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The small leucine-rich repeat proteoglycans in tissue repair and atherosclerosis

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Abstract. Hultgårdh-Nilsson A, Borén J, Chakravarti S (Lund University, Lund; University of Gothenburg and Sahlgrenska University Hospital, Gothenburg, Sweden; Johns Hopkins University School of Medicine, Baltimore, MD, USA). The small leucine-rich repeat proteoglycans in tissue repair and atherosclerosis (Review Symposium). *J Intern Med* 2015; **278**: 447–461.

Proteoglycans consist of a protein core with one or more covalently attached glycosaminoglycan (GAG) side chains and have multiple roles in the initiation and progression of atherosclerosis. Here we discuss the potential and known functions of a group of small leucine-rich repeat proteoglycans (SLRPs) in atherosclerosis. We focus on five SLRPs, decorin, biglycan, lumican, fibromodulin and PRELP, because these have been detected in atherosclerotic plaques or demonstrated to have a role in animal models of atherosclerosis. Decorin and biglycan are modified post-translationally by substitution with chondroitin/dermatan sulphate GAGs, whereas lumican, fibromodulin and PRELP have keratan sulphate side chains, and the core proteins have leucine-rich repeat (LRR) motifs that are characteristic of the LRR superfamily. The chondroitin/dermatan sulphate GAG side chains have been implicated in lipid retention in atherosclerosis. The core proteins are discussed here in the context of (i) interactions with collagens and their implications in tissue integrity, fibrosis and wound repair and (ii) interactions with growth factors, cytokines, pathogen-associated molecular patterns and cell surface receptors that impact normal physiology and disease processes such as inflammation, innate immune responses and wound healing (i.e. processes that are all important in plaque development and progression). Thus, studies of these SLRPs in the context of wound healing are providing clues about their functions in early stages of atherosclerosis to plaque vulnerability and cardiovascular disease at later stages. Understanding of signal transduction pathways regulated by the core protein interactions is leading to novel roles and therapeutic potential for these proteins in wound repair and atherosclerosis.

Keywords: atherosclerosis, biglycan, decorin, fibromodulin, lumican, small leucine-rich repeat proteoglycan.

Introduction

The small leucine-rich repeat proteoglycans (SLRPs) are integral components of the collagenous extracellular matrix (ECM) that regulate a range of cellular functions important in innate immune responses and wound healing. Recent findings suggest involvement of many of the SLRPs in the development of atherosclerotic plaques. Atherosclerosis is a luminal narrowing of blood vessels caused by plaque formation that reduces blood flow to the heart, brain or peripheral organs, leading to coronary heart disease, stroke or peripheral vascular disease, respectively [1, 2]. The proteoglycans discussed in this review have distinct roles in the early steps of plaque formation, whilst the progressive changes in plaques in turn shape proteoglycan and ECM homeostasis, which further contribute to disease pathogenesis. Thus, plaques are initiated by localized changes in endothelial gene expression and accumulation of low-density lipoproteins (LDLs), mediated by the ECM (mainly

proteoglycans) and lipolytic enzymes such as lipoprotein lipase [3] and sphingomyelinase [4–6]. The interaction between LDL and proteoglycans promotes modification and aggregation of LDL [4], and uptake by macrophages leading to foam cell formation. The conversion of macrophages to foam cells stimulates the release of potentially atherogenic factors that further alter proteoglycan metabolism. Retained and altered lipoproteins, together with neighbouring macrophages, stimulate chemotaxis and transformation of smooth muscle cells (SMCs) from the contractile to the proliferative state, which causes increased synthesis of proteoglycans [4].

In this review, we present the current understanding of SLRPs in the context of injury and wound healing in general and, where possible, in relation to specific changes in atherosclerosis. We focus on five SLRPs that have either been detected in atherosclerotic plaques or for which links to atherosclerosis have been demonstrated in animal models: decorin, biglycan, proline-/arginine-rich end leucine-rich repeat protein (PRELP), fibromodulin and lumican. The role of collagens and collagen-binding proteins in the developing atherosclerotic lesions [7], as well as the structure and functions of the SLRPs [8–13], has been reviewed recently. The functions of the SLRPs reviewed here and their associations with human disease are summarized in Table 1.

