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Soluble biglycan as a biomarker of inflammatory renal diseases

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Abstract

Chronic renal inflammation is often associated with a progressive accumulation of various extracellular matrix constituents, including several members of the small leucine-rich proteoglycan (SLRP) gene family. It is becoming increasingly evident that the matrix-unbound SLRPs strongly regulate the progression of inflammation and fibrosis. Soluble SLRPs are generated either via partial proteolytic processing of collagenous matrices or by *de novo* synthesis evoked by stress or injury. Liberated SLRPs can then bind to and activate Toll-like receptors, thus modulating downstream inflammatory signaling. Preclinical animal models and human studies have recently identified soluble biglycan as a key initiator and regulator of various inflammatory renal diseases. Biglycan, generated by activated macrophages, can enter the circulation and its elevated levels in plasma and renal parenchyma correlate with unfavorable renal function and outcome. In this review, we will focus on the critical role of soluble biglycan in inflammatory signaling in various renal disorders. Moreover, we will provide new data implicating proinflammatory effects of soluble decorin in unilateral ureteral obstruction. Finally, we will critically evaluate the potential application of soluble biglycan vis-à-vis other SLRPs (decorin, lumican and fibromodulin) as a promising target and novel biomarker of inflammatory renal diseases.

Keywords

Decorin; lumican; fibromodulin; Toll-like receptor; matrix metalloproteinases

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1. Introduction

Accumulation within the tissues or release into the circulation of the extracellular matrix (ECM) derived small leucine-rich proteoglycan (SLRP), biglycan is a common feature of a large number of renal pathologies. This has led to the proposal that this SLRP could directly contribute to the progression of renal diseases (Anders and Schaefer, 2014, Moreth et al., 2010, Moreth et al., 2014, Schaefer, 2011, Thompson et al., 2011, Yokoyama and Deckert, 1996).

A member of the class I family of SLRPs, biglycan consists of a 42 kDa protein core containing leucine-rich repeats (LRRs) and one or two covalently-linked glycosaminoglycan (GAG) chains at the N-terminus, consisting of either dermatan sulfate (DS) or chondroitin sulfate (CS) (Choi et al., 1989, Roughley and White, 1989). Notably, biglycan is also present in a non-proteoglycan form in extracts of human articular cartilage and intervertebral disc. The unglycanated form of biglycan is found only in a small proportion in newborn cartilage and has a wider abundance in adults. In contrast, the closely related decorin exists only in its proteoglycan form at all ages (Roughley et al., 1993).

Through either the protein core or GAGs, biglycan is able to interact with various ECM components such as collagen types I, II, III, and VI or elastin, contributing to the organization and stabilization of the matrix (Douglas et al., 2006, Reinboth et al., 2002, Schonherr et al., 1995, Wiberg et al., 2001, Wiberg et al., 2002, Wiberg et al., 2003). Consequently, biglycan was considered for long time only as a quiescent ECM derived-molecule. However, the last decade of research has brought biglycan to light as a bioactive molecule with key roles in signaling. In this capacity, biglycan interacts with bone morphogenic protein (BMP)-2, -4, -6 and chordin (Chen et al., 2004, Miguez et al., 2011, Moreno et al., 2005), transforming growth factor (TGF)- β (Hildebrand et al., 1994), tumor necrosis factor (TNF)- α (Tufvesson and Westergren-Thorsson, 2002), Wnt-1-induced secreted protein 1 (WISP1) (Desnoyers et al., 2001) and vascular endothelial growth factor A (VEGF) (Berendsen et al., 2014). Biglycan can also serve as a ligand for different receptors or complexes such as Toll-like receptors (TLRs) 2 and 4 (Schaefer et al., 2005), Activin like kinase 6 (ALK6), purinergic receptors P2X₇/P2X₄ (Babelova et al., 2009), the class A scavenger receptor (SR-A) (Santiago-Garcia et al., 2003), low-density lipoprotein receptor-related protein 6 (LRP6) (Berendsen et al., 2011), MuSK (Amenta et al., 2012), and dystrophin-glycoprotein complex (Bowe et al., 2000, Rafi et al., 2006).

