevated levels of GAGs in the lipid-enriched areas compared to the lipid-poor areas [63]. While these studies did not prove cause and effect, they did highlight an important relationship between lipid accumulation in vascular lesions and GAG content and composition.

Proteoglycans in cardiovascular disease

With the advent of extraction techniques, which preserved the protein moieties of the PGs and provided a means to recover intact PGs from vascular lesions [64-67], together with the use of antibodies, which recognized different core proteins, it became clear that the different GAGs within the lesions were attached to specific core proteins. Thus, it is now recognized that the bulk of the CS chains present in atherosclerotic lesions are present as the PG versican, although other CS-containing PGs such as aggrecan [68, 69] and NG2 [70] have been found as well! The DS chains within blood vessels and atherosclerotic lesions are distributed between two highly homologous core proteins of the small leucine rich repeat (SLRP) family and form the PGs decorin and biglycan while KS is also present in the SLRP family as fibromodulin and lumican [6, 7]. HS chains within vascular lesions are found on several different core proteins that make up the perlecan, syndecan, and glypican gene families [4, 9, 19, 71, 72]. All of these PGs are present in different amounts and locations within vascular lesions and differentially and/or similarly affect a wide variety of events that contribute to vascular disease.

Chondroitin sulfate proteoglycans (CSPGs)

Versican is a large CS-containing PG with a core protein of approximately 260 kDa with 15-20 attached CS chains [73, 74]. Versican exists in at least four isoforms (V0-3) due to the alternative splicing of the major exons that code for the GAG attachment regions in the core protein. Versican is a major PG present in the ECM of normal human blood vessels [75] and increases dramatically in all forms of human vascular disease (reviewed in [31, 76]) (Fig. 2A-F). Moreover, the versican content of vessels, such as saphenous veins used in vascular bypass surgery, has been linked to the propensity of the vessel to fail following engraftment [77]. Versican is present in early intimal hyperplasia especially following balloon angioplasty [30, 32, 33, 36, 78, 79] and is primarily associated with arterial SMCs (ASMCs). As lesion severity progresses, lipid and macrophages accumulate along with versican [15, 80] (and reviewed in [31]). Versican is also prominent at the edges of the necrotic core in more advanced plaques [81] and in close proximity to some of the deposited lipoproteins within the lesions [15, 21, 82], suggesting a possible role for versican in lipid retention. In vitro binding assays show that versican isolated from cultured ASMCs binds to low-density lipoprotein (LDL) with saturable kinetics and with a binding affinity of 2.3×10^{-8} M [83, 84]. Estimating the stoichiometry of this interaction reveals that at least five LDL

particles can bind to a single CS chain on versican [84]. Conditions that promote CS chain elongation in ASMCs, such as cell proliferation [85, 86], oxidized LDL (oxLDL) [87] and TGF- β [88] also cause increased binding of LDL to versican. The composition of the CS chains of versican may affect LDL binding as well. CS enriched in the 6-O sulfate isomer binds LDL more avidly than those enriched in the 4-O sulfate isomer [89]. Furthermore, the charge density on the GAG influences LDL interactions in that increases in the degree of sulfation of CS increases lipoprotein interaction with these PGs [90]. A PG binding site in apoprotein B in LDL has been identified [22]. Replacing the positively-charged amino acids at this site with neutral amino acids eliminates versican binding to LDL in vitro [83] and inhibits the development of atherosclerosis in a lipid-induced mouse model of atherosclerosis despite elevated levels of serum cholesterol [91].

Degradation products of versican as well as fragments of other PGs such as decorin and biglycan accumulate in human vascular lesions [92] (and reviewed in [93]). Versican can be degraded by a number of enzymes including matrix metalloproteinases (MMPs), serine proteases, and at least 6 members of the ADAMTS family including ADAMTS1, -4, -5, -9, -15 and -20 [93-96]. ADAMTS1, -4, and -5 expression is elevated in vulnerable human carotid atherosclerotic plaques and in the serum of patients with carotid artery stenosis [97-99] and in mouse models of atherosclerosis [98, 100]. *ApoE*^{-/-}*ADAMTS4*^{-/-} double knockout mice have a significant reduction in plaque burden at 18 weeks of age confirming that ADAMTS4 contributes to diet-induced atherosclerosis in *ApoE*^{-/-} mice [100]. Of interest, a polymorphism in the ADAMTS1 gene has been associated with an increase in vascular disease in two separate studies [101, 102]. The fragments generated by the ADAMTS enzymes and other proteases have also been observed to build up in lesions in both human [92] and in several different animal models of vascular disease [93, 98, 103]. In fact, in a primate model of blood flow-induced intimal atrophy, Kenagy and colleagues found a selective increase in ADAMTS4 and fragmented versican coinciding with ASMC death and lesion regression [93, 103-106]. Furthermore, this group went on to show that Fas ligand induced ADAMTS4 synthesis by ASMCs in vitro and that ADAMTS4 colocalized with TUNEL-positive SMCs in the vascular graft [93, 103]. In an extension of these studies, Kenagy and colleagues compared the neointimal atrophy that occurs in the baboon model of vascular graft failure to a tight external wrap graft failure model and found that of 23,000 genes, only three ECM-modifying genes were common to both models with ADAMTS4 being one of them [106]. Such findings implicate this enzyme and versican turnover as important players in vascular disease. Fragments of versican have been shown to regulate cell apoptosis in mammalian interdigital web regression [107]. Other ADAMTS enzymes may be important as well. For example, ADAMTS5 is markedly reduced in atherosclerotic aortae from *ApoE*^{-/-} mice and this reduction is accompanied by increases in versican and biglycan, which lead to an increase LDL retention in this animal model [108]. Perhaps a balance between intact vs. fragmented versican is a critical element in promoting vascular disease. While there is abundant literature on specific proteases involved in PG breakdown in vascular disease, there is little data available to indicate whether the enzymes that degrade CS or DS GAGs such as chondroitinases are altered in vascular disease. This is in contrast to heparanase, which is elevated in vascular disease and degrades HS GAGs (see below).