SLRP classification and evolutionary relationships

The SLRPs are a subfamily of the large (>300 members) leucine-rich repeat (LRR) superfamily

Table 1 *Cellular functions, gene-targeted mice and human disease associations of SLRPs*

Proteoglycan	Gene symbol	Cellular functions	Gene-targeted null mice and references[c]	Human disease association and references[d]
Lumican Class II SLRP	LUM	Proliferation↓ Apoptosis↑ Migration↑ Differentiation↑ TLR4 signals↑	[a]*Lum*^{tm1Chak} [58] [a]*Lum*^{tm1Wwk} [60]	Cancer [12, 86] Systemic lupus erythematosus [88] Myopia [90]
Fibromodulin Class II SLRP	FMOD	Complement functions↑	*Fmod*^{tm1Aol} [63]	Chronic lymphocytic leukaemia Mantle cell lymphoma [83]
PRELP Class II SLRP	PRELP	Complement functions↓	[b]Prelp$^{tm1(KOMP)Vlcg}$	Progeroid symptoms [107]
Decorin Class I	DCN	TLR2, 4 signals↑ Proliferation↓ Apoptosis↑ Autophagy↑ Differentiation↑ Fibrosis↑ Angiogenesis↑↓ Tumorigenesis↓	*Dcn*^{tm1Ioz} [61]	Congenital stromal corneal dystrophy [153, 154, 156] Marfan syndrome [155] Progeroid symptoms [107] Cancer [12]
Biglycan Class I	BGN	TLR2, 4 signals↑ Inflammasome ↑ lipid retention	[a]Bgn^{tm1Mfy} [57]	Lupus nephritis [12, 164, 165] Turner syndrome

Up and down arrows indicate activation or inhibition of a biological process.
[a]Mouse strains donated to The Jackson Laboratory (http://jaxmice.jax.org).
[b]Targeted ES cell line available through VelociGene (http://www.regeneron.com/velocigene).
[c]Key references describing the gene-targeted mice are included, additional information available through the Mouse Genome Informatics (http://www.informatics.iax.org).
[d]Additional information on disease association available through OMIM (http://omim.org) and GeneCards (http://www.genecards.org).

that includes the toll-like receptors (TLRs) and NOD-like receptors [14]. The LRR superfamily is characterized by tandem repeats of leucine-rich motifs of 21, 24 or 26 amino acids, classified into seven different types based on conserved amino acids. The N-terminal and C-terminal ends of the SLRPs form disulphide-bonded caps as deduced from the crystal structures of decorin and biglycan [13, 15–17]. The last two LRR motifs in SLRPs are characteristically longer than the other LRRs, and the penultimate motif forms an extended loop (often referred to as an ear extension, or the LRRCE motif [18]), which is specific to chordates. Insights into the evolution of the SLRP subfamily came from multiple sequence alignment studies of the LRRCE motif. This subfamily appears to have evolved from an ancestral SLRP through large-scale gene and genome duplication and loss of genes, and the modern SLRPs retain clustered syntenic localization on specific chromosomes [18, 19]. The functional implications of these conserved structures in health and disease remain to be elucidated.

The SLRPs are subdivided into five classes based on sequence alignment and the spacing of four cysteine residues at the N-terminus [13, 20]. The Class I SLRPs includes biglycan and decorin, and the Class II comprises fibromodulin, lumican and PRELP. The core proteins of these five SLRPs are small, ranging in size from 40 to 60 kDa, and contain 11–12 LRR motifs. The crystal structure of decorin (at a resolution of 2.7 Å) indicates an antiparallel dimer structure of two curved solenoid monomers [15], but biochemical approaches suggest that the biologically active form is a monomer in solution [16]. The crystal structure of biglycan (at a resolution of 3.4 Å) also indicates dimerization of curved solenoid monomers [17].

Interactions between glycosaminoglycans and LDLs in atherogenesis

Decorin and biglycan are post-translationally modified with either one or two chondroitin/dermatan sulphate side chains, respectively [21]. Lumican and fibromodulin are modified by the addition of keratan sulphate side chains [22–26]. The numbers of keratan sulphate side chains can vary, or these proteoglycans can be present as glycoproteins either permanently in some tissues or transiently in newly synthesized or remodelled ECM [27, 28]. The involvement of the glycosaminoglycan (GAG) components of proteoglycans in atherosclerosis was recognized even before the functions of the individual core proteins were understood. Thus, according to the lipid retention hypothesis, the GAGs in the subendothelial matrix promote localized retention of LDL in the vessel wall [4, 29–33]. In atherosclerotic plaques, LDL colocalizes mainly with chondroitin sulphate and dermatan sulphate associated with the biglycan core protein [34], as decorin does not generally colocalize with retained lipoproteins even though it can interact with lipoproteins *in vitro* [34, 35].