Given the magnitude of biglycan interactome, it is not surprising that this SLRP leads to organ-specific effects encompassing renal diseases (Kuroda et al., 2004, Moreth et al., 2010, Moreth et al., 2014, Schaefer et al., 2004, Stokes et al., 2000), bone formation and healing (Berendsen et al., 2014, Chen et al., 2004, Desnoyers et al., 2001, Miguez et al., 2014), muscular dystrophy (Bowe et al., 2000, Rafii et al., 2006), control of neuromuscular synapses (Amenta et al., 2012) and autoimmune perimyocarditis (Popovic et al., 2011). In addition, biglycan has been recently linked to cancer cell proliferation (Hu et al., 2014, Niedworok et al., 2013). It is assumed that mainly the soluble form of biglycan, released from the matrix via partial proteolytic processing during tissue injury, binds and induces signaling through different receptors (Anders and Schaefer, 2014, Moreth et al., 2010,

Moreth et al., 2014, Nastase et al., 2014, Zeng-Brouwers et al., 2014). Thus, selective targeting of soluble biglycan could be accomplished without disrupting tissue homeostasis in several pathological conditions. In this review we will focus on the role of circulating biglycan in the aggravation of different renal diseases, will address the possibility of using soluble biglycan as a biomarker in progressive renal pathologies, and critically assess the importance and the modalities of neutralizing matrix-unbound biglycan in therapeutics.

2. Biglycan expression and signaling in the kidney

Biglycan is expressed as a component of the ECM in all organs (Bianco et al., 1990, Ungefroren et al., 1998), a feature it shares with decorin (Iozzo, 1998). However, the expression patterns of these two SLRPs are not overlapping, suggesting different roles in pathology (Stokes et al., 2000).

2.1. Distribution and regulation of biglycan in the kidney

In normal adult rat kidneys, biglycan is found in collecting ducts, distal tubules and vessel walls as well as within the glomeruli, mainly associated with capillaries, but also within the mesangium and around podocytes (Schaefer et al., 1998). In contrast, decorin is expressed only in trace amounts in the mesangium (Merline et al., 2009a, Schaefer et al., 1998).

In human renal cortex, biglycan is expressed mainly in the tubulointerstitium, in the peritubular mesenchymal cells and distal tubules as well as in endothelial and weaker in mesangial and epithelial cells of the glomeruli (Schaefer et al., 2000). Notably, *in situ* hybridization studies have shown that endothelial cells are the main source of *BGN* (Schaefer et al., 2000). In the tubulointerstitium *BGN* mRNA is found in peritubular fibroblasts, distal tubules, and collecting ducts as well as in endothelial cells, smooth muscle cells, and the adventitia of blood vessels (Schaefer et al., 2000).

Biglycan overexpression is a common feature in the progression of several renal pathologies (see Table 1 and Section 4). Early studies demonstrated that the expression of biglycan in different types of cells in the kidney can be increased by growth factors or cytokines (Davies, 1995). For example, in rat glomerular mesangial cells, TGF- β induces biglycan and decorin, mostly detected in the conditioned media rather than bound to the ECM (Border et al., 1990). By comparison, platelet-derived growth factor (PDGF), interleukin-1 β (IL-1 β) or TNF- α induce lower proteoglycan production in mesangial cells (Border et al., 1990).

Biglycan is also produced by glomerular epithelial cells in response to TGF- β (Nakamura et al., 1992), and diabetic rats biglycan mRNA levels are elevated together with increased TGF- β mRNA and protein (Yamamoto et al., 1993). Injection of antimesangial serum into rats leads to injury of mesangial cells and acute mesangial proliferative glomerulonephritis with a concurrent enhanced levels of glomerular biglycan and TGF- β mRNA (Yamamoto et al., 1994). Other studies have shown that rats with chronic renal transplant rejection develop a large number of autoantibodies against mesangial cell focal adhesion plaques, vinculin and proteins secreted by these cells in culture, including biglycan and decorin (Paul et al., 1998). The autoantibodies strongly contribute to the local damage and interfere in the tissue repair process after injury (Paul et al., 1998).

