Small Leucine-Rich Proteoglycans in Kidney Disease

Liliana Schaefer

Pharmazentrum Frankfurt, Institut für Allgemeine Pharmakologie und Toxikologie/ZAFES, Klinikum der JW Goethe-Universität Frankfurt am Main, Frankfurt am Main, Germany

ABSTRACT

Research over the past 2 decades provides ample evidence that small leucine-rich proteoglycans (SLRPs; such as decorin, biglycan, fibromodulin, and lumican) of the extracellular matrix are deeply involved in the regulation of inflammatory and fibrotic renal disorders. Initial efforts in SLRP research focused on the interaction between decorin and TGF-β because it had been unequivocally demonstrated that decorin treatment exerts beneficial effects in fibrotic disorders involving TGF-β overproduction in the kidney. This was followed by a paradigm shift in our understanding of SLRP biology, with new evidence showing that in addition to their role as structural matrix components, soluble SLRPs also act as signaling molecules regulating various complex biologic processes in a molecule- and cell-specific manner. With the identification of SLRP-derived endogenous ligands of Toll-like receptors, the general question regarding the mechanisms of SLRP-derived signaling in pathogen-dependent and independent renal inflammation arose. This led to the fascinating concept of SLRPs as autonomous triggers of sterile renal inflammation in response to renal stress or injury. This review focuses on the key biologic roles of SLRPs in the normal and diseased kidney with special emphasis on newly described signaling events triggered by these proteoglycans.


Small leucine-rich proteoglycans (SLRPs), named for their small size (up to 42 kD) and the leucine-rich repeats of the protein core, are biologically active components of the extracellular matrix (ECM).1–4 They are covalently substituted with a varied number and type of glycosaminoglycan (GAG) side chains, namely chondroitin sulfate (CS), dermatan sulfate (DS), or keratan sulfate (KS). On the basis of the arrangement of the cysteine clusters, ear repeats, intron/exon organization, and homologies at the protein and genomic levels, the SLRPs are divided into five distinct classes. Detailed structural characteristics of SLRPs have been provided in recent reviews.1,5 The leucine-rich repeats of the protein core along with the GAG side chain allow for a wide range of matrix-matrix and matrix-cell interactions. SLRPs are long known to bind to various types of collagens and elastic fibril components, thereby regulating the kinetics, assembly, and special organization of fibrils in skin, tendons, and the cornea.6,7 Thus, SLRPs over many years were thought to be mere structural components of the ECM.

The initial finding of Yamaguchi et al.8 indicating that decorin, a class I CS/DS SLRP, binds the profibrotic cytokine TGF-β and neutralizes its activity attracted a lot of attention in the field of nephrology.9 Similar to decorin, the structurally related class I CS/DS SLRP biglycan and the class II KS SLRP fibromodulin interact through their protein cores with all three isoforms of TGF-β, exhibiting dissociation constants in the nanomolar range.10 Both observations were followed by many studies addressing the potential mechanisms of the decorin/TGF-β interaction and the therapeutic effects of these proteoglycans in TGF-β-overexpressing renal disorders. The recent discovery that soluble (not matrix-bound) decorin and biglycan act as signaling ligands for various receptors, thereby directly regulating cell behavior, provides a new paradigm in understanding the biologic role of these SLRPs.1,2,5

DECORIN

Decorin, the most widely studied member of the SLRP family, participates in ECM assembly and influences cell adhesion, proliferation, differentiation, and apoptosis.1,4 In the normal kidney decorin is expressed mainly by renal fibroblasts and therefore as a secreted molecule is primarily present in the peritubular space. In contrast, the normal glomerulus contains only trace amounts of decorin synthesized by mesangial cells.11–15 In the absence of collagen type I, a well known binding partner, decorin is not retained at the site of formation and may contribute to matrix remodeling.