The direct interaction between LDL and negatively charged GAG chains on the proteoglycans involves positively charged amino acids in apolipoprotein (apo) B, the main protein moiety on LDL [4, 36]. ApoB is a large protein (4536 amino acids) that wraps around the LDL particle and, unlike other apolipoproteins, is not exchangeable [37, 38]. In studies of delipidated apoB100, eight clusters of positively charged residues were identified that interact with proteoglycans [39–43]. Subsequent studies of transgenic mice expressing human recombinant LDL with specific mutations in those sites identified residues 3359–3369 (Site B) as the functional proteoglycan-binding site in native LDL. The other binding sites are probably buried in the surface lipid layer and are therefore nonfunctional [3, 29, 43]. Subendothelial retention of LDL can be enhanced by sphingomyelinases (SMases) [5] and the SMase activator apo CIII [6]. Furthermore, subendothelial retention of atherogenic lipoproteins to GAGs can also be facilitated by lipoprotein lipase (LPL) [3, 44]. The binding between LPL and LDL is mediated through an interaction between LDL lipids and LPL [45]. LPL facilitates the interaction between GAG chains and extensively oxidized LDL (which cannot bind directly to GAG because of the reduced number of positive charges) [46, 47].

The importance of Site B in the retention of atherogenic lipoproteins has been tested *in vivo* [32]. Mice expressing human recombinant control LDL or LDL with defective proteoglycan binding (i.e. LDL with a Site B mutation that abolishes the binding to proteoglycans) were fed a cholesterol-rich diet for 20 weeks [32]. The results showed that the vessel wall area covered by atherosclerotic lesions correlated with the plasma cholesterol level in both groups of transgenic mice. However, the extent of atherosclerosis differed dramatically. Transgenic mice expressing a form of LDL that is defective in binding proteo-

glycans had a considerably milder degree of atherosclerosis than mice expressing the wild-type recombinant LDL form [32]. These findings show that LDL with decreased proteoglycan binding has a markedly reduced atherogenic potential, and provide direct experimental evidence that binding of LDL to artery wall proteoglycans is an early step in atherogenesis.

Interactions of SLRP core proteins with collagens

The core proteins of SLRPs have two main functions. First, they regulate collagen fibril architecture and assembly to control tissue strength and biomechanics [9]. Secondly, studies show that these proteins can regulate cellular properties such as proliferation, migration, phagocytosis and innate immune responses through specific interactions with cytokines, chemokines, ligands and receptors [9, 13, 48–52] as discussed in the next section.

To determine the impact of SLRP-collagen interactions in atherosclerosis, it is important to understand functions of collagens in healthy vascular tissues and how these functions can potentially shape plaque properties. The fibrillar collagen types I and III, the fibril regulatory collagen type V, basement membrane collagen type IV and filament-forming collagen type VI are all abundant in plaques. Collagens regulate the structural integrity of vessel walls, influence lipid retention and regulate proliferation and migration of SMCs (for recent review, see [7]).

The five SLRPs considered here can affect the structural integrity of plaques by modulating collagen fibril assembly and interactions of lipids with bioactive sites on collagens and SMCs. There are multiple lines of evidence to suggest that these SLRPs interact with collagens. First, early electron microscopy analyses showed an association between dermatan sulphate and keratan sulphate proteoglycans on specific collagen fibril bands [53, 54]. Secondly, in *in vitro* fibrillogenesis assays, inclusion of lumican or decorin core proteins modified the kinetics of fibril formation and reduced the diameter of assembled fibril [55, 56]. Thirdly, *in vivo* experiments using gene-targeted mice deficient in lumican, decorin, biglycan and/or fibromodulin demonstrated collagen fibrils with irregular contours and diameter, and impaired biomechanical strength of multiple connective tissues, confirming the role of these SLRPs in collagen fibril structure and function [57–63].

Lumican and fibromodulin bind collagen type I *in vitro*; a stronger binding site unique to fibromodulin resides in LRR11, whilst both SLRPs show weaker binding at LRR7 [64]. This indicates that fibromodulin competes with lumican for collagen binding during collagen fibrillogenesis and replaces collagen-bound lumican as fibril growth progresses [64]. In the developing mouse tendon, peaks in SLRP expressions suggest that lumican is mainly active during early fibrillogenesis and that fibromodulin, although active throughout fibrillogenesis, contributes most significantly during the later stages [65]. Atherosclerotic plaques are dynamic structures, as are the collagen fibrils in the plaques. The fibrils are remodelled continuously by synthesis of the constituent collagen proteins and their degradation. Thus, atherosclerotic plaques contain collagen fibrils at different stages of maturation, some at the early lumican-driven and others at the late fibromodulin-driven stages, and fibromodulin and lumican are found in overlapping areas within human carotid atherosclerotic plaques (Hultgårdh-Nilsson A, unpublished data). In addition, lumican and fibromodulin are likely to affect collagen turnover and synthesis through their role in transforming growth factor (TGF)β signal regulation [66–68]. Fibromodulin is negatively correlated with the degree of fibrosis; it is downregulated in hypertrophic scars, and in fibromodulin-deficient mice, wound healing is impaired with increased fibrosis and altered ECM collagen composition [69–71]. Moreover, fibromodulin can directly reprogram human fibroblasts to a multipotent quiescent stem cell-like state [72].