Aortic aneurysms are a frequent complication of severe atherosclerosis and constitute an important clinical problem. Versican V0 mRNA is decreased by 40% in human abdominal aortic aneurysms, with significantly reduced immunostaining for versican [43] and with evidence of versican degradation and CS composition modifications [109, 110]. A proteomic approach has confirmed the loss of versican in abdominal aortic aneurysms along with a number of other ECM proteins [111]. Some of this loss could be due to selective versican degradation as versican degradative enzymes, such as plasmin [93, 104], increase in association with aneurysmal expansion [112, 113]. Most recently, ADAMTS4 was linked to the development of sporadic aortic aneurysm and dissection in mice; and elevated expression of ADAMTS4 was found in patients with sporadic ascending thoracic aortic aneurysms and dissection [114]. Thus, late phase aneurysmal development may involve selective loss of versican. Psuedoaneurysms of the temporal artery show increased immunostaining for versican in areas where there is elastic fiber interruption and breakage [46], indicating a possible early involvement of versican in ECM remodeling involving elastogenesis (Fig. 2C, D). In addition, a gene mapping study of familial thoracic aortic aneurysms and dissections identified a gene locus for this disease at 5q13-14 [115], which is the chromosomal segment that contains the versican gene [73, 116]. However, sequencing of the versican gene from affected individuals failed to reveal any variation that could account for the phenotype. On the other hand, single nucleotide polymorphisms (SNPs) and haplotype analyses of the versican gene in intracranial aneurysms revealed a strong association, suggesting a potential role for versican in this type of aneurysm [117]. Of interest, the versican intracranial aneurysm susceptibility gene does not appear to be associated with abdominal aortic aneurysms [118]. We did find, however, that in a mouse model where there has been a post-natal deletion of TGF β R2, significant elastolysis was associated with an enrichment of versican [119].

Versican and its binding partner, hyaluronan, are present at the plaque/thrombus interface in advanced human atherosclerotic lesions [16], suggesting a possible role in thrombosis. For example, versican promotes platelet adhesion at low shear rates and cooperates with collagens to promote platelet aggregation [120]. In addition, versican at the plaque/thrombus interface may, in part, regulate the water content of this region of the lesion, and in turn promote coagulation, since water transfer is linked to anticoagulation activity of molecules, such as the CS in versican [121]. In addition, the G3 domain of versican can bind to tissue factor pathway inhibitor-1 (TFPI-1) promoting blood coagulation through the extrinsic pathway [122]. Thus, multiple pathways exist for versican to have a role in thrombosis as part of vascular disease.

Versican is also prominent in most forms of vascular disease in experimental animals [21, 123-128] although it appears not to be enriched in diet-induced murine atherosclerotic lesions created using genetic ablation models such as *ApoE*^{-/-} and or *Ldlr*^{-/-} animals [129], possibly explained by the compositional differences in vascular PGs between mice and humans. We did find, however, using a congenic mouse strain exhibiting the atherosclerosis susceptibility QTL (*Athsq1*) gene, a significant enrichment of versican in enlarged atherosclerotic lesions compared to non-congenic littermates [128] (Fig. 2E, F). Interestingly, bone marrow transplantation studies suggested that both accelerated lesion formation and versican accumulation in this congenic mouse strain were mediated by

macrophages [128]. Monocytes/macrophages do synthesize versican in a variety of different conditions [130-132], including conditions that promote atherogenesis, such as hypoxia [133, 134] and with subsequent LDL accumulation [135]. Additionally, versican is upregulated in macrophages within myocardial infarcts [136] and in peripheral blood mononuclear cells in patients with coronary artery stenosis [137], and a cleaved fragment of versican is elevated in plasma from patients with atherosclerotic disease [138]. In addition, CSPG-LDL complexes are taken up rapidly by both macrophages [139, 140] and SMCs [141, 142], suggesting that PGs attached to lipid particles may play fundamental roles in foam cell formation in atherogenesis. Such studies suggest important crosstalk between macrophages and ASMCs in regulating versican and lipid accumulation in vascular disease.

ECM remodeling takes place throughout the different phases of atherosclerosis as part of an injury and inflammatory response. Elastic fiber degradation is a major histological feature in atherosclerotic lesions and cleavage products of elastin are proinflammatory, exacerbating atherosclerosis in animal models [143-145]. Elastic fibers are very long-lived, with a half-life of well over 70 years, and fail to reassemble after being destroyed [146]. In fact, elastic fibers are conspicuously absent from restenotic and atherosclerotic lesions! One factor that appears to inhibit elastic fiber assembly is CS, which is the type of GAG attached to the versican core protein [147]. Galactosamine carbohydrates such as CS are known to interact with the elastin binding protein (EBP) on the surface of elastin-producing cells where they interfere with elastic fiber assembly. In ASMCs, we have found that overexpression of the versican variant V3 that lacks CS leads to changes in tropoelastin expression and assembly of elastic fibers in long-term ASMC cultures [148]. In addition, when V3-transduced ASMCs are seeded into balloon-injured rat carotid arteries, a compact and highly structured neointima, enriched in elastic lamellae develops [148]. Furthermore, injecting rabbit V3-transduced ASMCs into injured rabbit carotid arteries in animals placed on a lipid-rich diet prevents lipid and macrophage buildup over an 8-week period [126]. The 8-week lesions are devoid of lipid and macrophages, but enriched in elastic fibers (Fig. 3)! Previous studies had shown that monocytes do not adhere well to elastic fibers, but adhere avidly to collagen [149], suggesting that elastin is a poor adhesive substrate for monocytes. We do know that V3 expression by ASMCs modulates several signaling pathways important in generating an ECM that resists monocyte adhesion and is anti-inflammatory [150-153]. Thus, by manipulating the PG content of the vascular ECM, we have inhibited two major events in the vascular wall that typify atherosclerosis development, i.e., lipid accumulation and macrophage accumulation.