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Correspondence: Dr. Liliana Schaefer, Pharmazentrum Frankfurt, Institut für Allgemeine Pharmakologie und Toxikologie, Klinikum der JW Goethe-Universität Frankfurt am Main, Haus 74, Z. 3.108a, Theodor-Stern-Kai 7, 60590, Frankfurt am Main, Germany. Phone: +49 69-6301-7899; Fax: +49 69-6301-83027; E-mail: Schaefer@med.uni-frankfurt.de

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but is removed by diffusing into the circulation or alternatively by endocytosis. Decerin accumulates in areas of tubulointerstitial fibrosis in several experimental and human nephropathies and may be a good predictor of progression in chronic kidney disease. Recent progress in SLRP research indicates the amount of decorin in tissue sections does not necessarily reflect its biologic effect because it represents mainly that which has been sequestered in the ECM and therefore may not be available to neutralize TGF-β or act as a ligand to various receptors (Figure 1).

Decorin-Mediated Neutralization of TGF-β
A great deal of attention has been focused on the antifibrotic properties of decorin as a neutralizing factor of TGF-β, and decorin treatment exerts salutary effects in renal disorders involving TGF-β overproduction regardless of the mode of decorin administration. Two decades of investigations confirmed the antifibrotic effects of decorin in several organs, such as kidney, lung, heart, skeletal muscle, liver, blood vessels, skin, and conjunctiva. Several mechanisms of decorin-mediated inactivation of TGF-β are postulated, including physical interaction with TGF-β and interference with TGF-β signaling, either directly or indirectly by regulating other modulators of TGF-β activity, particularly fibrillin-1, myostatin, and formation of decorin/TGF-β complexes. The latter are eliminated from the tissue through the circulation, by urinary excretion, or in the presence of collagen type I sequestration by the ECM (Figure 1). Taken together, it appears it is not the direct physical interaction of decorin with TGF-β but rather its interference with TGF-β signaling that plays a key role in neutralizing this cytokine. The various modes of interaction between decorin and TGF-β have been described at length in earlier reviews.

Decorin as a Signaling Molecule in Renal Disease
The generation of decorin-deficient mice considerably improved our understanding of how renal fibrosis is slowed down by decorin. Decorin-null mice have no apparent renal phenotype. Challenging decorin-deficient mice by unilateral ureteral obstruction (UUO), a well-established model of renal inflammation and fibrosis, provided clear-cut evidence that the absence of decorin markedly aggravates renal fibrosis. Surprisingly, the major reason for the acceleration of fibrogenesis was a massive loss of tubular epithelial cells because of apoptotic cell death. Importantly, tubular apoptosis occurred at the onset of UUO before up-regulation of TGF-β, indicating that this

Figure 1. Soluble versus matrix-bound decorin in healing and fibrosis. Soluble decorin acts as a signaling ligand of the IGF-IR, thereby protecting tubular epithelial cells against apoptosis or inducing synthesis of fibrillin-1 in renal fibroblasts. In addition, decorin is capable of neutralizing, directly or indirectly through fibrillin-1, the activity of TGF-β. In renal fibrosis most decorin is bound to matrix components, particularly collagen type I, as a part of the fibrotic scar and is therefore unable to act as a signaling molecule. However, matrix-bound decorin is still able to sequester TGF-β in the matrix, thereby withdrawing the cytokine from its cell membrane receptors.
mechanism is directly triggered by the lack of decorin. A similar effect of decorin deficiency on epithelial apoptosis was reported in streptozotocin-induced diabetes. Nevertheless, TGF-β activity is enhanced in UUO and diabetic nephropathy, stressing the importance of decorin in TGF-β inactivation.