SLRPs and their interactions with ligands, growth factors, and cell surface receptors: potential therapeutic targets in the developing atherosclerotic lesion

Lumican (LUM) and fibromodulin (FMOD)

Using *in situ* hybridization and immunohistochemistry, Onda and co-workers detected lumican transcript and protein, respectively, in normal arterial tissue and four categories of atherosclerotic plaques (diffuse intimal thickening, fatty streak, complete atheroma with fibrous plaques and complicated lesions) [73]. The authors detected positive immunostaining for lumican in the adventitia of the normal coronary artery. In thickened intima, immunohistochemical staining of lumican was weak in the medial and thickened intima, and it was associated with the matrix surrounding the SMCs. In fibrolipid lesions, lumican staining was

associated with the matrix around foamy macrophages, and in atherosclerotic plaques, it was found in the thickened intima, but not in cholesterol-rich or calcifying areas. In the same study, the lumican transcript was detected in intimal SMCs of atherosclerotic plaques indicating that these cells are the major source of lumican in plaques. Mouse model studies have primarily been used to investigate the expression of lumican under healthy conditions. In developing mouse embryos (E9.5), we have shown that the lumican transcript is detectable in the head and lateral mesenchyme; at a later stage, lumican is expressed in the heart, pulmonary and aortic valves, and arterial walls [58, 74]. Arterial lumican is present in its lactosaminoglycan-modified form [28]. Accordingly, lumican from arterial extracts and cultured vascular endothelial cells in immunoblots appears as a 50 kDa band and not a GAG-containing diffuse 50–90 kDa band as seen in corneal extracts [28, 49], implying that the lumican core proteins may be more important than the GAG-containing proteoglycan form in atherosclerosis.

Lumican and fibromodulin proteins are detectable in human arteries as reported by Talusan *et al.* [75]. The intima of the atherosclerosis-prone internal carotid artery showed increased deposits of lumican compared to the more resistant internal thoracic artery. However, fibromodulin levels were similar in the two types of arteries [75]. On the other hand, *LUM* gene expression was elevated in arteries from patients with coronary artery disease compared to healthy control subjects [76], as well as in femoral arteries with atherosclerotic plaques from patients with peripheral occlusive arterial disease [77] and in aortic valves from patients with degenerative aortic stenosis [78]. In addition, lumican and fibromodulin was detected in carotid atherosclerotic plaques from symptomatic and asymptomatic patients (A Hultgårdh-Nilsson A, unpublished data). In this unpublished study, fibromodulin was significantly higher in plaques obtained from patients with diabetes and in those with an increased incidence of post-operative neurological events. In addition, there was a positive correlation with fibromodulin and plaque lipids, proinflammatory cytokines, low SMC content and the anti-inflammatory cytokine interleukin (IL)-10.

In accordance with the studies of fibromodulin in human atherosclerotic plaques, *ApoE*×*Ldlr* knockout mice accumulate fibromodulin in macrophage-rich areas [79, 80]. Moreover, Shami *et al.* showed that the extent of atherosclerosis generated by a shear stress-modifying carotid artery cast is reduced in *ApoE*×*Fmod* double-null mice, with reduced lipid retention, smaller plaques and decreased plaque burden [79]. In the same study, the authors showed that ECM extracts from SMCs of wild type compared to fibromodulin-null mice promoted increased production of cytokines and uptake of lipids in a cultured macrophage cell line, suggesting a role for fibromodulin in lipid uptake [79]. Fibromodulin has also been shown to activate the classical and alternative complement pathways through interactions with the first subcomponent of the C1 complex (C1q) and the soluble inhibitor C4b-binding protein (C4BP) [51, 81, 82]. It is possible that complement activation mediated by fibromodulin and may influence functions of macrophages, such as adhesion and uptake of cell debris, lipids and other factors to ultimately influence plaque formation or progression. It is likely that fibromodulin, at sites of fibrolipid lesions, could affect the innate immune response, production of proinflammatory cytokines, accumulation and activation of macrophages and subsequent plaque formation. Fibromodulin has been associated with chronic lymphocytic leukaemia, and $Fmod^{-/-}$ mice (Table 1) show phenotypic features of osteoarthritis [83, 84].