Versican and the vascular cell phenotype

Accumulation of versican impacts the phenotype of a number of vascular cells involved in atherosclerosis progression, reviewed in [31, 154]. Versican is produced when ASMCs are stimulated to proliferate and migrate, forming a viscoelastic coat around the cells that facilitates their division and migration [85, 154-159]. Inhibition of this coat formation blocks the proliferation of ASMCs in response to growth factors such as PDGF [154, 156, 157]. The synthesis of versican by ASMCs is also regulated by microRNAs (miRNAs) [160]. For example, overexpression of miRNA-143 and -145 in ASMCs decreases versican expression in ASMCs [161] and neointimal formation in a rat model of acute vascular injury [162].

Versican can also influence endothelial cell phenotype. For example, human stromal stem cells that produce elevated levels of versican when co-cultured with vascular endothelial cells promote endothelial cell tubulogenesis in vitro and a 50-fold increase in vessels in vivo when patches containing these cells are transplanted onto the hearts of athymic rats [163]. A similar role for stromal cell-derived versican in regulating angiogenesis in a tumor growth model has recently been reported [164]. Furthermore, versican is upregulated and degraded in an in vivo mouse model of VEGF-induced angiogenesis [165]. While the mechanism responsible for versican's effect on endothelial cells is not known, it most likely has something to do with the ability of versican to interact with HA and modify the pericellular microenvironment around endothelial cells to allow destabilization of cell adhesion and a change in cell shape to promote angiogenesis. HA is a well-known ECM regulator of angiogenesis in vascular disease [166, 167].

Versican and inflammation

Inflammatory responses require the emigration of leukocytes from the vasculature into damaged underlying tissue areas as part of the innate immune response, usually driven to some extent by hypoxia and acidification of the intimal fluid [168]. Such changes in the ECM help create an acidic microenvironment which dramatically impacts several key events in the intima that promote lesion development [168]. Upon extravasation into the subendothelial compartment, leukocytes encounter the ECM, which then influences their adhesion, migration, activation, and retention (reviewed in [169]). High resolution confocal microscopy shows that versican/hyaluronan complexes synthesized by cultured stromal cells such as SMCs and fibroblasts form cable-like structures enriched in hyaluronan and versican within the ECM that bind monocytes/macrophages [170-175]. Such structures can be visualized within the ECM in atherosclerotic lesions associated with clumps of macrophages [174]. In addition, versican interacts with the surface of macrophages in specific ways. For example, fragments of the G1 domain of versican promote cable formation by stromal cells in culture [176]. Also, the G3 domain of versican can interact with P-selectin glycoprotein-1 (PSGL-1) on the surface of macrophages and cause macrophage aggregation [177]. Macrophages can also bind CSPGs and DSPGs through the class A scavenger receptor to mediate macrophage adhesion [178]. Versican, either intact or degraded, can interact with macrophage TLR2 to promote inflammation through induced secretion of inflammatory cytokines, such as tumor necrosis factor- α (TNF α) and IL-6 [179, 180]. Several studies have suggested that the highly sulfated CS GAG chains of versican may be critical for promoting inflammatory cytokine release and activity [169, 180-185]. Furthermore, once bound to the versican-containing ECM, leukocytes may degrade versican [93, 94, 186] and generate fragments such as “versikine” that further impact the inflammatory response [187]. Recently, we generated a tamoxifen-inducible versican-deficient mouse and showed that inflammation was dramatically decreased in the lungs of mice exposed to a viral mimetic [152]. No studies have yet been initiated using this mouse to interrogate vascular disease. Thus, versican is part of a pro-inflammatory ECM [174, 188] and may be a useful and novel therapeutic target to control the immune response associated with inflammation in a variety of diseases, including vascular disease [174, 189, 190]. However, we were intrigued to find that when we knocked out versican production specifically in macrophages and tested the

inflammatory response in the lungs of mice treated with the viral mimetic as described above, inflammation was increased in the lungs of these mice, suggesting that macrophage-derived versican may be operating in an anti-inflammatory manner [131]. Other studies have shown that versican synthesized by stem cells in a corneal wound model is immunosuppressive as well [191]. Such studies raise interesting questions about the role of versican and its source in inflammation in general and in atherosclerosis specifically.

The small leucine-rich repeat proteoglycans (SLRPs)

Members of the SLRP family have core proteins ranging in size from 40 - 60 kDa, possessing 11-12 leucine-rich repeat (LRR) sequences with either attached CS, DS, or KS chains. These PGs are a subfamily of a large superfamily of LRR proteins, which include a number of protein receptors, such as the toll-like receptors (TLRs) and nucleotide oligomerization domain (NOD)-like receptors [4]. Slight differences in protein core structure identify five distinct classes of SLRPs with a wide range of interactive capabilities that can impact ECM assembly, cell behavior, and cell signaling [4, 6, 7, 192]. The major SLRPs identified in blood vessel disease are decorin, biglycan, lumican, and fibromodulin (see reviews [5-7, 193, 194]).