The underlying mechanisms of decorin-dependent regulation of apoptosis are explained by decorin acting as a signaling molecule through the canonical IGF signaling pathway. Binding of decorin core protein to the IGF type I receptor (IGF-IR) is followed by phosphorylation of the receptor and activation of Akt/protein kinase B with subsequent induction of p21WAF1 by a mitogen-activated protein kinase-independent pathway (Figure 1). These effects were confirmed in several noncarcinoma cell types including tubular epithelial cells, renal fibroblasts, and endothelial cells. Interestingly, even by triggering the same pathway decorin may give rise to distinct biologic outcomes depending on cell type and biologic context. In renal fibroblasts, decorin binds to IGF-IRs and triggers phosphorylation of phosphoinositide-3 kinase, Akt/protein kinase B, and mammalian target of rapamycin, and p70 S6 kinase, resulting in the translational regulation of fibrillin-1 (Figure 1). These findings therefore provide a novel concept, showing that a matrix component (decorin) directly regulates the synthesis of another matrix constituent (fibrillin-1). In UUO and in streptozotocin-induced diabetes, the IGF-IR is upregulated in the kidney of decorin-null mice. However, this could not compensate for decorin deficiency, resulting ultimately in decreased fibrillin-1 content. The respective affinities of decorin and IGF-I for binding to the IGF-IR only differ by a factor of 10- to 20-fold depending on the cell type. Thus, it is conceivable that abundant decorin might compete and displace IGF-I from the IGF-binding proteins to trigger canonical IGF signaling.

There is now good evidence that decorin signals by multiple pathways through its interactions with four different tyrosine kinase receptors. In normal cells decorin triggers IGF-IR and lipoprotein-receptor-related protein-1. In contrast, interaction of decorin with EGF receptor (EGFR) and c-Met have been reported mainly in carcinoma cells. For details regarding decorin signaling, several recent reviews are recommended. In the kidney, in addition to interactions of decorin with the IGF-IR as described above, there is one report indicating involvement of decorin/EGF/caspase-3 pathway in mesangial cells upon decorin overexpression. In light of the great potential of decorin signaling to be involved in the pathogenesis of kidney disease, the scarcity of available data is surprising and clearly shows a need for further research.

**Complex Role of Decorin in Renal Diseases**

Several other findings indicate that the role of decorin in renal disease is even more complex. In the UUO model, collagen type I deposition is diminished in fibrotic decorin-null kidneys although synthesis was enhanced, suggesting that decorin is necessary to protect collagen fibrils from proteolytic digestion. However, reduction of collagen type I deposition did not protect the decorin-deficient kidney against accelerated fibrosis, indicating that early uncontrolled apoptosis of tubular epithelial cells might be the key event in the progression of renal fibrosis. There are several reports suggesting that decorin delays the development of albuminuria by not fully defined mechanisms. In streptozotocin-induced diabetes, decorin-deficiency accelerates renal manifestations showing increased albuminuria and proteinuria, impaired renal function, enhanced apoptosis of tubular epithelial cells, overexpression of TGF-β1 and connective tissue growth factor, ECM accumulation, upregulation of the proinflammatory proteoglycan biglycan, and enhanced infiltration of mononuclear cells with increased levels of Nox-4. Increased proteinuria is associated with enhanced cyclin-dependent kinase inhibitor p27Kip1, a downstream mediator of decorin, in podocytes and tubular epithelial cells. Diabetic decorin-null mice suffer from enhanced mortality probably because of low levels of adiponectin. Another possibility of how decorin might affect ECM remodeling and renal fibrosis is the effect on the expression of matrix metalloproteinases by mechanisms that are still unresolved.

The ability of decorin to regulate adhesion and migration in a cell-dependent manner further underlines its complex role in fibrogenesis. More details have been described in a recent review by Merline et al. Thus, decorin appears to be a potent antifibrotic molecule, influencing renal fibrogenesis directly through receptor-mediated signaling and indirectly in TGF-β-dependent and -independent ways.

**BIGLYCAN**

Biglycan, a class I SLRP structurally related to decorin, is covalently linked with up to two CS/DS side chains. Like decorin, biglycan is present mainly in the interstitium in the normal kidney, preferentially in the peritubular and perivascular space. Unlike decorin, biglycan is expressed at low levels in epithelial cells of the distal tubule and the collecting duct. In the normal glomerulus, biglycan is mainly present in endothelial cells. Mesangial cells and podocytes express only small amounts of biglycan under physiologic conditions. Distinct expression patterns most likely reflect different pathophysiological roles for these SLRPs.