To date, lumican has not been investigated in mouse models of atherosclerosis. However, studies of lumican in the context of immune, inflammatory and fibrotic responses provide key insights into its potential role in atherosclerosis and tissue repair as discussed below. Lumican expression is induced in fibroblast cultures by proinflammatory signals such as lipopolysaccharides or IL-1β and suppressed by immunosuppressive TGFβ [52, 85]. Polymorphisms in *LUM*, and changes in its expression levels have been associated with multiple diseases, ranging from cancer to systemic lupus erythematosus and myopia [86–90]. Table 1 shows the key cellular functions associated with each of the five SLRPs, as well as the available gene-targeted mouse models and key associations with some human disease (this is not a complete list of all disease associations).

Interactions between lumican and the cell surface influence cellular migration, proliferation and apoptosis, which are all important to wound healing and immune responses and should be considered in atherosclerosis plaque biology [49, 52,

91–93]. Chemotactic migration of neutrophils and macrophages is aided by the interaction of lumican with β2 integrin receptors [49], whereas lumican-β1 integrin receptor interactions may be important for epithelial cell migration [94]. Lumican's role in epithelial cell migration is further supported by the finding of delayed healing of corneal and dermal epithelial wounds in lumican-deficient mice [60, 91] and of expedited wound healing by administration of soluble lumican glycoprotein [95]. With respect to functions in leucocytes, lumican interacts with CD14 [52, 96, 97], a glycosylphosphatidyl inositol-linked cell surface LRR adaptor protein that promotes TLR4-mediated innate immune and inflammatory responses to bacterial lipopolysaccharides. This lumican–CD14 interaction enhances phagocytosis of nonopsonized bacteria by macrophages, which may facilitate clearance of complement-resistant bacteria and possibly dead and damaged cells. Thus, lumican-null mice show poor clearance of *Pseudomonas aeruginosa* infections and inefficient resolution of inflammation [85, 93, 98]. Lumican in the ECM has also been reported to bind the proinflammatory cytokine CXCL1, providing a chemokine gradient for migration of leucocytes in the wound bed [48]. Similarly, lumican binds to Fas ligand (FasL), a member of the tumour necrosis factor family [91], and may help to retain soluble FasL in the ECM and enhance its proinflammatory functions [99]. In addition, lumican-null mouse fibroblasts have marked reductions in Fas protein levels, and Fas–FasL mediated cellular apoptosis [91, 92]. These properties are important in cancer but may also affect leucocyte recruitment, amplification and clearance in atherosclerosis.

Intraplaque angiogenesis is another important phenomenon that is associated with atherosclerosis plaque vulnerability [100]. Gene expression analysis of endothelial cells grown on Matrigel matrices shows that lumican can regulate angiogenesis by inhibiting endothelial cell activation through p38 MAPK, as well as invasion, sprouting and vessel formation in mice [101]. It has been suggested that these effects involve interference with integrin α2β1 receptor activity as well as downregulation of matrix metalloprotease (MMP)-14 expression [102, 103]. Jian *et al.* have shown that fibromodulin enhances human endothelial cell adhesion, spreading, actin stress fibers and formation of tube-like structures *in vitro* [104]. These results are supported by observations that fibromodulin is a key regulator of angiogenesis in multiple *in vivo* systems [104, 105]. The specific roles of lumican and fibromodulin in intraplaque angiogenesis remain unclear.

PRELP

Bengtsson *et al.* isolated the 58 kDa PRELP protein from bovine articular cartilage and cloned the human PRELP cDNA from an articular chondrocyte cDNA library [106]. The *PRELP* gene encodes a 382 amino acid polypeptide with a calculated molecular mass of 42 kDa. Similar to other SLRPs, the core protein contains 10-11 LRR motifs, ranging in length from 20 to 26 residues, and that carry several N-linked oligosaccharides. The N-terminal region is unusually rich in arginine and proline residues. PRELP shares the highest sequence identity with fibromodulin (36%) and lumican (33%). There have been no reported studies using *Prelp*-null mice, but gene-targeted *Prelp*-null mouse embryonic stem cell lines are available (Table 1). PRELP may have a role in Hutchinson–Gilford progeria, a disease characterized by premature ageing [107].

PRELP is normally expressed in the ECM of collagen-rich tissues such as the skin, sclera, tendon, lung and heart [108, 109]. The N-terminal domain of PRELP, which is unusual in that it is basic and rich in arginine and proline [106], has been shown to bind both heparin and heparan sulphate proteoglycans [110]. This may indicate that PRELP anchors basement membranes to connective tissues [111]. The N-terminal domain has also been implicated in bone metabolism [112]; after uptake of a synthetic peptide representing the N-terminal domain of PRELP by osteoclast precursors through an annexin II- and chondroitin sulphate-dependent mechanism, the peptide translocates to the nucleus where it prevents transcription of osteoclast-specific genes [112]. This group subsequently showed that the N-terminal peptide of PRELP could ameliorate osteolytic changes in a mouse model of bone loss [113].