Biglycan production is not restricted to renal resident cells. For example, in a unilateral ureteral obstruction (UUO) model, at day 4 post operation, biglycan expression is primarily found in epithelial cells of dilated tubules. After 7 days, however, biglycan expression in tubular epithelial cells decreases and instead infiltrating and interstitial cells strongly express biglycan. At later stages, biglycan expression at both mRNA and protein levels in the kidney is mostly detected in the infiltrating and interstitial cells (Schaefer et al., 2002). In chronic renal allograft dysfunction due to kidney transplantation, biglycan is upregulated and synthesized by interstitial infiltrating cells (Bedke et al., 2007, Kiss et al., 2010). Moreover, inflammatory stimuli such as interleukin-6 (IL-6), chemokine (C-C motif) ligand (CCL) 5 or IL-1 β can trigger in macrophages the synthesis and release of biglycan (Bedke et al., 2007, Schaefer et al., 2005). In agreement with these studies, and in contrast to decorin treatment (Border et al., 1992), biglycan treatment in Thy-1 nephritic rats leads to more severe glomerular lesions, enhanced mononuclear infiltrates, overexpression of glomerular α 1 chains of collagen types I and IV and elevated albuminuria (Schaefer, 2011).

2.2. Soluble biglycan: Mechanisms of production

The finding that biglycan can be detected in a soluble form in plasma of different renal inflammatory and autoimmune pathologies (Moreth et al., 2010) raises interesting questions concerning the mechanism of biglycan production, fate and its role in disease progression. Biglycan is produced by macrophages and resident renal cells by the concurrent activity of IL-1 β , IL-6, and TNF- α (Schaefer et al., 2005) or TGF- β and PDGF (Border et al., 1990), respectively. When overexpressed, biglycan mostly deposits in the extracellular matrix and is found in collagen enriched-areas (Stokes et al., 2000, Stokes et al., 2001). A potential mechanism of action is that when the ECM becomes saturated with biglycan, then biglycan becomes soluble and reaches the extracellular fluid and subsequently the blood circulation (Moreth et al., 2010). Whether biglycan is also present in the lymphatic system is not yet known. A contributing factor to the solubility of biglycan is the proteolytic processing of collagenous matrix which liberates this SLRP or its bioactive fragments (see Section 3 and Table 2). Either of the two mechanisms can be operative depending on the type of pathology.

Non-inflammatory glomerular diseases are usually associated with less proteinase expression leading to extensive matrix accumulation (Lenz et al., 2000). However, in diabetic nephropathy, the accumulation of matrix is due to massive synthesis and decreased degradation by matrix metalloproteinases (MMPs) (Lenz et al., 2000). The biglycan levels in the blood of patients with manifest diabetic nephropathy are slightly higher than normoglycemic healthy controls, and this correlates with levels of CCL5 (Moreth et al., 2010). These data favor the first hypothesis, that is, release of soluble biglycan is due to ECM oversaturation rather than proteinase expression and activity.

It is well established that inflammatory glomerular diseases are associated with enhanced MMP expression (Lenz et al., 2000) such as in lupus nephritis where MMP-1, -2 and -3 are overexpressed (Nakamura et al., 1995). In patients with diffuse proliferative lupus nephritis type IV, high plasma levels of biglycan correlate with albuminuria and elevated chemokine (C-X-C motif) ligand (CXCL) 13 levels (Moreth et al., 2010). In plasma of patients with

acute renal allograft rejection, high soluble biglycan levels associate with TNF- α , IL-1 β , CCL2, CCL5 and CXCL13 (Moreth et al., 2010). Soluble biglycan and specific fragments generated in the injured kidney can be released in the bloodstream and be transported to other organs (Figure 1). At the same time soluble biglycan can also be produced in other organs, reach the bloodstream and consequently the kidney (Leeming et al., 2013, Moreth et al., 2010, Zeng-Brouwers et al., 2014).