**Biglycan and Renal Fibrosis**

Compared with decorin, the role of biglycan in renal disease has been studied to a much lesser extent. Several studies describe enhanced interstitial and to a lesser degree glomerular expression and deposition of biglycan in several fibrotic renal disorders. This upregulation is not surprising in light of the observation that, in all renal cell types studied in vitro and in vivo so far, TGF-β stimulates the expression of biglycan. The bulk of newly synthesized biglycan escapes from the kidney through plasma and presumably also after proteolytic fragmentation of the pro-
tein core in the urine or it becomes sequestered in the kidney by binding to various other ECM components.\textsuperscript{12,13,16}

In fibrotic conditions, the increasing deposition of potential binding partners (collagen types I or VI)\textsuperscript{54,55} and reduced proteolytic degradation results in progressive renal accumulation of biglycan within the fibrotic scar.\textsuperscript{16}

Biglycan is required for stable ECM formation by regulating collagen and elastic fibrils.\textsuperscript{36,57} In UUO, biglycan deficiency is associated with reduced expression of fibrillin-1 and a concurrent loss of elastic properties of renal tissue, as evidenced by cystic dilation of Bowman’s capsule and proximal tubules as well as hemorrhaging into the renal pelvis.\textsuperscript{56} However, the role of biglycan in fibrogenesis is not well understood. Although biglycan, like decorin, physically interacts with all TGF-\(\beta\) isoforms,\textsuperscript{10} no beneficial effects are seen in pulmonary fibrosis when biglycan instead of decorin is induced adenovirally.\textsuperscript{25} In the kidney, Thy-1 nephritic rats treated with biglycan in an analogous setting to decorin\textsuperscript{9} develop more severe glomerular lesions associated with enhanced infiltration of mononuclear cells, overexpression of glomerular \(\alpha1\) chains of collagen types I and IV, and elevated albuminuria (Schaefer and Kresse, unpublished data).

**BIGLYCAN: ENDOGENOUS LIGAND OF TOLL-LIKE RECEPTORS-2/4 AND REGULATOR OF THE NLRP3 INFLAMMASOME**

Research over the last 6 years gave rise to a fascinating new concept suggesting two distinct functions of biglycan. As an ECM component, biglycan plays a crucial role in matrix assembly, whereas soluble biglycan (not matrix-bound) acts as a proinflammatory signaling molecule.\textsuperscript{2,58,59}

Two key observations that biglycan is a nitric-oxide-regulated gene in mesangial cells\textsuperscript{60} and that renal overexpression of biglycan in a model of UUO is associated with enhanced macrophage infiltration\textsuperscript{38} indicate involvement of this proteoglycan in renal inflammation. Importantly, in UUO, upregulation of biglycan in the epithelial cells of distal tubules and collecting ducts occurs before macrophage infiltration, suggesting that biglycan is involved in the initiation of the inflammatory response.\textsuperscript{58} Furthermore, soluble biglycan acts as an endogenous ligand of the innate immunity receptors, Toll-like receptors (TLR)-2/4, in macrophages (Figure 2).\textsuperscript{58} The ligand form of biglycan may come from the pool of matrix-sequestered biglycan liberated by proteolytic degradation.\textsuperscript{5} Because biglycan is abundant in most tissues, rapid liberation of large amounts of this TLR ligand might occur under conditions of tissue stress or injury without the need for de novo synthesis. In addition, resident cells and infiltrating macrophages\textsuperscript{61} are capable of delivering newly synthesized biglycan.\textsuperscript{58} It is tempting to speculate that de novo synthesis of the intact molecule not only increases overall amounts of biglycan but also ensures biglycan-mediated signal transduction because it is likely that not all biglycan fragments will be able to bind to TLR2/4. By binding to TLR2 and TLR4, biglycan activates p38, Erk, and nuclear factor-\(\kappa\)B\textsuperscript{62} with subsequent expression of proinflammatory cytokines such as TNF\(\alpha\), macrophage inflammatory protein (MIP)-2, and pro-IL-1\(\beta\) (Figure 2).\textsuperscript{58,59} This will cause further recruitment of new macrophages, which also will start to produce biglycan, creating a feed-forward cycle that is able to drive the inflammatory response forward in an autocrine and paracrine manner.\textsuperscript{58}

