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# Small leucine-rich proteoglycans and matrix metalloproteinase-14: Key partners?



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## Abstract

Small leucine-rich proteoglycans (SLRPs) are important regulators of extracellular matrix assembly and cell signaling. They are a family of proteoglycans that are present in extracellular matrix and that share in common multiple repeats of a leucine-rich structural motif. SLRPs have been identified as inhibitors of cancer progression by affecting MMPs, especially MMP-14 activity. Lumican, a member of the SLRPs family, and its derived peptides were shown to possess anti-tumor activity. Interestingly, it was demonstrated recently that lumican interacts directly with the catalytic domain of MMP-14 and inhibits its activity. The aim of this review was to summarize the interactions between SLRPs and MMPs with a special interest to lumican.

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## Introduction

Small leucine-rich proteoglycans (SLRPs) are a family of eighteen proteoglycans that are present in the extracellular matrix. SLRPs are important regulators of extracellular matrix assembly and cell signaling. They have been implicated in the regulation of cancer growth and progression. Some of the SLRPs were described to regulate MMP-14 activity by a direct interaction [1,2].

## Small leucine-rich proteoglycans

The SLRPs are a family of proteoglycans that are present in the extracellular matrix and that share in common multiple Leucine-Rich Repeats (LRRs).

The first crystal structure of a leucine-rich protein was obtained for pancreatic ribonuclease inhibitor (RNI) [3]. RNI shows an overall right-handed, solenoid-like morphology, where each turn of the solenoid corresponds to a single LRR. The crystallographic structure of decorin and biglycan [4,5] is also solenoid-like, but with an arch shape.

The SLRPs have similar central LRR domain flanked by conserved cysteine motifs on N- and C-terminal. This central LRR domain consists of tandem repeats rich in leucine and other small hydrophobic residues. These repeats contain a characteristic pattern of 11 amino acids LxxLxLxxNxL (x being any amino acid), where consensus leucine can be substituted by isoleucine, valine or less frequently by other hydrophobic amino acids [6]. Lengths of individual repeats in different SLRPs vary from 20 to 39 amino acids. The LRR

class I, II and V show typical model following a short–long–long regular pattern throughout the entire LRR domain [4]. The LRR are composed of elements of secondary structures such as  $\alpha$ -helix, polyproline type II helix,  $3_{10}$  helix,  $\beta$ -turn and  $\beta$ -sheets.

The SLRP family contains 18 members, which can be classified into five classes based on criteria like conservation and homology at the protein and genomic levels, the presence of characteristic N-terminal cysteine-rich clusters with defined spacing, and chromosomal organization [7].

### Classification of SLRPs

- Class I: Decorin, biglycan, asporin and ECM2 belong to this class. All class I SLRPs have a similar exonic organization (eight exons), with highly conserved intron/exon junctions. This class is characterized by a cluster of N-terminal cysteine signature CX3CXCX6C that forms two disulfide bonds. Although decorin and biglycan can be substituted with either one or two chondroitin/dermatan sulfate chains, asporin lacks the typical Ser-Gly dipeptide and flanking amino acids required for glycanation. ECM2 is characterized by 13 LRRs and 3 N-glycosylation sites.
- Class II: Five members of this class can be divided into three subgroups based on their amino acid sequence homology. Fibromodulin and lumican comprise the first sub-family with 48% identity of the protein sequence, keratocan and PRELP constitute the second subfamily with 55% homology of structure, while osteoadherin forms a separate subfamily with 37–42% homology with other members of the class II. Their respective genes have a similar exonic organization (three exons), with a large central exon encoding most of LRRs. This class has the cysteine-rich consensus sequence CX3CXCX9C and they contain clusters of tyrosine sulfate residues at their N terminal parts that could contribute to the polyanionic nature of SLRPs. Class II members contain primarily keratan sulfate (KS) and poly lactosamine, an unsulfated form of KS.
- Class III: This class contains opticin, epiphycan and osteoglycin characterized by a relatively low number of LRRs (opticin (6 LRRs; 1 N-linked glycosylation site); epiphycan (6 LRRs; 3 O-linked glycosylation and 2 N-linked glycosylation sites); osteoglycin (7 LRRs; 1 O-linked and 2 N-linked glycosylation sites)) and a genomic organization comprising seven exons. The members of this class include the consensus N-terminal cysteine sequence CX2CXCX6C.
- Class IV: It is composed of chondroadherin, nyaltopin and tsukushi. This class of SLRPs,

compared to the other classes of SLRPs (I–III), is not canonical since these SLRPs share structural homology and functional properties with other classes but do not contain glycosaminoglycan (GAG) and thus are not real proteoglycans. Also the cluster of N-terminal cysteine differs between members of this class (CX3CXCX6-17C).

- Class V: This is another non-canonical class of SLRPs. It contains two genes, podocan located on chromosome 1, and a highly homologous podocan-like protein 1 located on chromosome 19. These two proteins have different cysteine-rich cluster (CX3-4CXCX9C). Podocan is a glycoprotein but not a proteoglycan.

### Three dimensional structure of SLRPs

The structural and functional analysis of fibromodulin and chondroadherin has been recently reported by Paracuellos and collaborators [8]. They determined crystal structures at  $\sim 2.2^\circ\text{A}$  resolution of human fibromodulin and chondroadherin, two collagen-binding SLRPs and showed that their overall fold was similar to that of the prototypical SLRP, decorin, whereas neither fibromodulin nor chondroadherin formed a stable dimer. SLRPs morphology (curvature) seems to be determined by the length of the LRR. The curvature of the SLRPs defines a convex side and a concave face to the core protein. The solvent-exposed concave face provides an ideal surface for interaction with macromolecular ligands and has been shown to be involved in protein-protein interaction of the leucine-rich protein. GAGs and other glycosylations are mostly located on the convex side of SLRPs. The structural characteristics of the four main SLRPs are presented in Table 1. The glycosylated fibromodulin presented in Fig. 1 is modeled considering the inner core protein and by grafting on its surface the classical biantennary glycan structure such as the one described by [9]. This distribution and orientations of the glycosylations confer asymmetric conformation to the molecule and may impact its properties and interactions. Lumican interaction with MMP-14 is a good example to illustrate how the post-translational modifications of lumican as keratan sulfate proteoglycan (KSPG), or as glycoprotein, or as core protein alone, will impact its direct effect on MMP-14 activity [2]. Thus, one can infer that the number of glycans and their position on the SLRPs surface might affect the dynamical properties and behaviours of the macromolecules and have a crucial role on the overall structures and function of the SLRPs.