Decorin and biglycan

Decorin and biglycan are members of Class I SLRPs and contain an N-terminal domain that is usually substituted with 1 (decorin) or 2 (biglycan) CS/DS GAG chains [4, 195]. Morphological, biochemical, immunochemical, and immunohistochemical studies have shown these two molecules are located throughout atherosclerotic lesions with some overlap [6, 7, 14, 15, 18, 20, 21, 193, 196, 197]. Decorin appears to be more prominent in primary atherosclerotic plaques as compared to restenotic lesions [196] although decorin does appear around stent wires in stent implantation models in experimental rabbits [198]. Microarray analysis of senescent vascular SMCs shows that decorin is enriched in senescent cells and associates with vascular calcification of SMCs [199]. Decorin is found in association with collagen deposits together with other collagen-binding SLRPs in vascular lesions as well as in the necrotic core of the plaques. Decorin is enriched also in the collagen-rich adventitia of both normal and atherosclerotic vessels [197]. Transfection of rat ASMCs with the decorin gene followed by the transfer of these cells into injured rat carotid arteries reduces lesion formation and creates thinner lesions enriched in collagen type I [200]. Such results may indicate that decorin could serve to stabilize plaques by increasing collagen fibrillogenesis as plaques develop. However, decorin, along with biglycan, is found in the outer layer of diffuse intimal thickenings in developing human lesions that appear to precede lipid buildup, suggesting that these PGs may serve as a depot for infiltrating lipoproteins before the accumulation of macrophages [14, 20, 201]. Decorin is often associated with TGF β -rich regions of vascular lesions, suggesting a possible relationship between these two molecules [21]. Decorin is known to bind to and inhibit TGF β activity in other disease model systems, such as in glomerulonephritis [202] and in lung fibrosis models [203]. Decorin-overexpressing ASMCs have a markedly dampened response to TGF β -responsive gene activation [204]. Decorin also appears to play a role in the biomineralization of atherosclerotic plaques. For example, decorin is strongly upregulated in mineralizing

cultured ASMCs and overexpression of decorin by ASMCs in culture or adding decorin to mineralizing ASMC cultures significantly enhances calcium deposition in these cultures [205]. Further support for the role of decorin in calcification is seen in the co-localization of decorin and calcium in human coronary atherosclerotic lesions [205]. Decorin also associates with microvessels within lesions characterized by excessive inflammation such as in temporal arteritis [46], indicating a potential role in angiogenesis in developing vascular lesions. A role for decorin in vascular angiogenesis is well documented [206-209]. Confluent monolayers of bovine aortic endothelial cells synthesize biglycan, but upregulate decorin expression when they are induced to form tubes in vitro [207, 208, 210]. Finally, the SLRP family of PGs, including decorin, binds C1q and effectively inhibits the complement cascade, possibly providing an atheroprotective effect by minimizing pro-inflammatory cellular responses triggered by C1q [211].

Biglycan is also located throughout atherosclerotic lesions as well, but is associated with smooth muscle-containing regions of the plaque both in atherosclerosis and restenosis [14, 20, 21, 196, 198, 212]. Biglycan is also present in elevated amounts in plasma in patients with acute coronary syndrome and in *ApoE*^{-/-} mice, and appears to be involved in the regulation of thrombin, suggesting that biglycan may play an unappreciated protective role by inhibiting thrombin activity, platelet activation, and macrophage-mediated inflammation [213]. Biglycan is found associated with the macrophage and in the lipid-filled core of the plaque [82] and is extensively co-localized with ApoE- and ApoB-containing lipoproteins in humans, as well as in experimental animal model lesions [14, 15, 20, 82, 214, 215]. Biglycan also appears to be involved in the retention of remnant lipoproteins as well and thus may play a role in insulin resistance [216]. In humans, biglycan increases in early lesions primarily in the outer area of the intima and localizes with lipids before the appearance of macrophages, supporting the hypothesis that lipids are retained in the vascular wall before the involvement of macrophages [14, 20, 201]. The fact that the lipid builds up in the outer layer of the intima and not within the intima close to the lumen raises questions as to whether circulating lipid may be coming from the vasa vasorum rather than the luminal side of the vessel [217]. *Ldlr*^{-/-} mice infused with Ang II have increased vascular content of biglycan and perlecan and retain twice as much LDL when infused, compared to saline controls [218]. In addition, lipoproteins accumulate in arteries from biglycan-expressing transgenic mice on an atherogenic-rich diet [193] as well as in AngII-induced lesions. Long-term treatment with antagonists to the angiotensin II AT-1 receptors decreases biglycan accumulation and atherosclerosis in *ApoE*^{-/-} mice on a Western diet without affecting blood pressure or the amount of collagen within the lesion [219]. Such results indicate that pharmacological treatments using AT-1 antagonists and/or ACE-inhibitors may have beneficial effects in protecting against atherosclerosis by affecting PG accumulation. However, biglycan deficiency in an Ang II aneurysm model in mice fed a high-fat diet versus controls showed that absence of biglycan was not atheroprotective, possibly due to the upregulation of other lipid-binding PGs such as perlecan [42]. Both perlecan and biglycan increase in lipid-induced lesions in mouse models of atherosclerosis [129]. Lipoprotein accumulation increases in cultures of biglycan-expressing ASMCs [220] and this accumulation can be blocked by expressing mutated biglycan, which does not have CS/DS chains. In addition, inhibition of TGF β levels using a TGF β -neutralizing antibody

inhibited vascular biglycan accumulation and reduced atherosclerotic lesion area in Ang II-treated *Ldlr*^{-/-} mice [221].