Although PRELP, like fibromodulin, interacts with C1q and C4BP [51], its mechanism of biological activity is through complement inhibition [114]. Thus, PRELP may hinder the formation of complement attack complex on cell membranes in damaged cartilage and therefore limit pathological complement activation in inflammatory diseases such as rheumatoid arthritis and in age-related macular degeneration [115].

Decorin (DCN)

Decorin, one of the most well characterized SLRPs, contains a protein core with 12 LRRs and one tissue-specific chondroitin sulphate or dermatan sulphate GAG chain, covalently bound to its N-terminus. The protein is a stromal proteoglycan synthesized chiefly by fibroblasts, stressed vascular endothelial cells and SMCs. Its name is derived from the fact that it 'decorates' collagen, and it was initially characterized by its high-affinity interactions with collagen fibres [116] and its role in the regulation of collagen fibrillogenesis [61, 117–119].

Decorin was the earliest collagen regulatory SLRP to be recognized as a modulator of cell proliferation [120]. Based on its structural and signal transduction functions, decorin is described as a bifunctional proteoglycan [121, 122], acting both as a signalling molecule and a structural ECM component [50, 123–125]. The LRR motifs are generally considered to be sites of protein–protein interactions; in the decorin core protein, these sites interact with several receptor tyrosine kinases, including the epidermal growth factor receptor (EGFR), the insulin-like growth factor 1 receptor (IGF-1R), MET (proto-oncogene) and the vascular endothelial growth factor receptor 2 (VEGFR2), as well as the low-density lipoprotein receptor-related protein 1 (LRP1) and innate immunity receptors (see [125, 126] for review), as discussed below.

Early studies of decorin were focused primarily on its antiproliferative and antifibrogenic/antiscarring functions (reviewed in [125, 126]). In the 1990s, decorin was shown to interact with TGFβ [127, 128], and its antifibrotic functions were investigated in a number of biological systems [50, 129–135]. The last LRR motif of decorin also interacts with connective tissue growth factor and this interaction was shown to restrict production of fibronectin and collagen type III, thus influencing turnover and production of the ECM [136]. The antiproliferative, antitumorigenic and pro-apoptotic functions were attributed to decorin core protein-EGFR interactions and downregulation of EGFR signal transductions [137]. Studies using exogenous decorin and gene-targeted mice deficient in decorin further indicated the modulation of cyclin-dependent kinase inhibitor-1 (p21/CIP) signalling pathways and suppression of proliferation by decorin [138–140].

Decorin, in addition to biglycan and lumican, has roles in the innate immune response and inflammation. Circulating decorin levels increase during inflammation in patients with sepsis as well as in a septic mouse model and, as shown in pull-down assays in cell culture-based expression systems, decorin interacts with both TLR2 and TLR4 [141]. The results indicate that decorin promotes TLR2- and TLR4-mediated downstream induction of the proinflammatory cytokines tumour necrosis factor-α and IL-12 at the protein level [141]. An intermediary in this pathway appears to be decorin-driven upregulation of the proinflammatory programmed cell death 4 (PDCD4) protein, which is a translational repressor of IL-10. In addition, the lowering of IL-10 was suggested to be due to a decorin-associated decrease in TGFß and the resultant reduction in the microRNA miR-21, which itself contributes to elevating IL-10. Additional inflammation-related functions of decorin include its role in downregulating the expression levels of intercellular adhesion molecule (ICAM)-1 and syndecan-1 and inhibition of polymorphonuclear leucocyte adhesion to the endothelial layer of blood vessels [142]. Decorin has also been reported to drive autophagy in endothelial cells through its interactions with VEGFR2 [143]. The proinflammatory functions of decorin, together with its role in attenuating immunosuppressive TGFβ and autophagy, may be particularly relevant to the development of an inflammatory environment during the formation of atherosclerotic plaques.

Early studies examined proteoglycan distribution in normal and atherosclerotic coronary arteries and identified low levels of decorin in the intima of normal coronary arteries and high levels in the fibrous caps of atherosclerotic lesions and in native and restenotic atherosclerotic segments [144–147]. Decorin colocalized with profibrotic TGFβ and platelet-derived growth factor (PDGF) and macrophages in a diet-induced atherosclerosis model in primates [147] and in fibrous caps of atherosclerotic lesions in an *Apoe*×*Ldlr* knockout mouse model of accelerated atherosclerosis [80]. In a recent mass spectrometric analysis of proteins extracted from the aortic valve and renal arteries, decorin and biglycan were amongst the group of proteins retained in a LDL-affinity column [148]. The enhanced presence of decorin and biglycan was also confirmed in lesion-prone areas of the subendothelial intimal ECM [148].