### Biological functions of SLRPs

The biological functions of SLRPs were mostly elucidated by the characterization of mutations occurring in patients suffering from genetic diseases

**Table 1.** Structural characteristics of human SLRPs.

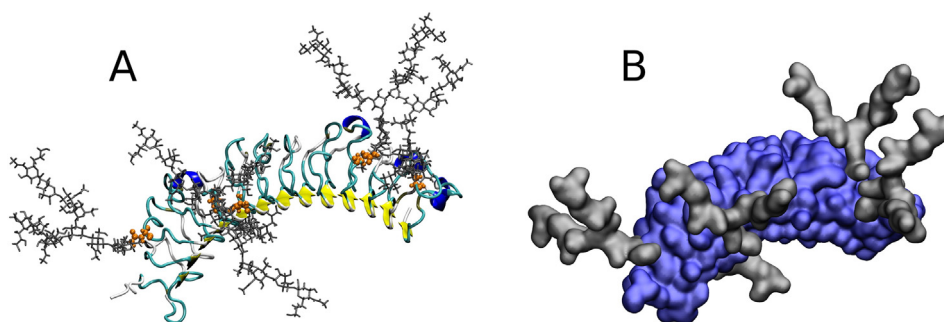
Human SLRPs	UniProtKB AC	Length (chain)	PDB ID	% of identity	N-linked glycosylation	O-linked glycosylation	Disulfide bond
Biglycan	<a href="#">P21810</a>	368 (38–368)	–	94,58 (biglycan bos taurus)	<b>N270</b> <b>N311</b>	– <b>S42</b> <i>S180</i> <b>S47</b> <i>S198</i>	C63–C69 C67–C76 C321–C354
Decorin	<a href="#">P07585</a>	359 (31–359)	–	89,44 (decorin bos taurus)	<b>N211</b> <b>N262</b>	<i>N303</i> <b>S34</b>	C54–C60 C58–C67 C313–C346
Fibromodulin	<a href="#">Q06828</a>	376 (19–376)	5MX0	–	– <i>N127</i> <i>N166</i> <i>N201</i> <i>N291</i> <i>N341</i>	– – – – –	C76–C82 C80–C92 C334–C367
Lumican	<a href="#">P51884</a>	338 (19–338)	–	39,58 (fibromodulin human)	<b>N88</b> <b>N160</b>	<i>N127</i> <i>N252</i> – –	C37–C43 C41–C53 C295–C328

Structural data information concerning SLRPs and their related 3D structures can be found on UniProtKB/Swiss-Prot database (UniProt Consortium, [155]). The second column gives the accession numbers. The third column gives the number of amino acids contained in the full length core protein of the SLRPs and that of the protein without the signal or propeptide portion. Among the four SLRPs our study focuses on, only fibromodulin 3D structure is solved (fourth column). The fifth column gives the percentage of identity between the whole sequences of the core protein in amino acids and a potential resolved template. N-linked and O-linked glycosylation sites are indicated in bold (glycosylation sites experimentally evidenced) or in italic (putative glycosylation sites – sequence analysis) characters.

and of specific SLRP-deficient mice. Mutations of decorin, fibromodulin, PRELP, opticin or keratocan lead to ocular symptoms in humans, characterized by either corneal opacity, high myopia or hyperopia and astigmatism [10,11]. Mutations can be located either in the LRR repeats or in other parts of the SLRP sequences, as demonstrated for keratocan for instance [12]. Keratocan is responsible for autosomal recessive cornea plana. In this disease, the forward convex structure is flattened, leading to a decrease of refraction. Two out of the four reported mutations are nonsense mutations but the remaining two mutations affect LRR8 and LRR9 [12]. Mutations in the decorin gene lead to connective tissue disorders such as the congenital stromal corneal dystrophy [13,14]. The heterozygous mutations affect the ear region of decorin [15] causing bilateral corneal opacities in patients [13].

The deletions of SLRP induced several defects in mice. In biglycan-deficient mice, a reduced bone mass was observed with decreased production of bone marrow stromal cells and larger irregular collagen fibrils indicating an osteoporosis-like phenotype as well as spontaneous aortic dissection and rupture [16,17]. Decorin-deficient mice presented skin fragility phenotype with loosely packed collagen networks resembling Ehlers-Danlos syndrome [18]. In lumican-deficient mice, skin laxity and corneal opacity were observed [19]. SLRPs have been long known to be able to bind to various types of collagens (at least I, II, V and VI) thereby regulating the kinetics, assembly, and spatial organization of fibrils in skin, tendons, and cornea [20–23].

However, the biological functions of SLRPs extend far beyond their interactions with collagens. SLRPs interact with various cytokines, including transforming



**Fig. 1.** Secondary structure, surface representation and N-glycosylation positions on human fibromodulin. (A) Cartoon representation: the fibromodulin backbone is colored according to the secondary structure of the core protein. The residues bearing the N-glycosylations are displayed using orange Van der Waals motifs. Bi-antennary glycosylated chains were modeled with gray licorice. (B) Surface representation: it considers the occupancy of the glycosylated chains which were modeled with gray surfaces.

growth factor-beta (TGF- $\beta$ ) (biglycan, decorin, lumican), bone morphogenic proteins (biglycan, tsukushi), Wnt-I-induced secreted protein-I (biglycan, decorin), von Willebrand factor (vWF) (decorin), platelet-derived growth factor (PDGF) (biglycan, decorin) and tumor necrosis factor-alpha (TNF- $\alpha$ ) (biglycan, decorin) leading to modulation of their diverse biological functions [24].

As extracellular compounds, SLRPs can interact with various signaling receptors, like Toll-like receptors [25], insulin-like growth factor receptor [26], epidermal growth factor receptor [27], low density lipoprotein receptor related protein [28], integrin  $\alpha 2\beta 1$  [29–31], and c-Met [32,33], thereby acting as direct triggers of signal transduction. These interactions result in modulation of cellular growth, proliferation, differentiation, survival, adhesion and migration under developmental, physiological and pathological conditions [7]. In addition, decorin regulates autophagy [33]. Decorin was reported to be not only an inducer, but also a prerequisite for autophagy. It was shown that the stimuli which induced autophagy provoked simultaneously the decorin expression both at the mRNA and protein levels, both *in vitro* and *in vivo*, through a transcriptional mechanism *via* inhibiting the canonical mTOR signaling pathway. As autophagy is a misleading process, promoting cell survival under stressful conditions, but also leading to apoptosis when apoptotic pathways are not efficient, it may have an association with cancer [34]. Taking into account the ability of decorin to induce endothelial cell autophagy, the anti-cancer features of decorin may contribute to the regulation of autophagy during pathological conditions.