In vitro binding studies show that biglycan binds to LDL with saturable kinetics and with an association constant of 1.7×10^{-7} M [84]. The direct interaction of LDL with biglycan involves positively-charged amino acids in the apoprotein portion of LDL at residues 3359-3369 (SiteB) [23, 83]. Transgenic mice overexpressing a defective PG-binding apoprotein, when fed a high-fat diet, failed to develop atherosclerosis after 20 weeks, even though serum cholesterol levels were significantly elevated [91]. These results indicate that subendothelial retention of apoprotein B-100-containing lipoproteins by PGs is a critical step in the early stages of atherosclerosis, as observed by Nakashima and colleagues in human arteries [14, 20, 201]. Such results provide direct experimental evidence that binding of LDL to artery wall PGs is an early step in atherogenesis. In addition, the nature of the cholesterol ester within the LDL particle also has influence on PG binding [222, 223]. Biglycan also binds lipoprotein lipase and promotes native and oxidized lipoprotein retention within the ECM [84]. Furthermore, lipoproteins bound to PGs are more sensitive to modifications such as oxidation and enzymatic hydrolysis, thereby affecting their potential atherogenicity [139, 224, 225]. Biglycan is synthesized by ASMCs and agonists such as TGF β which stimulates DS GAG elongation provide biglycan with an increased capacity to bind lipoproteins [88]. In addition to serving as a depot for lipid, biglycan may also influence lipid metabolism within the developing lesion since this PG binds phospholipase A2 and enhances the activity of this enzyme and the generation of proinflammatory products [83, 226, 227].

In addition to binding and retaining lipoproteins in vascular lesions, and in contrast to decorin, which has a tendency to localize to collagen with blood vessels and lesions, biglycan associates with other ECM components such as elastin and tends to localize around vascular cells in the pericellular matrix. In fact, manipulation of biglycan expression in ASMCs promotes elastic fiber expression and assembly in vitro and in injured arteries in animal models of experimental atherogenesis [228]. Since elastic destruction is a hallmark for developing atherosclerotic lesions, controlling elastic fiber formation may be a therapeutic approach to decreasing atherosclerotic lesion formation [146, 190]. Like other SLRPs, biglycan is expressed by multiple cell types in vascular disease, such as endothelial cells [210] and macrophages, and behaves as a danger-associated molecular pattern (DAMP), promoting an increase in inflammatory cytokines in a TLR2-/4-dependent manner [4, 229-231].

While decorin impacts angiogenesis, biglycan also appears to be modified as endothelial cells sprout and form tubes. For example, we found that human umbilical vein endothelial cells when induced to sprout in vitro upregulated ADAMTS4 and biglycan, generating 37 kDa, 33 kDa, and 30 kDa fragments of biglycan. Furthermore, both biglycan and ADAMTS4 co-localized to surface invaginations (podosomes) of the endothelial cells [232], identifying a site that may be operative in the proteolytic processing of PGs involved in promoting endothelial cell angiogenesis (Fig. 4). Previous studies have established that biglycan is a substrate for ADAMTS4 [233].

Lumican and fibromodulin

Fibromodulin and lumican are two KS-containing PGs that are found in human arteries and human atherosclerotic plaques (see review [6]). Both mRNA transcripts and core proteins of these KSPGs have been identified in vascular lesions, including diffuse intimal thickening, fatty streaks, complete atheroma with fibrous plaque and complicated lesions [6, 234, 235]. While there is considerable overlap in the distribution of these two PGs, it is of interest that lumican is deposited adjacent to lipid-filled macrophages in fibrolipid-filled lesions. The association of lumican with macrophages may suggest a role in regulating macrophage phenotype in the lesion, since other studies have shown that lumican can interact with $\beta 2$ integrin on the surface of neutrophils and macrophages, possibly aiding chemotactic migration of these or other cells into the atherosclerotic plaque [236, 237]. Arterial lumican appears to be present as a lactosaminoglycan-modified form [238] and does not contain KS chains as is present in other tissues. This observation indicates an important potential role of the core protein, and not the KS chain in mediating events as part of the atherogenic process. An interesting proteomic study of human arteries showed that the intima of the atherosclerotic-prone carotid artery was enriched in versican, biglycan, and perlecan, but contained elevated levels of lumican core protein compared to non-atherosclerotic-prone vascular areas [68]. Furthermore, lumican gene expression is elevated in arteries from patients with coronary artery disease compared to healthy controls [239, 240].

Fibromodulin also accumulates in atherosclerotic plaques and like lumican accumulates in macrophage-rich areas [234, 241]. Fibromodulin, like decorin, interacts with TGF β 1 and, when expressed in a vein graft failure organ culture model, fibromodulin caused a decrease in TGF β 1 and a reduction of neointimal hyperplasia of the vein graft [242]. Fibromodulin expression is significantly elevated in symptomatic plaques and is higher in plaques from patients with diabetes [234]. Fibromodulin is associated with plaque lipids as well. A potential role of fibromodulin in atherosclerosis is its ability to interact with C1q [243] potentially impacting complement activation and leukocyte invasion. Furthermore, these KSPGs are capable of binding chemokines and cytokines which can impact the angiogenic phenotype during atherosclerotic plaque formation [234, 244, 245]. Finally, fibromodulin, like decorin, binds collagen I but at different sites along the collagen fibril [246]. Studies in mice lacking both ApoE and fibromodulin show reduced lipid retention, smaller plaques, altered collagen, and decreased plaque burden [241]. Excellent reviews on the role of KSPGs in atherosclerosis are available [6, 247].

Heparan sulfate proteoglycans (HSPGs)

Perlecan

Perlecan is a PG with a large core protein (467 kDa in humans) divided into five distinct domains with attachment sites for three HS chains [19, 248-250]. HS chain extension is catalyzed by a family of transferases and modified by N-deacetylation and N-sulfation and also by epimerization and variable O-sulfation, giving rise to complex HS chain composition [251, 252]. This complexity results in selective binding capabilities for a number of HSPG ligands. Since the binding sequences in the HS GAGs exist independent of binding sequences in the HSPG core, perlecan can be viewed as a highly interactive PG [4, 71].