2.3. Biglycan signaling in inflammation

Following production in macrophages or mesangial cells, soluble biglycan, in turn, acts as a signaling molecule on these cells (Figure 1). In macrophages, soluble biglycan serves as a ligand for TLR2, TLR4 and P2X₄/P2X₇ and induces receptor clustering and cooperativity (Babelova et al., 2009, Schaefer et al., 2005). By signaling through TLR2/4, biglycan activates nuclear factor- κ B (NF- κ B), p38 and extracellular signal-regulated kinase (Erk) in macrophages (Schaefer et al., 2005). As a consequence a series of cytokines and chemokines are transcribed and released, (Moreth et al., 2010, Schaefer et al., 2005, Zeng-Brouwers et al., 2014). Moreover, activation of TLR2/4 signaling pathway induces Nod-like receptor (NLR) family, pyrin domain containing 3 (NLRP3) and pro-IL-1 β (Babelova et al., 2009). Through the interaction with the P2X₄/P2X₇ receptors biglycan activates the NLRP3 inflammasome assembly and Caspase-1 activation, leading to the maturation and release of IL-1 β . The latter process depends on reactive oxygen species (ROS) and heat shock protein 90 (Hsp90) (Babelova et al., 2009). In a UUO mouse model generated in a genetic background lacking *Bgn*, the levels of active Caspase-1, mature IL-1 β and the infiltrating macrophages in the ligated kidney are lower than in wild-type mice (Babelova et al., 2009). Biglycan induces the B cells chemoattractant CXCL13 in primary murine macrophages and spleen dendritic cells in a TLR2/4/NADPH oxidase-dependent manner (Moreth et al., 2010). Notably, the intact biglycan proteoglycan is required for the CXCL13 induction, insofar as either the protein core or the GAG chains alone are unable to induce the signaling (Moreth et al., 2010).

In a transient transgenic mouse model where soluble biglycan was *de novo* synthesized specifically in hepatocytes, the biglycan released in the bloodstream targets the kidney (Moreth et al., 2010, Zeng-Brouwers et al., 2014). Once there, biglycan induces the chemoattractants CXCL1, CXCL2 and CCL2 and recruitment of neutrophils and macrophages via the TLR2/4/myeloid differentiation factor 88 (MyD88) pathway. At the same time, biglycan-dependent CCL5 release and T cell infiltration occur via the TLR4/TIR-domain-containing adaptor-inducing interferon beta (TRIF) pathway (Zeng-Brouwers et al., 2014). In agreement with these findings, transient overexpression of soluble biglycan exacerbates renal inflammation in an ischemia reperfusion injury mouse model via physically interacting and signaling through TLR2/4 (Moreth et al., 2014).

Collectively, the studies summarized above have demonstrated that biglycan expression is dynamically regulated in various pathologies, suggesting a central role of biglycan in renal inflammation and immunomodulation.

3. Matrix metalloproteinases involved in the generation of biglycan fragments

Biglycan can bind to the N-terminal triple helix of collagen VI (Wiberg et al., 2001) and other collagen subtypes (Douglas et al., 2006, Reinboth et al., 2002, Schonherr et al., 1995, Wiberg et al., 2001, Wiberg et al., 2002, Wiberg et al., 2003), thereby promoting the assembly of supramolecular collagen complexes (Melchior-Becker et al., 2011, Reinboth et al., 2006, Wiberg et al., 2002). Zinc-dependent endopeptidases such as MMPs can degrade a broad-spectrum of ECM components including proteoglycans (Gross and Lapiere, 1962, Sternlicht and Werb, 2001, Stocker and Bode, 1995). These enzymes are synthesized in an inactive form; the zinc moiety in the activation site is stabilized by interaction with a Cys residue in the N-terminal propeptide. Disruption of this interaction triggers the cleavage of the N-terminus, leading to enzyme activation (Van Wart and Birkedal-Hansen, 1990). There are more than 25 members currently in the MMP family (Phatharajaree et al., 2007).

MMP-mediated ECM remodeling is critical in numerous developmental and disease-related processes (Sternlicht and Werb, 2001). This activity is not only able to alter the microenvironment of the ECM and thus affect cellular behavior, but also modulates the activity of biologically active molecules (Vu and Werb, 2000). The metalloproteinase bone morphogenetic protein-1 (BMP-1) can remove the N-terminal propeptide from the biglycan molecule, resulting in a mature form present in all tissues (Scott et al., 2000). In the last decade, various MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) have been shown to cleave biglycan within its central LRR region (Melching et al., 2006, Monfort et al., 2006, Stegemann et al., 2013, Zhen et al., 2008). These MMPs have different affinities for specific ECM components; for instance, MMP-12 tends to degrade proteoglycans rather than collagen (Zhen et al., 2008). Mass spectroscopy studies have shown that biglycan yields a great number of peptides compared to other cleaved ECM components, implying its susceptibility to MMP cleavage (Zhen et al., 2008). Not only metalloproteinases, but also the pro-apoptotic serine protease Granzyme B (GrB) can cleave biglycan, leading to release of active TGF- β 1 (Boivin et al., 2012). Additional details on biglycan cleavage by these proteases are presented in Table 2.