### SLRPs in cancer

Some SLRPs have been implicated in regulation of cancer growth and progression (Table 2). The highest number of reports concerned decorin, lumican, fibromodulin and biglycan. Decorin is the most studied protein. Its expression is almost always suppressed in cancer cells whereas it is abundant in activated stromal fibroblasts [35]. Its gene expression was found highly upregulated in pancreatic cancer tissue in comparison to normal pancreas [36]. In order to understand the important association of decorin with solid malignancies, it is worth noticing that the absence of decorin in breast tumor is an established clinical indicator of invasive breast cancer [37–39]. On the contrary, decorin expression is found downregulated by at least 50% in 2 types of non-small cell lung cancer, adenocarcinoma and squamous cell carcinoma [40]. Decorin inhibits melanoma cell migration and invasion [41]. In a mammalian model, treatment with decorin core protein reduced primary tumor growth and eliminated metastases [39]. Mechanisms of decorin-dependent tumor repression are linked to its

ability to potently induce the endogenous synthesis of p21, a key inhibitor of cyclin-dependent kinases. This is carried out by soluble decorin binding in a paracrine fashion to several receptor tyrosine kinases (RTK) including the epidermal growth factor receptor (EGFR), insulin growth factor-insulin receptor (IGF-IR) and Met [37]. Met is an RTK of high affinity, and when decorin binds it, a cascade of multiple oncosuppressive properties, *i.e.*: apoptosis, mediation of cell cycle, growth inhibition, tumor cell mitophagy, and angiostasis are activated [42,43]. Thus, decorin is a natural RTK inhibitor and systemic delivery of recombinant decorin inhibits the growth of various tumors [44,45]. The receptor binding activates the initiation of signaling pathways downstream of vascular endothelial growth factor receptor 2 (VEGFR2). This process is successively coupled with Peg3/Beclin 1/LC3-dependent cell endothelial autophagic program. Another accompanying phenomenon with the newly-introduced autophagic program is the significant effect of decorin on capillary morphogenesis, tumorigenic growth and cell migration. Decorin is not only reported as regulator of the innate immune response, but also to alter the macrophage function and cytokine secretion, rendering this SLRP a pro-inflammatory one [28,32]. Moreover, decorin was shown to be cleaved by MMP-14 during corneal angiogenesis [46]. Altogether, there is plethora of publications proving the anti-tumorigenic, anti-angiogenic, and anti-metastatic roles of decorin both *in vivo* and *in vitro*, rendering this SLRP a potent therapeutic candidate in cancer [32].

Aberrant expression of another member of SLRP, biglycan, has been linked to a number of pathological conditions such as osteoporosis, glomerulonephritis, pancreatic cancer and mesothelioma [47–49]. Significantly higher expression levels of biglycan were shown in tumor tissues from the ovary, colon and liver compared with adjacent normal tissues [50,51]. In addition, it was found that biglycan is over-expressed in pancreatic cancer tissues *via* the induction of cyclin-dependent kinase inhibitor p27KIP1 [52]. Biglycan expression was also found to be higher in tumor endothelial cells in comparison to normal endothelial cells [53]. Biglycan is the closest SLRP to decorin, sharing 63% of identity in amino acid sequence, and many similar characteristics, like binding to TGF $\beta$  [54,55], inducing the Wnt pathway [56]. With regards to TGF $\beta$ , biglycan was also reported to regulate differentiation and cell growth *via* altering TGF $\beta$ /Smad2 signaling pathway [57]. In contrast to decorin, biglycan is considered as a pro-angiogenic SLRP, as it binds to VEGFA and subsequently activates the VEGFR2 signaling pathway [58]. This pro-angiogenic SLRP is reported to be a pro-inflammatory one, binding to Toll-like receptors (TLR)-2 and -4. The high production and secretion of biglycan from the macrophages render this pro-inflammatory SLRP a ligand for the (TLR)-2 and -4 of the innate immune system [25,59,60], resulting in inflammatory damage.



**Table 2.** Roles of decorin, biglycan, fibromodulin and lumican in cancer.

SLRPs	Roles in cancer	References
Decorin	Regulator of the innate immune response	[28]
	Pro-inflammatory role, by altering the macrophage function and cytokine secretion	[28,32,43]
	Natural RTK inhibitor	[44,45]
	Potential induction of the endogenous synthesis of p21	[37]
	Inhibition of melanoma cell migration and invasion	[41]
	Reduction of primary tumor growth and elimination of metastasis	[39]
	Binding to Met and activation of multiple oncosuppressive properties, <i>i.e.</i> : apoptosis, mediation of cell cycle, growth inhibition, tumor cell mitophagy and angiostasis	[32,42,43]
Biglycan	High expression in tumor tissues of ovarian, colon and liver cancer	[50,51]
	Overexpression in pancreatic cancer tissues <i>via</i> the induction of p27KIP1 inhibitor	[52]
	High expression in tumor endothelial cells, compared to normal endothelial cells	[53]
	Regulation of differentiation and cell growth <i>via</i> altering TGF $\beta$ /Smad2	[57]
	Pro-angiogenic property, by binding to VEGFA, and subsequent activation of VEGFR2 signaling pathway	[58]
	Pro-inflammatory property, by binding to (TLR)-2 and -4	[59,60]
Fibromodulin	Substrate of MMP-13	[63]
	Promotion of angiogenesis both <i>in vitro</i> and <i>in vivo</i>	[60,76]
	Binding to EGFR and VEGFR, due to its N-terminal domain	[73]
	Binding to TGF $\beta$ , and specifically to the collagenous part of C1q complement, activating the classical pathway of the complement	[74,75]
Lumican	Alteration of the balance of fluids in the tumor stroma, influencing the chemotherapy response	[67]
	Downregulation of apoptosis of stromal cells in Lum <sup>-/-</sup> mice	[99]
	Decrease in p21WAF/CIP1 expression, and a consequent increase in cyclins A, D1, and E in Lum <sup>-/-</sup> fibroblasts	[100]
	Downregulation of p53 in Lum <sup>-/-</sup> fibroblasts	[100]
	Suppression of tumorigenic transformation of rat fibroblasts, induced by v-src and v-K-ras and decrease of subcutaneous tumor formation <i>in vivo</i> , with a simultaneous decrease of cyclin D1 expression	[101]
	Inhibition of tumor growth in an <i>in vivo</i> mouse model	[30]
	Increase in cell adhesion	
	Binding to $\beta$ 2 integrin and promotion of neutrophils migration	[105]
	Inhibitory role in prostate cancer is mediated <i>via</i> $\alpha$ 5 $\beta$ 1 integrin	[103,104]
	Direct binding to the catalytic domain of MMP-14 and inhibition of its activity	[2,111]
	Direct binding to $\alpha$ 2 $\beta$ 1 integrin, leading to inhibition of melanoma cell migration, and activating FAK	[31,106]
	Alteration of cell migration of the EMT-like B16F1 cells, induced by elevated level of Snail expression	[111]
	Regulation of migration of human colorectal cells	[110]
	Correlation of pancreatic cancer expression with advanced stage of retroperitoneal and duodenal invasion	[119]
	Exhibition of angiostatic properties and inhibition of endothelial cell invasion, angiogenic sprouting and vessel formation in mice	[109,117]
Inhibition of invasion of human pancreatic cells	[119]	
Regulation of osteosarcoma cell	[121,122]	
Attenuation of cell functional properties, <i>i.e.</i> : proliferation, migration and invasion in breast cancer cell models of different estrogen receptor status, provoking EMT reprogramming and affection of major MMPs expression	[123]	