Surprisingly, biglycan triggers the release of mature IL-1\(\beta\) without any further need for other co-stimulatory factors in addition to its role as a TLR2/4 ligand in the synthesis of pro-IL-1\(\beta\). On the basis of its complex structure consisting of a protein core with two covalently bound GAG side chains, biglycan is capable of clustering of TLR2 and TLR4 with purinergic P2X\(_{7r}\)/P2X\(_{4}\) receptors, leading to the activation of the NLRP3 inflammasome\textsuperscript{63} and caspase-1 with subsequent release of mature IL-1\(\beta\) (Figure 2).\textsuperscript{59} Thus, autonomous signaling of bi-
glycan through several alternative pattern recognition receptors sensing gram-negative (TLR4) and gram-positive (TLR2) pathogens with subsequent activation of the NLRP3 inflammasome emphasizes the key role of this proteoglycan in the innate immune response.

**Biglycan in Sterile and Pathogen-Mediated Kidney Diseases**

The observation that soluble biglycan represents a danger signal that is picked up by innate immune receptors sheds a new light on the pathophysiological mechanisms of noninfectious, inflammatory kidney diseases. UUO with subsequent upregulation of biglycan is associated with enhanced levels of active caspase-1 and mature IL-1β and therefore might serve as a good model to test whether biglycan acts *in vivo* as an endogenous inducer of inflammation. In fact, biglycan deficiency markedly attenuates the increase in active caspase-1 and mature IL-1β and results in lower numbers of infiltrating mononuclear cells in the renal interstitium and less damage to the obstructed kidney. Studies in various experimental models of non-pathogen-derived inflammatory kidney disease, including ischemia/reperfusion injury, chronic allograft rejection, and streptozotocin diabetes, reveal a striking concurrence of biglycan expression and the extent of renal injury. Some reports further correlate this link with the expression and activation of TLR2/4, indicating *in vivo* relevance of biglycan/TLR2/4 interactions in mediating sterile inflammatory kidney disease. On the other hand, the role of biglycan in pathogen-mediated renal inflammation has not been investigated yet. However, in LPS- (TLR4-dependent) and zymosan-induced (TLR2-dependent) sepsis, biglycan deficiency results in a clear survival benefit associated with lower levels of circulating TNFα and IL-1β as well as a reduction of active caspase-1 and mature IL-1β associated with lower numbers of infiltrating macrophages in the lung, a major target organ of sepsis in mice. It is tempting to speculate that in pathogen-mediated inflammation biglycan potentiates the inflammatory response by signaling through a second TLR that is not involved in pathogen sensing; however, in sterile inflammation soluble biglycan is capable of acting as an autonomous trigger of inflammation by clustering TLRs and purinergic P2X<sub>7</sub>/P2X<sub>4</sub> receptors. Thus, biglycan appears to be a danger signal and a crucial trigger of inflammation in sterile and pathogen-mediated renal disorders.