Modifications in MMP expression and activity in the kidney can imbalance the ECM synthesis and degradation, thus altering the cellularity, progressively leading to glomerular scarring and various renal disorders (Arthur, 1998, Giannelli et al., 1997, Lenz et al., 2000, Turck et al., 1997) (Table 2). Moreover, MMPs may regulate certain growth factors thereby indirectly contributing to ECM turnover (Fowlkes et al., 1995, Kalluri et al., 1997). Thus, biglycan activity is highly regulated and depends on both transcriptional and post-transcriptional events leading to its solubility and signaling during inflammation and remodeling.

4. Biglycan abundance in renal disease progression

Biglycan synthesis and expression are altered in a wide variety of renal disorders. Primary injuries in either glomeruli or renal tubules and interstitium may be the result of infections, environmental toxins, or allergic reactions to medication or systemic diseases (Jimenez et

al., 2008, Perazella and Markowitz, 2010, Praga and Gonzalez, 2010, Rodriguez-Iturbe and Musser, 2008, Vinen and Oliveira, 2003). These damages can acutely or gradually decrease renal function leading to deterioration and renal failure (Corrigan and Stevens, 2000, Joss et al., 2007).

4.1. Augmented expression of biglycan in glomerular diseases

Increased glomerular ECM can solidify part of the tufts, efface the capillaries, and cause sclerosis (Brenner et al., 1982). In primary glomerular disease, biglycan expression is increased in fibrous crescents and sclerotic glomeruli of IgA or membranous nephropathy patients, but is unchanged in patients with minimal change disease (Kuroda et al., 2004, Stokes et al., 2001). This upregulation is likely associated with elevated TGF- β in glomeruli and correlates to albumin excretion and progression of glomerular diseases (Ebefors et al., 2011). A similar distribution is also present in experimental renal injury models, in which biglycan is localized at glomerulosclerotic lesions.

Biglycan, as well as decorin and their endocytosis receptor are more abundant and they co-localize in the kidney in different rat models of tubulointerstitial fibrosis and glomerulosclerosis compared to healthy controls (Table 1) (Border et al., 1990, Schaefer et al., 1998). Thus, it is presumable that their amount in the kidney tissue is controlled by synthesis and secretion as well as their endocytosis and degradation (Schaefer et al., 1998). For instance, biglycan-cleaving proteinases MMP-2 and MMP-9 are overexpressed in various glomerular diseases (Table 2) (Binder et al., 1999, McMillan et al., 1996). During glomerular inflammation, mesangial cells overexpress biglycan in response to IL-1, PDGF (Figure 1), while NO production correlates with reduced biglycan expression (Kastner et al., 2007, Schaefer et al., 2003, Shihab et al., 2000, Williams et al., 2007). However, strong immunostaining for both decorin and biglycan is observed in the mesangium of injured glomeruli (Schaefer et al., 1998). The overexpressed decorin has an antifibrotic role as a negative regulator of TGF- β (Iozzo, 1998); however this is not the case for biglycan whose role in fibrosis is not clear.

4.2. Accumulation of biglycan in tubulointerstitial nephropathy

Changes in the tubulointerstitium may be due to degenerative progression, necrosis, or reversible damages (Nath, 1992). Elevated levels of biglycan mRNA and protein are often observed in fibrous areas of the tubulointerstitium in human and rat tubulointerstitial diseases (Stokes et al., 2001) (Table 1). Synthesis of biglycan increases during the development of chronic cyclosporine (CsA) nephrotoxicity, a tubulointerstitial fibrosis model (Shihab, 1996, Shihab et al., 2002). Consequently, pirfenidone treatment improves CsA-induced fibrosis by downregulating synthesis of biglycan (Shihab et al., 2002). In a UUO model, biglycan overexpression in epithelial cells of distal tubules appears before macrophage infiltration (Leemans et al., 2009, Moreth et al., 2010, Schaefer et al., 2002, Schaefer, 2011). Biglycan deficiency in UUO lowers active caspase-1 levels and IL-1 β production in the circulation and other organs (Babelova et al., 2009). In the same model, biglycan induces the production of fibrillin-1 and this could contribute to cystic dilatation of Bowman's capsule and proximal tubules (Schaefer et al., 2004). In such fibrotic disorders, the accumulation of biglycan binding partners, such as the fibrillar collagens type I or VI

(Wiberg et al., 2002), together with limited proteolytic degradation could predispose to an accumulation of this SLRP within the scar during the progression of renal injury (Schaefer et al., 2001). Collectively, these data reveal a correlation between biglycan and renal disorders, and further point to a potential participation of biglycan in triggering and sustaining renal disease progression.