Perlecan is localized to basement membranes below endothelial cells and basal laminae around SMCs and diffusely throughout atherosclerotic lesions in both humans and in experimental animal models of atherosclerosis [9, 21, 253-259]. Like other PGs, perlecan is upregulated in response to arterial injury in experimental animals [124]. However, perlecan is downregulated in human atherosclerosis [255]. Interestingly, perlecan and biglycan are the prominent PGs in diet-induced atherosclerosis in mice [129] possibly reflecting PG compositional differences in the vasculature between mouse and man. We found that perlecan expression was low in the media and early neointima at time of maximal ASMC proliferation in the rat carotid injury model [253]. Despite the reduction in perlecan expression, the late neointimal lesions remained perlecan-rich coincident with lack of ASMC proliferation [253]. Tran et al. and Tran-Lundmark et al. confirmed that the HS chains of perlecan contribute to SMC growth control both in vitro and in vivo during intimal hyperplasia [256-258]. SMCs that populate the neointima following arterial injury progressively lose proliferative capacity and growth factor responsiveness by 4-6 weeks. There is a considerable literature that suggests that HSPGs, including perlecan and GAGs, such as heparin, are potent inhibitors of ASMC proliferation in vitro and in vivo [254, 256, 260-265]. A question arises as to whether perlecan or other HSPGs in some way regulate ASMC proliferation in vivo. Kinsella and colleagues treated vascular explants of 35- to 42-day induced rat neointimal lesions with heparin lyase I and II which degrade HS followed by mitogenic PDGF BB stimulation. Removal of the endogenous HS from the late lesion explants caused a dramatic recovery of the proliferative responsiveness of the neointimal SMCs [253, 266]. Heparin's role in influencing ASMC proliferation was later shown to be partially due to its interaction with FGF-2 [266]. More recent studies demonstrated that retinoic acid, which is an inhibitor of ASMC proliferation, stimulated the expression of perlecan in murine SMCs and both cell proliferation and increased expression of perlecan were reversed in SMC cultures from transgenic mice expressing HS-deficient perlecan [257]. Interestingly, accompanying the effect of heparin inhibiting SMC proliferation in vivo, heparin also has a dramatic effect on the remodeling of the ECM in that it decreases elastic fiber and collagen formation, potentially altering the mechanical properties of the lesion in the rat carotid injury model [267]. In addition, endothelial cell-derived perlecan inhibits thrombosis and intimal hyperplasia in a porcine carotid injury model [268].

Heparanase, the enzyme that degrades HS GAGs, has also been implicated in atherosclerosis. Heparanase immunostaining in human coronary arteries with atherosclerosis increases with vascular disease [269-271] and these changes were prominent in the regions of the plaque that were prone to rupture. Heparanase is also enriched in regions of the plaque that contain inflammatory cells, such as macrophages, potentially impacting macrophage activation, clotting, and thrombosis [270, 272, 273]. Additionally, heparanase is also capable of degrading HS GAG chains which have been shown to impact the ability of FGF2 to stimulate vascular cells [274, 275]. Thus, heparanase is a multifaceted protein that consists of enzymatic and non-enzymatic activities that may play critical roles in the pathogenesis of vascular disease.

It is important to note that under certain conditions heparin/HS and perlecan can stimulate cell growth. For example, low concentrations of heparin support fibroblast growth factor receptor (FGFR) activation by FGF-2, particularly in the absence of endogenous HS, thus

promoting cell growth associated with angiogenesis [276]. It has been noted that perlecan isolated from different vascular beds bears HS chains that differentially bind FGF-2 and FGFR [277]. Recent studies show that perlecan made by SMCs contains primarily HS attached to its core protein, while perlecan isolated from endothelial cells contains both HS and CS chains. Furthermore, the perlecan from these two different cell sources exhibited differences in their ability to bind FGF-2 and had different signaling capabilities [71]. Proteolytic cleavage of basement membrane PGs such as perlecan also release C-terminal globular domains such as endorepellin that have important inhibitory activities for vascular cell growth and angiogenesis [278-280]. Such fragments may influence endothelial cell adhesion and survival [279, 281] by causing cytoskeleton and focal adhesion disassembly through integrin destabilization [279]. Macrophages also synthesize multiple HSPGs. Modifying the sulfation pattern of these PGs impacts cytokine sequestration, promoting atherosclerosis in mouse models of atherosclerosis [282].