Gathering all these data, biglycan could be used as a biomarker of inflammation [61] and as a tool with many possible applications in innate and adaptive immunity, in tumorigenesis and in inflammation [62]. Biglycan is also described to be a substrate of MMP-13 [63].

Fibromodulin is also homologous to decorin (48% of identity in amino acid sequence) and its mRNA has been detected in a variety of clinical malignancies, such as lung, breast, and prostate carcinomas [64–66]. Fibromodulin is associated with dense collagen matrix in tendons and ligaments, as well as in fibrotic tissues, tumors and atherosclerotic plaques [67–69]. In fibromodulin-deficient mouse tendons, collagen fibrillogenesis is dysregulated: collagen fibrils are misassembled in parallel to altered lumican

deposition [70]. These mice also exhibit an increase in lysyl oxidase-mediated cross-linking of tendon collagen I [71]. More recently, fibromodulin was shown to interact with collagen cross-linking sites and to activate lysyl oxidase [72]. Furthermore, it might be added that a role in collagen II cross-linking has been suggested for chondroadherin [8].

The role of the N-terminal tyrosine sulfated domain of fibromodulin in collagen fibril formation and the inhibitory effect of its isolated leucine-rich repeat domain on fibril formation was described [73]. The tyrosine-sulfated domain and the leucine-rich repeat domain both bound to three specific sites along the collagen type I molecule, at the N terminus and at 100 and 220 nm from the N terminus. The N-terminal

domain shortened the collagen fibril formation lag phase and tyrosine sulfation was required for this effect. The isolated leucine-rich repeat domain inhibited the fibril formation rate, and full-length fibromodulin showed a combination of these effects. The fibrils formed in the presence of fibromodulin or its fragments showed more organized structure. Fibromodulin and its tyrosine sulfate domain remained bound on the formed fibre. Taken together, this suggests a novel, regulatory function for tyrosine sulfation in collagen interaction and control of fibril formation [73]. As known, functionality depends on structure and in the case of fibromodulin, its charged N-terminal domain may contribute to collagen cross-linking, and eventually binds to growth factors, such as EGF and VEGF or inflammatory cytokines during pathophysiological conditions [73].

Fibromodulin as homologous to decorin, binds to TGF $\beta$  [54] and specifically to the collagenous part of the complement C1q, but exceptionally activates the classical pathway of the complement [74,75]. In the case of solid malignancies, fibromodulin alters the balance of fluids in the tumor stroma, by enhancing the extracellular fluid and decreasing the interstitial one and consequently influencing the chemotherapy response [67]. Recent findings reveal that fibromodulin promotes angiogenesis both *in vitro* and *in vivo*, a property associated with wound repair, cancer cell growth and embryonic development. Under specific circumstances of enhanced levels of collagen I and III, angiopoietin (Ang)-2 and VEGFR, accompanied by decreased ratio of Ang-1/Ang-2, fibromodulin favored an angiogenic behavior. All these data introduce fibromodulin as potent therapeutic effector in cases of impaired angiogenesis [60,76]. Fibromodulin is also described to be degraded by MMP-13 [77].

## Lumican

Lumican was identified for the first time in the cornea as a keratan sulfate proteoglycan [78]. However, lumican is expressed in the extracellular matrix of different tissues. It was detected in arteries [79], skin [19], kidney [80], lung [81], breast [82], colon [83], pancreas [84], bone [85], intervertebral disc [86], and cartilage [87]. Its structure varies not only according to the tissues but also during ageing [88,97]. In addition, growth factors and cytokines can modulate the molecular form of lumican [89].

### Lumican structure

The transcript of human lumican (1014 bp) encodes a protein of 338 amino acids including a 18 amino acids signal peptide (permitting secretion of this SLRP into the ECM) and three major domains [87,90]: a negatively charged N-terminal domain

containing sulfated tyrosine and cysteine residues; a central part which contains 9 leucine-rich repeats; a C-terminal domain of 66 amino acids which contains two conserved cysteines and two LRRs.

Chakravarti and collaborators [90] described 11 LRRs motifs (LxxLxLxxNxL) in human Lumican. The alignment of lumican sequences from different species shows that the central part is highly conserved. Four potential sites for the substitution by N-linked KS or oligosaccharides are situated at position 87, 126, 159, 251 of the core protein of human lumican [90–93]. The secondary and tertiary structures of lumican are characteristics of the SLRPs [4,5]. LRRs are characterized by a common molecular architecture adapted to protein-protein interactions. More recently, Yamana et al. [94] showed that lumican could interact with the TGF- $\beta$  receptor-1 (ALK5). Moreover, they confirmed that these effects of lumican did not depend on its glycan moiety. They also showed that a fragment of the lumican core protein, composed of its last 13 amino-acids (named LumC13 or lumikine), reproduced the stimulating effects of the complete lumican molecule on corneal epithelial wound healing *in vitro* and *in vivo*. Recently, this same group demonstrated that LumC13 forms a stable complex with ALK5 and confirmed that instillation of this peptide on mice eyes significantly promoted corneal epithelium wound healing [95].