4.3. Imbalanced biglycan synthesis and degradation in diabetic nephropathy

Diabetic nephropathy, a major risk of end-stage renal disease, is associated with dyslipidemia which in turn aggravates the progression of renal disorders (Sato et al., 1991, Takemura et al., 1993, Thompson et al., 2011). Both patients with type II diabetic nephropathy and streptozotocin (STZ)-induced diabetic mouse models show a marked induction of biglycan expression especially within the tubulointerstitial compartment. This change often correlates with a marked recruitment of monocytes and macrophages and an increase in the degree of interstitial fibrosis and proteinuria (Merline et al., 2009a, Moreth et al., 2010, Schaefer et al., 2001). Notably, this SLRP upregulation in the kidney occurs in all stages of diabetic nephropathy (Schaefer et al., 2001, Thompson et al., 2011). However, in the glomerular space, biglycan is detected only in advanced stages of the disease (Schaefer et al., 2001). This is compatible with the hypothesis that the overproduced biglycan is first (during earlier stages of the renal disease) is mostly released in the circulation and urine.

As mentioned above, both diabetes and hyperlipidemia enhance renal biglycan content in preclinical animal studies. This SLRP co-distributes with apolipoprotein B and thus contribute to lipid retention within blood vessels as biglycan binds to lipoproteins and aggravates disease progression (Thompson et al., 2011). Mechanistically, the enhanced TGF- β levels in diabetes cause increased renal biglycan synthesis and tissue deposition, which, in turn, causes renal lipid retention/accumulation, thereby contributing to the development of glomerular injury (Hayashi et al., 2012, Thompson et al., 2011). This pathophysiological process could be further facilitated by ECM-cleaving enzymes (Table 2).

4.4. Circulating biglycan evokes lupus nephritis

As discussed above, the non-matrix bound, soluble form of biglycan is generated by ECM degradation and/or by de novo synthesis by infiltrating macrophages and resident cells upon tissue damages (Schaefer et al., 2005, Schaefer, 2010, 2011, Wang and Harris, 2011). Notably, MMP-2 and MMP-3, two proteinases capable of degrading biglycan, are upregulated in a murine lupus nephritis model (Table 2)(Nakamura et al., 1995). Levels of biglycan are strikingly increased in the circulation and kidneys of patients with human systemic lupus erythematosus (SLE) and lupus-prone (MRL/*lpr*) mice (Moreth et al., 2010). SLE is an autoimmune disease characterized by a variety of organ disorders, particularly reflected by glomerular lesions. Soluble biglycan recruits macrophages, B cells and T cells to the kidney by triggering the production of their chemoattractants CXCL13, CCL2, CCL3, and CCL5 respectively. Biglycan-triggered CXCL13 expression in resident macrophages and dendritic cells from the spleen is TLR2/4-dependent and causes recruitment of Chemokine (C-X-C motif) receptor (CXCR) 5⁺ B cells to the kidney. Lymphoid follicle-like clusters gradually form together with infiltrating T cells and macrophages. Recent studies have consistently shown that transient overexpression of circulating biglycan is markedly

pro-inflammatory and induces severe renal damage (Moreth et al., 2010). Consequently, biglycan deficiency ameliorates renal and systemic outcomes by reducing renal damage, albuminuria, lowering levels of autoantibodies and decreasing the expansion of the spleen and lymph nodes (Moreth et al., 2010, Nastase et al., 2014, Schaefer, 2011).