Like all PGs, lipoproteins are known to bind to HS chains borne on perlecan [283]. To test the importance of perlecan in lipoprotein retention in mouse models of atherosclerosis, mice that had a heterozygous deletion of perlecan were crossed into *ApoE* and *Ldlr*^{-/-} backgrounds [259]. Examination of atherosclerosis in *ApoE*^{-/-} with a heterozygous perlecan deletion did not reveal altered lipoprotein profiles [259]. However, these studies confirmed the previously-reported distribution of perlecan in developing vascular lesions, although the presence of a perlecan-null allele did not significantly alter the size of the lesion that was ultimately formed. Perlecan was detected in subendothelial regions adjacent to small fatty streaks that formed early in the disease pathogenesis in mice. In fact, HSPGs in the subendothelial ECM can mask binding sites for other LDL ligands and inhibit lipid accumulation [284]. LDL binds weakly to HS in vitro and it is likely that other molecules such as lipoprotein lipase act as bridges between perlecan and the lipoprotein [285-287]. In a follow up study, Tran-Lundmark and colleagues investigated the role of HS chains of perlecan in lipoprotein retention by using mice deficient in the HS chains of perlecan crossed to *ApoE*^{-/-} mice [258]. Removing the HS chains from perlecan resulted in significantly reduced atherosclerosis at both early and late stage lesions in *ApoE*^{-/-} mice, with significant reduction of lipid accumulation and an increase in SMC actin. Such results support a pro-atherogenic role for HS chains of perlecan, confirming an earlier study [259]. Serum amyloid is another plasma lipoprotein (HDL)-associated molecule that interacts with HS-containing PGs such as perlecan [288] and accumulates in lesions of atherosclerosis [289]. In fact, elevated serum amyloid A (SAA) levels are associated with increased cardiovascular risk in humans. SAA is a major acute phase reactant that increases dramatically with inflammation in human vascular disease [290, 291]. SAA is deposited in murine atherosclerosis and is co-localized with extracellular perlecan [289]. Also, HDL isolated from LPS-injected mice contained elevated SAA levels and bound purified perlecan, isolated from human ASMC cultures, to a greater extent than HDL isolated from normal mice [289]. More recent studies show that other PGs such as versican may play a role in both the pro- and anti-inflammatory properties of HDL [292]. In summary, perlecan may have “two faces” – one that is pro-inflammatory (binding lipoproteins) and one that is anti-inflammatory (inhibiting SMC proliferation and migration)!

A role for HSPGs impacting the formation of pro-atherogenic lipoproteins outside the blood vessel and within the liver also exists, contributing to changes in lipid profiles and atherosclerosis susceptibility [293-295]. This topic will be covered in another review in this collection from Jeff Esko's group at UC San Diego.

Therapeutic implications (*PGs as therapeutics or targets for therapeutics?*)

Vascular diseases are responsible for 30% of all deaths worldwide each year with atherosclerosis responsible for the global rise in mortality [296, 297]. Current therapies that target this disease include strategies to reduce risk factors (lifestyle changes) such as smoking, hypertension, hypercholesterolemia, hypertriglyceridemia, and hyperglycemia. The maximum efficacy of such approaches is limited to around 30% [298]. Treatments that target the pathological events in the vascular wall in humans are almost non-existent [299]! Since PGs impact many of these pathogenic events, there may be merit in targeting specific PG components in the prevention and treatment of vascular disease. There are some intriguing examples of such approaches in animal models of atherosclerosis. For example, some success has been achieved by targeting lipid/PG interactions. Soto and colleagues have developed chimeric monoclonal antibodies that recognized sulfated GAGs that can block LDL interactions with CS GAGs. Using these antibodies to immunize NZ rabbits prevented diet-induced atherosclerosis in this animal model [300].

Inhibiting intimal hyperplasia using miRNAs targeting versican in ASMCs [160-162] or expressing the V3 variant of versican in ASMCs [126, 127, 148] has also been used to target the early stage of vascular lesion development with some success. Controlling ECM assembly and preventing its degradation is another approach. For example, both the V3 isoform of versican (lacking CS GAGs) and the core protein of biglycan (lacking CS/DS GAGs) promote ECM assembly by ASMCs and influence elastic fiber assembly, reversing the destruction of the ECM that accompanies vascular disease [126, 127, 146, 148, 190, 228]. These treatments also create an ECM that resists inflammation. Such studies highlight a potential for using PGs to stabilize the ECM [146].

Some success has been achieved by creating functional mimetics of PGs and GAGs to be used as therapeutics to treat specific events associated with vascular disease. For example, Panitch and colleagues have fabricated small molecules using molecular engineering approaches to create peptidoglycans which contain a GAG backbone covalently linked to peptides that have selective affinity for components involved in regulating key events in the pathogenesis of vascular disease [301, 302]. This group developed a synthetic biomimetic by conjugating multiple collagen binding peptides to a DS backbone, taking advantage of the anti-platelet activity of DS and the collagen binding activity of the peptides [303, 304]. The goal was to interfere with platelet binding to exposed collagen following balloon angioplasty [302]. In vitro studies demonstrated that the synthetic biomimetic effectively bound fibrillar collagens and prevented platelet adhesion both in vitro and when delivered to animals such as the pig following balloon angioplasty. Furthermore, this treatment blocked intimal hyperplasia in this pig model. These results indicate that synthetic glycopolymers may be a useful approach in targeting those vascular events that have a PG or GAG component.

Finally, there is the possibility that already approved drugs for treatment of vascular disease could impact the PG or GAG component of vascular disease beyond their primary targets and create a pleiotropic effect that is beneficial for reducing vascular lesion formation [189, 299]. For example, we found that rapamycin (sirolimus), which is an effective anti-mitogenic drug used following balloon angioplasty in humans to block intimal hyperplasia and restenosis, also negatively impacts the generation of a hyaluronan/PD-rich ECM generated by ASMCs, blocking the adhesion of monocytes to this ECM [305]. Drugs that are effective in blocking the angiotensin II AT-1 receptor, such as telmisartan, reduce biglycan accumulation and atherosclerosis in a lipid-induced mouse model of atherosclerosis [219].

There is presumably no disease without quantitative and/or qualitative changes in the PGs in the ECM

The challenge, however, is to distinguish between PG changes that cause disease and PG changes that result from disease, because therapeutic strategies will differ depending upon primary or secondary causation. However, whether the effects are direct or operate through indirect means, the conclusion is clear that PGs, in part, play important roles in regulating several key events in the pathogenesis of vascular disease and should warrant careful consideration as potential therapeutic targets in the future treatment of vascular disease.