Lumican presents a high molecular heterogeneity according to the tissue and to glycosylation. There are four structural forms of lumican: the core protein of 38 kDa which is a non-glycosylated form, a form (57 kDa) possessing short N-linked oligosaccharides, a form in which the oligosaccharides are substituted with poly-lactosamine chains, and a form in which sulfation of the poly-lactosamine chains occurs to give KS [96].

### Biological roles and mechanisms of action

Many SLRPs, including members of class I (*i.e.* decorin, biglycan) and II (*i.e.* lumican, fibromodulin) are known to interact with ECM collagen [20]. It was shown that the LRR7 of lumican is involved in its binding to collagen type I. The well described function of lumican is to regulate collagenous matrix assembly. Lumican also protects against collagen matrix degradation, masking the cleavage sites for collagenases MMP-1 and MMP-13 [23]. Lumican-null mice showed abnormal collagen fibril assembly, with large and abnormally shaped collagen fibrils and an extremely loose and fragile skin [19]. Moreover, a strong decrease of lumican expression in aged skin was observed [97]. Lumican was detected in numerous normal tissues. Lumican is involved in the regulation of cell functions such as cell proliferation, adhesion, migration, invasion and differentiation in addition to its role in ECM structural organization [98].

### Proliferation/apoptosis

Some of the biological functions of lumican were elucidated by the characterization of lumican-deficient mice. In lumican knockout (Lum<sup>-/-</sup>) mice, apoptosis of stromal cells was down-regulated. The function of FasL on intra-ocular tumors was determined by the microenvironment in conjunction with the form and level of FasL expressed [99]. The Lum<sup>-/-</sup> fibroblasts have decreased p21WAF/CIP1 expression, a universal inhibitor of cyclin-dependent kinases, and a consequent increase in cyclins A, D1, and E. The tumor suppressor p53, an upstream regulator of p21, is down-regulated in Lum<sup>-/-</sup> fibroblasts. The regulation of p21 by lumican is a p53-dependent pathway [100]. Lumican overexpression suppresses tumorigenic transformation of rat fibroblasts induced by v-src and v-K-ras and decreases subcutaneous tumor formation *in vivo*, with a concomitant decrease of cyclin D1 expression [101]. Lumican was described to associate with CD14 on the surface of macrophages and neutrophils, and to promote CD14-TLR4 mediated response to bacterial lipopolysaccharides (LPS) [102].

### Membrane proteins mediating lumican activities

Lumican does not bind just one integrin. It was reported that the lumican inhibitory role in prostate cancer was mediated *via*  $\alpha 5\beta 1$  integrin [103,104]. On the surface of neutrophils, lumican was shown to bind to  $\beta 2$  integrin and promote the migration of these cells [105]. The inhibition of the melanoma cell migration was mediated by  $\alpha 2\beta 1$  integrin, to which lumican binds directly [31]. The interaction of lumican with integrins in melanoma cell was proposed to activate signaling pathways *via* focal adhesion kinase (FAK) which participates in focal adhesion turnover and actin cytoskeleton reorganization [106,107]. Overexpression of lumican has been shown to affect the migration of human colon cancer cells through up-regulation of gelsolin and filamentous actin reorganization [108]. Lumican inhibitory effect on the migration of endothelial cells was associated with a regulation of the expression and activity of MMP-9 and MMP-14 by integrins [109]. Similarly to decorin, lumican can be cleaved by MMP-14 [110]. In contrast to decorin, it was demonstrated that lumican can bind directly to the catalytic domain of MMP-14 to inhibit its activity [2,111]. The postulated key partners of the action of lumican to modulate key cellular functions are displayed in Fig. 2.

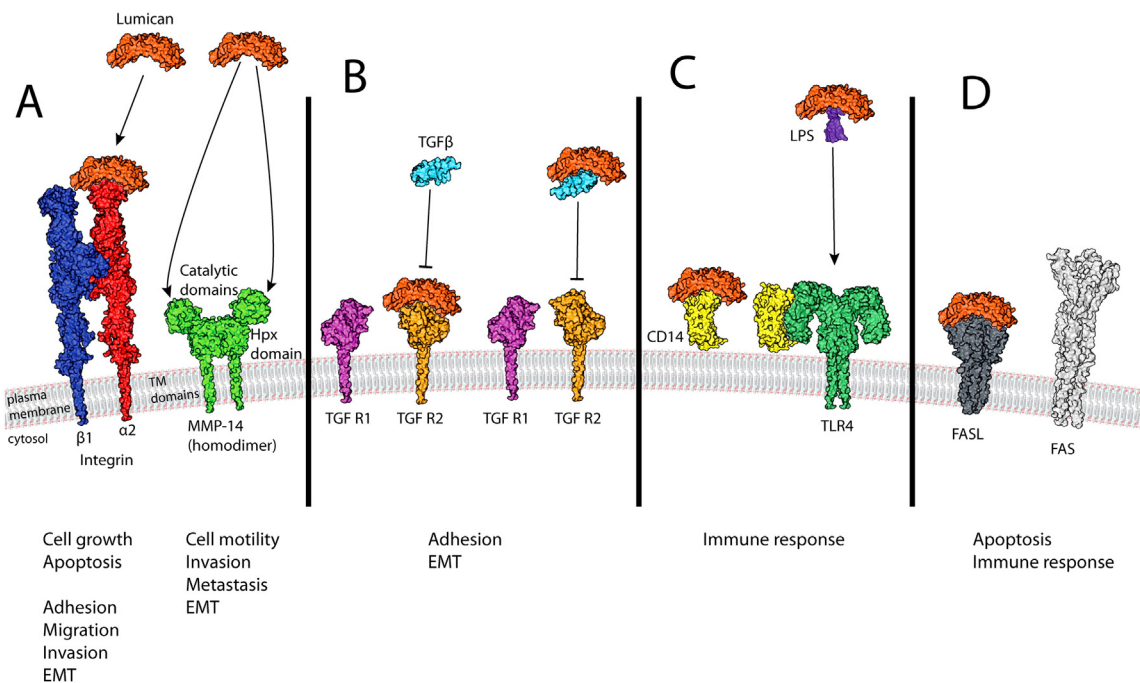
### Lumican in cancer

ECM degradation and proteoglycans play a major role in the control of tumor progression. Lumican is expressed in various tumor tissues but both positive and negative correlations with tumor aggressiveness