**BIGLYCAN: A LINK BETWEEN INNATE AND ADAPTIVE IMMUNE RESPONSE IN LUPUS NEPHRITIS**

In lupus nephritis (LN), a prototypical autoimmune disease affecting the kidney, soluble biglycan triggers the expression of the B cell chemoattractant CXCL13 by signaling through TLR2 and TLR4 in interstitial macrophages and dendritic cells. Elevated tissue levels of CXCL13 then cause recruitment of CXCR5<sup>+</sup> B cells, preferentially the B1 subset, into the kidney. Biglycan also induces the synthesis of RANTES (regulated upon activation, normal T cell expressed and secreted protein), monocyte chemoattractant protein (MCP)-1, and MIP-1α in macrophages, thereby attracting T cells and additional macrophages (Figure 2). These infiltrates eventually give rise to the formation of lymphoid follicle-like clusters of B cells, T cells, and macrophages in the kidney. Importantly, biglycan is markedly elevated in the plasma and kidneys from patients with LN and from lupus-prone (MRL/lpr) mice. Genetic elimination of biglycan in MRL/lpr mice improves systemic and renal outcomes by lowering levels of autoantibodies, reducing enlargement of the spleen and lymph nodes, and preventing renal damage and albuminuria. This amelioration is associated with reduced plasma and renal levels of CXCL13, RANTES, MCP-1, and MIP-1α as well as lower numbers of macrophages and B and T cells in the kidney. Furthermore, biglycan is a critical regulator of pro-IL-1β and the NLRP3 inflammasome as well as of the inflammasome/caspase-1-dependent maturation of IL-1β in LN. This transient overexpression of soluble biglycan provides *in vivo* proof for the involvement of TLR2/4 in biglycan-mediated signaling in the kidney. Thus, by bridging the innate and adaptive immune systems, endogenous soluble biglycan enhances the inflammatory response reaction and thereby aggravates the course of LN and perhaps other B cell-mediated inflammatory disorders.

**LUMICAN AND FIBROMODULIN**

Lumican and fibromodulin belong to the class II SLRPs and are referred to as KS proteoglycans because their GAG chains are composed of KS made up of repeating disaccharide units containing galactose (β1,3-galactose) and glucosamine (β1,3-galactose β1). Fibromodulin is known to interact with TGF-β, whereas at present there are no reports on the respective properties of lumican.

Basal expression of lumican and fibromodulin can be seen in the kidney, with more intense staining in the interstitium, preferentially in the peritubular space as compared with a weaker expression seen in the mesangial matrix. For reasons not understood, the expression pattern of lumican in normal kidney mimics that of decorin, whereas fibromodulin is present in epithelial cells of the distal tubules and the collecting ducts, displaying a staining pattern similar to biglycan.

Little is known about the role of KS SLRPs in renal disorders. In diabetic nephropathy, the tubulointerstitial and glomerular deposition of lumican and fibromodulin are mainly localized in areas of fibrotic scars and become progressively more pronounced with the extent of fibrosis in glomeruli and in the tubulointerstitium. Significant upregulation of lumican is detected in renal sections of chronic neonatal UUO. There is no direct evidence of lumican regulating apoptosis and infiltration in the kidney. However, taking into account its role in inducing apoptosis in tumor cells and mouse embryonic fibroblasts plus its ability to regulate macro-
phage and neutrophil infiltration in the eye,74–76 one might speculate that enhanced expression of lumican may be involved in apoptosis and interstitial infiltration in the UUO model.

CONCLUSIONS AND PERSPECTIVES

Two decades of research on the role of SLRPs in kidney disease provide numerous details regarding the expression pattern, localization, and metabolism mainly of decorin and biglycan. Decorin became firmly established as a potent antifibrotic molecule, influencing renal fibrogenesis by several distinct mechanisms, such as inhibition of TGF-β, regulation of ECM remodeling, and modulation of cell death, adhesion, and migration. A paradigm shift occurred when the soluble forms of decorin and biglycan were discovered to turn into receptor ligands, directly regulating cell behavior by signaling through receptor tyrosine kinases (e.g., EGFR, Met, and IGF-IR) and receptors involved in innate immunity and inflammation (e.g., TLRs and purinergic P2X receptors). These findings clearly demonstrate that the antifibrotic effects of decorin reach far beyond its interaction with the TGF-β system. The discovery that soluble biglycan will act as a danger signal improved our understanding of how pathogen-independent renal inflammation might be initiated and sustained. Data signifying the role of fibromodulin and lumican in renal disease are still quite sparse. Newer findings indicating a role in matricellular crosstalk might make them promising candidates for future research. With a better understanding of how SLRPs—in fact or fragments thereof—interact with various receptors, amplifying the beneficial effects of decorin or blocking the proinflammatory effects of biglycan with receptor antagonists could be developed for the treatment of inflammatory and fibrotic renal diseases.

DISCLOSURES

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BRIEF REVIEW