Concluding remarks

Once thought to only play structural roles in the pathogenesis of vascular disease and to represent nonspecific desmoplastic responses to vascular injury, it is quite clear today that specific components of the ECM, such as PGs, play important regulatory roles in this disease. Vascular disease involves several sequential events from the early lesion stage, through the late advanced plaque, and eventual plaque rupture. PGs play multiple roles “along the way” by influencing mechanotransduction pathways in the lesions through the regulation of ECM organization, stability and stiffness, facilitating intimal hyperplasia, promoting lipid accumulation and calcification, regulating leukocyte invasion and inflammation, and controlling clot formation and thrombosis. PGs are “keystone molecules” in the pathogenesis of vascular disease. And, as one of my esteemed colleagues suggested a while ago, “vascular proteoglycans and atherosclerosis: not over yet” [194]. Indeed, the importance of these ECM components in the pathogenesis of vascular disease has been overlooked and further mechanistic studies targeting specific PGs within the ECM are needed if we are to fully understand those factors that regulate the pathogenesis of vascular disease.

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Abbreviations footnote

ECM	extracellular matrix
PG	proteoglycan
GAG	glycosaminoglycan
CS	chondroitin sulfate
DS	dermatan sulfate
HS	heparan sulfate
KS	keratan sulfate

SMC	smooth muscle cell
ASMC	arterial SMC
LDL	low-density lipoprotein
oxLDL	oxidized LDL
MMP	matrix metalloproteinase
SNP	single nucleotide polymorphism
PSGL-1	P-selectin glycoprotein-1
TNFα	tumor necrosis factor- α
SLRP	small leucine-rich repeat proteoglycan
LRR	leucine-rich repeat
TLR	toll-like receptor
NOD	nucleotide oligomerization domain
DAMP	danger-associated molecular pattern
FGFR	fibroblast growth factor receptor
SAA	serum amyloid

Highlights

- Proteoglycans increase dramatically in the extracellular matrix of blood vessels in vascular disease.
- Proteoglycans accumulate in defined regions of vascular lesions.
- Proteoglycans interact with a large number of components in vascular disease and impact their atherogenicity.
- Proteoglycans interact with lipids to cause their accumulation in vascular disease.
- Targeting specific proteoglycans in animal models of vascular disease can interfere with vascular lesion development.
- Proteoglycans interact with vascular cells and alter their pathogenic phenotype.

Figure 1.

Composition of the extracellular matrix in atherosclerosis. Proteoglycans occupy a small percentage of the total extracellular matrix in a normal blood vessel (4%), but increase dramatically in the early lesion phase (~50%) with concomitant decreases in elastic fibers and collagen. As lesions become more advanced (late lesion), collagen predominates with continued decreases in elastic fibers.

Figure 2.

(A, B) Representative histologic sections of human coronary arteries showing pathological intimal thickening with macrophages stained for versican (brown). Sections kindly provided by Drs. Frank Kolodgie and Renu Virmani, CV Path Institute, Inc., Gaithersburg, MD [15]. (C, D) Sections from a human temporal pseudoaneurysm stained with a modified Movat's stain (C) which shows breaks in the intimal elastic membrane (purple) and an enrichment of proteoglycans (blue) contributing to lesion formation. Note the large number of microvessels within the adventitia and at the base of the forming lesion. (D) Immunostaining for versican of the adjacent section shows substantial involvement of this proteoglycan (black) [46]. (E, F) Histologic section of an atherosclerotic lesion immunostained for versican from an *Athsq1* Homozygote mouse fed a Western-style (high fat) diet. Intense versican staining (dark brown) is seen in the media of the aortic root lesion with some positive staining seen associated with the macrophage. Adjacent western blot shows enrichment of the versican (V0, V1) protein in the lesions in the congenic strain. (F) A higher magnification of the congenic strain lesion showing intense versican staining (brown) around the macrophages that had accumulated within the lesion [128]. Sections kindly provided by Dr. Carrie L. Welch, Columbia University Medical Center, New York, NY. Magnification: A and B, $\times 40$; C, D, and E, $\times 100$; F, $\times 300$.

Figure 3.

Impact of V3 versican expression on experimental lesion formation in balloon-injured carotid arteries from New Zealand white rabbits placed on a high-fat diet. Controlled expression of V3 with the injured carotid arteries causes increased elastic fiber formation and decreased accumulation of lipid, macrophages, and versican. Sections kindly provided by Dr. Mervyn J. Merrilees, Faculty of Medical and Health Sciences, University of Auckland, New Zealand. Magnification: $\times 80$.

Figure 4.

Colocalization of ADAMTS4 and biglycan in podosome-like structures formed by tubulogenic HUVECs cultured in 3-dimensional (3D) collagen gels. Panels A-C are generated from whole-mounts stained by immunocytochemistry and viewed by confocal microscopy. (A) Portion of a multicellular tube. Lumen (asterisks) and cell nuclei (N) are indicated. Rosettes and plaques (arrows) appear yellow, indicating colocalization of ADAMTS4 (red) and biglycan (green) in these structures. (B) En face detail of clustered ADAMTS4 (red), biglycan (blue), and the podosome component cortactin (green) in a HUVEC shows colocalization of: yellow – cortactin with ADAMTS4; cyan – cortactin with biglycan; magenta – ADAMTS4 with biglycan; white – all three molecules. (C) Z-stack of an area similar to "B" rendered in 3D and rotated 90° as a "side view" (arrow points toward the abluminal side of the cell). The image is digitally sectioned to reveal the central location of cortactin (e.g., white area, arrowhead) within an area of colocalized ADAMTS4 and biglycan (magenta). Scale bars = 10 μm (A), 5 μm (B), and 1 μm (C).

Figure 5.
Some of the key pathways involved in vascular lesion formation that are impacted by proteoglycans.