have been reported [96]. Lumican expression in pancreatic cancer correlates with an advanced stage and retroperitoneal and duodenal invasion [84]. The expression of lumican protein did not correlate with prognostic factors in breast carcinoma [82,112] but reduced lumican protein expression (40–175 kDa) is associated with poor outcome in breast cancer [38]. Data from our laboratory demonstrated antitumor properties of lumican *in vivo* in a mouse model of melanoma [113] and lung metastasis [114]. Melanoma cells were capable to adhere to lumican [30], resulting in the remodeling of their cytoskeleton and preventing their migration [106,107]. The above data were confirmed recently in an *in vivo* mice model, where lumican inhibited the tumor growth and was presented as a protagonist of ECM coherent assembly [115].

The antitumor mechanism of action of lumican was partially identified in our laboratory. Integrin  $\alpha 2\beta 1$  was characterized as a direct lumican receptor on melanoma cells [31]. The expression of lumican was preferentially located at the periphery of the tumor in stromal dermal fibroblasts [116]. In addition, it was shown that lumican exhibited angiostatic properties and inhibited endothelial cell invasion, angiogenic sprouting, and vessel formation in mice [109,114,117]. In lung cancer tissues, lumican was localized in the cytoplasm of cancer cells and/or stromal tissues adjacent to cancer cells [118]. In lung adenocarcinoma the expression level of lumican in cancer cells correlated with pleural invasion and larger tumor size. In squamous cell carcinoma, the expression level of lumican in cancer cells correlated with formation of a keratinized pattern, and stromal lumican expression correlated with vascular invasion. In squamous cell carcinoma and lung adenocarcinoma, the expression level of lumican in cancer cells did not correlate with patient prognosis. In pancreatic cancer, lumican protein was strongly localized in cancer cells, and in acinar and islet cells in chronic pancreatitis-like lesions adjacent to the tumor tissue [84]. It was also localized in fibroblasts and on collagen fibres close to cancer cells. Yamamoto and collaborators have shown that secreted 70 kDa lumican stimulated growth but inhibited invasion of human pancreatic cancer cells [119]. In colorectal cancer tissues, lumican was strongly localized in cancer cells in 75% of the cancer cases. The lumican protein was also localized in epithelial cells with mild reactive dysplasia and fibroblasts adjacent to cancer cells. These findings may indicate that the lumican protein synthesized by cancer cells, fibroblasts and epithelial cells with mild reactive dysplasia found adjacent to cancer cells may affect the growth of human colorectal cancer [83]. Overexpression of lumican has been shown to affect the migration of human colon cancer cells [108]. Human osteosarcoma cell lines were shown to express and secrete lumican, with the ability to regulate the growth and motility of these cells [120]. Lumican is able to regulate osteosarcoma cell adhesion [121,122].





**Fig. 2.** Schematic diagram demonstrating postulated mediators of the action of lumican in modulating key cellular functions. (A) The interaction of lumican with integrins was proposed to activate signaling pathways regulating cell growth, apoptosis, adhesion, cell motility, invasion, EMT and metastasis. Lumican is also involved in the inhibition of MMP-14 activity *via* a direct binding to the catalytic domain regulating cell motility, invasion, EMT and metastasis. (B) Lumican was shown to interact with TGF- $\beta$ , sequestering the growth factor in the ECM, in a way that modulates TGF- $\beta$  binding to the respective receptors and resulting in regulation of cell adhesion and EMT. (C) Lumican was also reported to interact with CD14 on macrophages, enhancing phagocytosis, or to interact with lipopolysaccharide and Toll-Like Receptor-4, regulating the immune response. (D) Lumican was also shown to interact with Fas-Fas ligand, mediating apoptosis and the immune response. Hpx, hemopexin domain. Adapted from a figure originally published in FEBS J, Brézillon et al. [96].

It was previously established that the altered activity of MMP-14 can serve as a potential mechanism of action of the anti-tumorigenic lumican [1,2,96]. Specifically, it was proven that the cell migration of the epithelial-mesenchymal transition (EMT)-like B16F1 cells, induced by elevated level of Snail expression, was altered by lumican [111]. This proposed mechanism can be even more complex, as MMP-14 affects cell migration, not only by adjusting the activity of downstream MMPs, but also by activating migration-implicated molecules, such as integrins and many related pathways. The direct synergism of membrane MMPs, especially of MMP-14 and integrins, was previously reported as a hallmark in tumor invasion and angiogenesis [109,111].

Besides the existing reports concerning the anti-cancer effect of lumican, its mechanism of action has not been fully elucidated. Lumican was shown to significantly attenuate cell functional properties including cell proliferation, migration and invasion in breast cancer models of different estrogen receptor status. It provokes an EMT reprogramming and affects the expression of major MMPs, implicated in breast cancer [123]. The effects of lumican may be due to different levels of action,

concerning either its interactions with ECM molecules or intermediation in the activity of membrane receptors [96,120] and MMP-14 [2,111] (see Fig. 2).

## Matrix metalloproteinases

Matrix metalloproteinases are a large family of proteases secreted by tumor and microenvironmental cells that are directly linked with invasion and metastasis through ECM. Moreover, they are associated with other carcinogenesis steps like tumor growth and promotion of angiogenesis [124–126].

## MMPs function in cancer progression

MMPs play key roles in physiological ECM remodeling, but also in cancer progression as they are implicated in invasion and metastasis [127]. There are several reports about cancer-promoting as well as cancer-inhibiting functions of MMPs, which are involved not only in invasion and metastasis, but also in other steps of carcinogenesis. MMPs can promote growth and survival of cancer cells by cleaving FAS ligand or growth factor binding proteins. They may also

participate to the shedding of transmembrane precursors, *i.e.* TGF- $\alpha$ .