Based on what is known of the molecular interactions of decorin and its presence in atherosclerotic lesions, an obvious question is: Does decorin have a beneficial or a detrimental role in atherosclerosis? However, the answer is not simple and may depend on the inflammatory milieu, cell type and disease stage [149]. Thus, decorin may promote differentiation and survival in endothelial cells, whereas it may increase inflammatory responses in leucocytes (Table 1). In arterial SMC cultures, decorin induces calcification and colocalizes with mineral deposition in human atherosclerotic plaques, suggesting that decorin functions as a promoter of intimal calcification [150]. It seems that the GAG chains are essential for the procalcification role of decorin: in *Extl2* knockout mice that overexpress GAGs, aortic calcification was more pronounced compared to wild-type mice after experimental induction of chronic kidney disease [151]. In agreement with this, Yan *et al.* demonstrated that oxidative stress-mediated mineralization of vascular SMCs *in vitro* involves the production of glycosaminoglycanated decorin and activation of TGFβ1 signalling [152]. Identifying the molecular mechanisms by which vascular calcification occurs has important clinical implications, as therapies can then be tailored to target those patients at most risk.

Mutations in *DCN* have been identified in families with congenital corneal stromal dystrophy (CCSD) [153, 154] and a decrease in the *DCN* encoded transcript has been reported in Marfan syndrome [155]. However, there are no clear associations between these *DCN* mutations and cardiovascular diseases. In CCSD, the *DCN* mutations yield truncated core proteins that disrupt the organization of collagen fibrils in the cornea and result in a loss of corneal transparency. Mouse models expressing truncated decorin transgenes in the cornea show similar disruptions of collagen fibril assembly [156]. Whether such dominant-negative *DCN* mutations contribute to dysregulated collagen fibrils in atherosclerotic plaques and their stabilities are unknown.

Biglycan (BGN)

In humans, biglycan is encoded by the *BGN* gene (Xq28) and expression of *BGN* is reduced in patients with Turner syndrome who are missing all or part of an X chromosome [157]. In addition, patients with an extra Y chromosome also show elevated *BGN* expression, even though *BGN* is X-linked, and there are no active Y chromosomal *BGN*. This is because gene(s) that regulate the transcription of *BGN* escape X inactivation under these conditions. Biglycan is synthesized as a precursor from which a 37 amino acid N-terminal peptide is cleaved off by bone morphogenetic protein (BMP) 1 to yield a 331 amino acid core protein with 12 tandem LRR motifs of 24 residues, and two chondroitin sulphate or dermatan sulphate GAG side chains attached at amino acids 5 and 10 in human biglycan [158, 159]. In contrast to decorin, which is a major collagen-interacting connective tissue component, biglycan was recognized as long ago as the late 1980s to have a strong pericellular localization [22, 158–160].

Individuals with Turner syndrome have low bone mineral density [161] and, similarly, mice deficient in *Bgn* display an osteoporosis-like phenotype [162]; these findings led to further studies of the role of biglycan in osteogenic stem cell fate [163]. Studies have shown that secreted and pericellular matrix biglycan is a modulator of multiple signalling centres that regulate innate immunity and inflammation. Thus, mouse macrophages stimulated with LPS, IL-6 or IL-1β upregulate expression of biglycan, whilst biglycan itself promotes innate immune responses and increases production of proinflammatory cytokines in a TLR4- and TLR2-dependent manner [164]. Tissue injury leads to the release of endogenous molecules acting as damage-associated molecular patterns (DAMPs). Biglycan appears to behave as a DAMP; its expression and both circulating and renal tissue levels increase in mouse models of lupus and in patients with lupus nephritis [165]. Additionally, biglycan enhances NOD-like receptor family, pyrin domain containing protein (NLRP)-3 inflammasome-mediated maturation of pro-IL-1β to IL-1β, and this is dependent on intact TLR2/4 and purinergic receptor P2X7 signalling [166].