MMPs also control proliferation signals by regulating the ECM composition, which promotes growth indirectly through interactions between ECM molecules and integrins. MMPs can inhibit cancer-cell growth by activation of TGF- $\beta$  and promote apoptosis, probably indirectly by changing the ECM composition, which influences integrin signaling [127]. MMPs display dual role in tumor angiogenesis, acting as positive and negative regulators. MMPs can promote angiogenesis by increasing the bioavailability of the pro-angiogenic growth factors, like VEGF, FGF-2, and TGF- $\beta$ , which stimulate proliferation and migration of endothelial cells. They were shown to liberate FGF-2 by cleavage of perlecan [128]. In addition, MMPs promote invasion of endothelial cells by cleaving structural components of the ECM, such as collagen types I, IV and XVIII, and fibrin. However, fragments of ECM produced by MMPs can in the same time serve as angiogenic inhibitors [129,130]. Cleavage of plasminogen by MMPs produces angiostatin and cleavage of collagen XVIII generate endostatin and both of them can reduce angiogenesis [131]. Specifically, the MMPs promote invasion and migration by cleaving laminin 332 and the adhesion molecules CD44 and E-cadherin. The released part of E-cadherin might then bind and inhibit the function of other uncleaved E-cadherin molecules. In addition, docking of MMP-9 to CD44 is required for cancer-cell invasion. MMPs might inhibit metastasis by cleavage of CXCL12, a chemokine of the CXC family that promotes breast cancer metastasis. MMPs promote the EMT that is associated with malignant behavior - by cleaving the cell-adhesion molecule E-cadherin and by liberating TGF- $\beta$  [132].

Reactive inflammatory cells provide some of the key MMPs involved in cancer progression, but MMPs also inhibit immune reactions against the cancer cells. MMPs cleave the interleukin-2 receptor- $\alpha$  on T lymphocytes, thereby inhibiting their proliferation. MMPs cleave various members of the CC and CXC chemokine families which are then unable to attract leukocytes [131].

### Matrix metalloproteinase 14

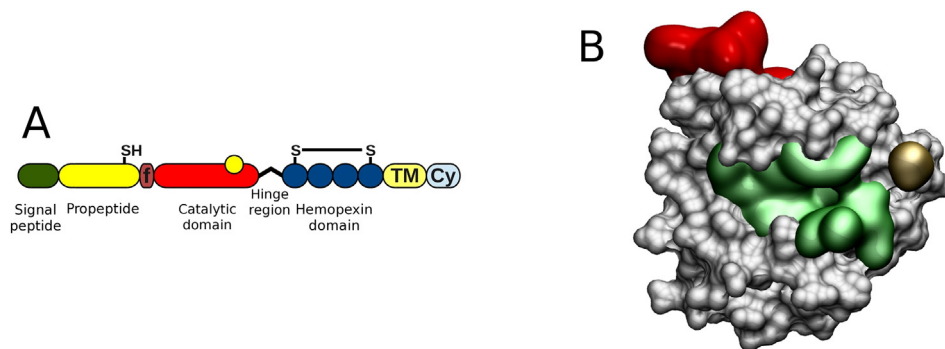
MMP-14 (MT1-MMP) was the first characterized membrane-type matrix metalloproteinase. It plays an important role in cell migration, not only by regulating the activity or expression of downstream MMPs but also by processing and activating migration-associated molecules such as integrins, ECM and a variety of intracellular signaling pathways [133]. During migration and invasion, MMP-14 localizes at lamellipodia, the migration front of the cells [134]. This localization is achieved by interaction of MMP-14 with CD44. It was also shown that MMP-14 can be co-localized with  $\beta$ 1 integrin [135]. MMP-14 was found to be highly

expressed in different cancers and its expression was shown to promote migration, invasion and metastasis of cancer cells *in vitro* as well as *in vivo* [136]. MMP-14 has a broad range of substrates, including the ECM components, like type-I, II, III collagen, fibronectin, laminins, vitronectin, decorin, lumican, elastin and aggrecan as well as non-ECM proteins including CD44, integrins, syndecan-1 and ICAM-1 [137,138].

### Structure of MMP-14

In addition to a specific transmembrane domain, MMP-14 (Fig. 3A) is characterized by multidomain structure consisting of a signal peptide, a pro-peptide, a catalytic domain, a hinge region, and a hemopexin domain. These domains are common with other MMP family members and like other MMPs, the enzyme is produced as a zymogen and requires an activation step to remove the pro-peptide proteolytically. Thus, activation of MMP-14 takes place during secretion in the Golgi and the enzyme is expressed on the cell surface as the active form [139]. Tochowicz and collaborators [140] described the complex dimerization of MMP-14; a critical step for the promotion of cellular invasion. As referred above, homodimerization is a prerequisite for the activation of proMMP-2 and the cell-surface degradation of collagen. The key domain leading this function is hemopexin (Hpx) domain, and the underlying mechanism was investigated through the analysis of its crystal structure. Mutagenesis in the monomers resulted in weakening of the dimer interactions of Hpx domain and in the long-term, inhibition of the exclusively dimer-dependent functions, like proMMP-2 activation and 3D invasion [140]. It was also demonstrated that the dimerization of MMP-14 Hpx domains was occurring during 3D-collagen invasion. Fluorescence resonance energy experiments showed that the dimerization is a constant and dynamic process occurring at the main edge of migrating cells, and is regulated by the actin cytoskeleton reorganization, mediated by the small GTPases, Cdc42 and Rac1 [141]. MMP-14 is located at the leading edge of migrating cells. In addition, a specific sequence, called MT-LOOP, was identified in the catalytic domain of MMP-14 [142]. It is critical for the enhancement of cellular invasion by regulating the cleavage of proMMP-2 to MMP-2 [141,143]. The deficiency of the MT-LOOP leads to the delocalization of the enzyme from  $\beta$ 1-integrin-rich cell adhesion complexes at the plasma membrane, and consequently inhibits both the dimer-dependent and independent functions of MMP-14 [142]. It is worth noticing that the localization of MMP-14 from the apical to the basal surface of epithelial cells enhances the collagen degradation [144,145].

The hemopexin-like domain of MMP-14 comprises the conserved domains responsible for dimerization of MMP-14 and its association with CD44. CD44 in turn is associated with F-actin through its cytoplasmic domain



**Fig. 3.** Structure of MMP-14. (A) Domain structure of MMP-14 (MT1-MMP). (B) Surface representation of MMP-14 catalytic domain. The coordinates extracted from the pdb structure 1BQQ present a catalytic site (green) and the MT-LOOP (red). The Asn229 is highlighted in yellow as a possible N-glycosylation site.

by interacting with Ezrin/Radixin/Moesin proteins. Thus, MMP-14 interacts indirectly with F-actin. Changes in the level of MMP-14 lead to intracellular cytoskeleton rearrangements and the processing of migration and invasion machinery, including proteolysis of proMMP-2 by MMP-14 [143]. Glycosylation of matrix metalloproteinases and tissue inhibitors was also reported. MMP-14 is O-glycosylated at Thr291, Thr299, Thr300, and/or Ser301 in the hinge region, and this modification was shown to be essential for the proMMP-2 activation on the cell surface. Incomplete glycosylation of MMP-14 stimulates extensive autocatalytic degradation and self-inactivation of enzyme [146]. The catalytic sites of MMPs do not contain O-linked glycans, but instead possess a conserved N-linked glycosylation site [147]. Thus, the interactions between SLRPs and MMPs might be regulated by the post-translational modifications of these two molecules. One can assume that the molecular interactions are mainly modulated through protein-protein interfaces but also through glycan-protein and glycan-glycan interfaces. Molecular modeling could be used to propose and test some interaction models and thus decipher the atomistic processes driving to the formation of these macromolecular complexes.

#### *Proteolytic processing of MMP-14*

It has been shown that the 60 kDa active MMP-14 undergoes further processing to 44–45 kDa forms by MMP-2 or MMP-14 itself. This removes the catalytic domain of MMP-14 making it inactive. Paradoxically, accumulation of this 44 kDa form has been associated with increased enzymatic activity *via* an inhibition of endocytosis of active MMP-14 [148]. A high level of 45 kDa form coincides with high proMMP-2 activation whereas no proMMP-2 activation occurs when 45 kDa form is not detected besides full length, mature MMP-14 on the cell surface. In some cells, the whole ectodomain of MMP-14 was shown to be shed. When

the cleavage occurs at Val524-Ile bond, functional MMP-14 is released from the cell surface [149].

The 3D structure of the catalytic domain of MMP-14 is illustrated in Fig. 3B. On this surface representation, three zones may be depicted: (i) the catalytic zone (in green) containing residues known to interact with TIMP2, (ii) the MT-LOOP area (in red) mentioned previously and (iii) a potential N-glycosylation site (in yellow).

#### *Trafficking and intracellular regulation of MMP-14*

MMP-14 was shown to be internalized by clathrin dependent and caveolae-dependent pathways [139]. This internalization from the cell surface suggests a mechanism of downregulation. Paradoxically, the internalization process appears to be essential for the enzyme to promote cell migration [150]. This may be connected with recycling of MMP-14 and insertion of recycled protease at the leading edge of migrating cells. Clathrin dependent internalization of MMP-14 is mediated by the C terminal cytoplasmic tail. MMP-14 is also internalized through caveolae. It was demonstrated that caveolae-mediated internalization of MMP-14 also plays an important role in MMP-14 mediated endothelial cell migration on a collagen substratum [151].

#### *Inhibition of MMP-14 activity*

The inhibition of the active form presented at the cell surface is one of the critical steps to regulate its activity. MMP-14 is inhibited by endogenous inhibitors TIMP-2, -3, and 4, but not by TIMP-1 [152]. RECK (reversion-inducing-cysteine rich protein with Kazal motifs), a GPI-anchored glycoprotein, is another inhibitor for MMP-14 [153]. Chondroitin/heparan sulfate proteoglycans, testican 1 and 3 and a splicing variant of testican 3, N-Tes, have also been shown to inhibit MMP-14 [154].

Lumican and its derived peptides were previously shown to regulate MMP-14 expression and activity [1,2]. The effect of lumican and decorin on MMP-14 activity was compared. In contrast to decorin, glycosylated form of lumican was able to significantly decrease the MMP-14 activity in B16F1 melanoma cells. Our results suggest that a direct interaction occurs between lumican and MMP-14. Lumican behaves as a competitive inhibitor which leads to a complete blocking of the activity of MMP-14. It binds to the catalytic domain of MMP-14 with moderate affinity (KD ~275 nM) [2]. Lumican may protect collagen against MMP-14 proteolysis, thus influencing cell-matrix interaction in tumor progression.

## Conclusion

*In silico* data, more particularly docking simulations, will be required to better understand the direct interactions between SLRPs and the catalytic domain of MMP-14. It seems crucial nowadays to use research protocols combining both *in silico* and *in vitro* methodologies. The procedure that aims at combining these complementary approaches will allow foreseeing great potentialities to decipher the interactions between various SLRPs and MMP-14. The challenges to be addressed in future studies will include taking into account the effect of glycosylations and other post-translational modifications in the fine description and characterization of the interactions. Taken together, the literature suggests that lumican *via* its direct interaction with the catalytic domain of MMP-14 could be considered as a good candidate in the future for potential therapeutic application.

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## Declarations of interest

The authors declare no competing interests.

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## Authors' contribution

S.Ba. and A.N. made the figures of the structure and molecular modeling of the fibromodulin and the catalytic domain of MMP-14. S.Ba. edited the modelization part of the text of the review. F.-X.M., M.D. supervised the review writing and editing.

K.P.-G., K.K., N.B., S.Ba. and S.Br. contributed to the review writing and editing. S.Br. prepared the draft and a figure, managed the overall supervision and submitted the review. All authors reviewed the manuscript.

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MMP-14;

Structure and molecular modeling;

Glycosylation

### Abbreviations used:

Ang, angiopoetin; ALK5, activin receptor-like kinase 5; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FGF, fibroblast growth factor; GAG, glycosaminoglycan; GPI, glycosylphosphatidylinositol; Hpx, hemopexin; IGF, insulin growth factor; IR, insulin receptor; KS, keratan sulfate; KSPG, keratan sulfate proteoglycan; LPS, lipopolysaccharide; LRR, leucine-rich repeats; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; PRELP, proline and arginine rich and leucine rich repeat protein; RNI, ribonuclease inhibitor; RTK, receptor tyrosine kinase; SLRPs, small leucine-rich proteoglycans; TGF- $\beta$ , transforming growth factor beta; TIMPs, tissue



inhibitors of metalloproteinases; TLR, toll-like receptor; TNF- $\alpha$ , tumor necrosis factor-alpha; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2; vWF, von Willebrand factor; Wnt, wingless-type.

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