Biglycan is present in the intima of normal human blood vessels, including coronary and other muscular arteries [145, 167]. Furthermore, of all the vascular proteoglycans tested, biglycan shows the best colocalization with apoB in experimental mouse models and human atherosclerosis [34, 168–170]. Biglycan and perlecan [171], a heparan sulphate proteoglycan (not discussed in this review), are the main proteoglycans synthesized by human arterial SMCs and both have been shown to bind apoB-containing lipoproteins *in vitro* [172]. The overlapping areas of biglycan

and lipid deposition in the intima have been reviewed by Nakashima *et al.* [173]. Further, Tannock and co-workers showed increased lipid retention and atherosclerotic lesions in biglycan-overexpressing transgenic mice that were maintained on an atherogenic diet [174]. The authors also showed a strong correlation between severity of atherosclerotic lesions and vascular biglycan content [174], indicating that vascular biglycan contributes directly to increased lipid retention and increased atherosclerosis development. However, biglycan deficiency alone is not atheroprotective, and it is possible that upregulated perlecan in these mice could compensate and maintain lipid retention properties [175]. Importantly, in the context of atherosclerosis, the biglycan-deficient mice demonstrated a reduction in dense collagen fibrils and increased aortic aneurysm formation [175].

Concluding remarks

There is accumulating evidence to support significant and diverse functions of SLRPs in the developing atherosclerotic lesion (see Fig. 1). These studies demonstrate that specific SLRPs can influence SMC and macrophage functions *in vitro* and, more importantly, that silencing or overexpressing genes encoding these SLRPs can greatly affect the atherosclerotic lesion. These findings are likely to stimulate new and exciting research in atherosclerosis leading to novel therapeutic strategies in humans. The proteoglycans discussed in this review have both demonstrated and proposed roles in atherosclerosis and are clearly emerging as key modulators of plaque formation and resolution. The GAG side chains have a major role in lipid retention at the early stages of atherosclerosis. The core proteins, on the other hand, may have independent and distinctive functions in plaque progression, through modulating immune responses, collagen turnover and tissue repair. Further molecular studies of the core proteins are likely to lead to the elucidation of their functions in plaques and help to develop targets for localized treatments in the future. In addition, increased awareness of the SLRPs will lead to their inclusion as significant candidate genes in genetic studies of atherosclerosis susceptibility. It is hoped that future studies of SLRPs will contribute to a better understanding of the mechanisms involved in atherosclerotic lesion development and stability.

Conflict of interest statement

The authors have no conflict of interests to declare.

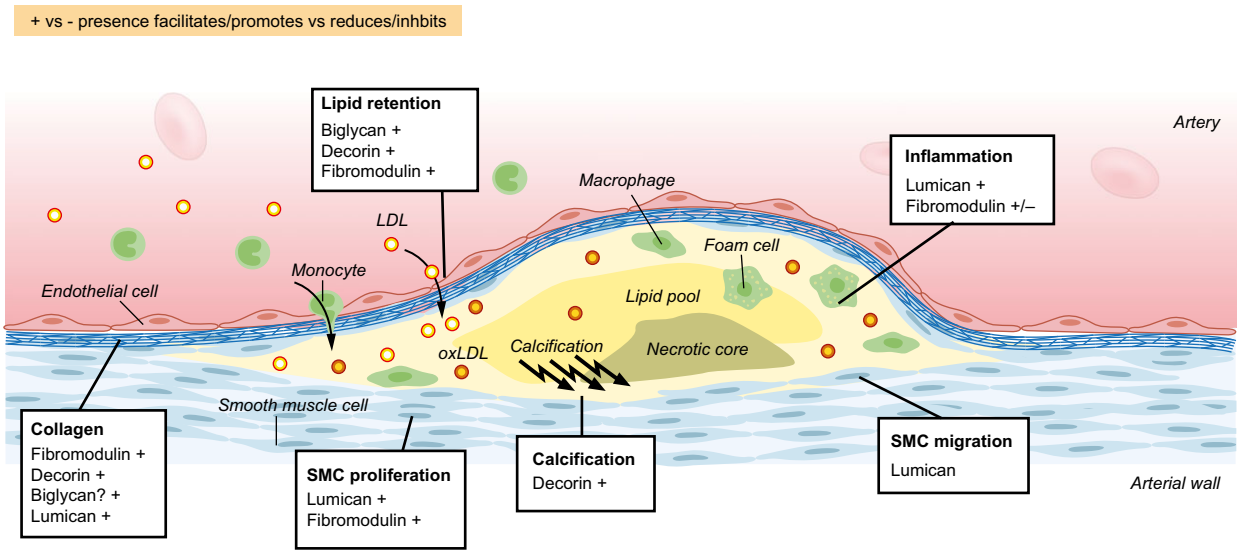

Fig. 1 *Small leucine-rich repeat proteoglycans (SLRPs) in atherogenesis. The atherosclerotic plaque (a) and an overview of the effects of SLRPs on plaque development (b). –, inhibitory or destabilizing effects; +, stimulatory or stabilizing effects; oxLDL, oxidized LDL; SMC, smooth muscle cell.*

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