4.5. Soluble biglycan aggravates renal ischemia reperfusion injury

During kidney transplantation, ischemia-reperfusion injury (IRI) negatively impacts both short and long-term graft survival (Chapman et al., 2005, Herrero-Fresneda et al., 2003, Tilney and Guttman, 1997). This initial injury leads to activation of an immune response causing various tissue damages (Bonventre and Zuk, 2004, Boros and Bromberg, 2006, de Vries et al., 2003, Zhou et al., 2000). Biglycan is upregulated in a Fischer to Lewis renal transplantation model (Bedke et al., 2007). Its expression is reduced together with that of other molecules associated with fibrosis (plasminogen activator inhibitor-1, PAI-1 and TGF- β 1) upon treatment with the BX471 Chemokine (C-C motif) receptor (CCR) 1 antagonist (Bedke et al., 2007). This correlates with less infiltrating and proliferating mononuclear cells. The CCR1 antagonism is further linked to the ability of BX471 to inhibit CCL5-induced secretion of biglycan by macrophages *in vitro* (Bedke et al., 2007).

In an IRI mouse model, TLR4 expression in tubular epithelial cells is increased, together with leukocyte infiltration within the kidney following ischemia (Wu et al., 2007b). Biglycan levels in plasma and kidney are highly elevated after injury (Wu et al., 2007b). This induction might be the consequence of massive ECM degradation by active MMP-8, MMP-14 in response to the injury (Table 2) (Covington et al., 2006, Vandenbroucke et al., 2012). In hepatocyte-specific experimental mouse model where biglycan is transiently overexpressed as circulating soluble proteoglycan, there is exacerbation of the inflammatory response following ischemia; this process is linked to an induction of serum TNF- α in a TLR2/4-dependent manner (Moreth et al., 2014). Chemokine CXCL1, CCL2, CCL5 are elevated in the kidney, leading to neutrophil, macrophage and T cell recruitment and infiltration. Thus, biglycan is one of the soluble molecules that contribute to a worsening of renal ischemia reperfusion and therapeutics directed at blocking this SLRP or its receptors could improve the clinical outcome. This hypothesis is indeed supported by the *in vivo* observation that proinflammatory signaling can be greatly retarded in a genetic background lacking both innate immune receptors, TLR2/4 (Moreth et al., 2014).

5. Soluble biglycan: ECM-derived danger signals in non-kidney-related pathogenesis

As an endogenous ligand of TLR2/4, soluble biglycan acts as damage-associated molecular pattern (DAMP) thus can trigger pro-inflammatory signaling in other organs where biglycan accumulates (Zeng-Brouwers et al., 2014). Along similar lines, experimental models, where soluble biglycan is transiently overexpressed, demonstrate aggravated pathological outcomes (Babelova et al., 2009, Moreth et al., 2010), showing its critical role in regulating disease progression (Frey et al., 2013, Moreth et al., 2014, Nastase et al., 2012, Nastase et al., 2014, Schaefer, 2011).

In addition to renal-associated pathology, increased level of circulating biglycan is also found in experimental models of sepsis and liver fibrosis (Babelova et al., 2009, Genovese et al., 2013). In the latter disease, the ability of biglycan to engage TGF- β (Schaefer et al., 2005) could play a role in regulating the fibrotic response (Droguett et al., 2006). Notably, patients with rheumatoid arthritis (RA) show elevated levels of antibodies targeting biglycan in the affected synovial fluid (Polgar et al., 2003), and this process has been recently linked to fragmentation of collagen fibers, thus contributing to the RA phenotype (Antipova and Orgel, 2012). In a collagen-induced RA experimental model, fragmented biglycan was detected in serum (Genovese et al., 2013), implying that degraded biglycan from the ECM is antigenic and can produce autoantibodies that gradually causes damage.

The fact that soluble biglycan can be detected in plasma and serve as a danger signal highlights its potential of being used as a biomarker in related disorders. Recently, a sequence-specific biglycan fragment ELISA has been developed using as antigen a peptide cleaved by MMP-9 and MMP-12 (Genovese et al., 2013). By monitoring serum samples from carbon tetrachloride-induced liver fibrosis and bile duct ligation (BDL) experimental models, it was shown that in both cases animals present elevated soluble biglycan compared to healthy controls (Genovese et al., 2013). This enhancement correlates with the extent of liver fibrosis determined by Sirius red staining, and could potentially be used as a marker in diagnosis.

Collectively, these findings indicate that the spectrum of biglycan biology is quite vast and not necessarily restricted to renal pathology.

6. Selected SLRPs in renal inflammatory diseases

6.1. Decorin: shifting seesaw between its antifibrotic and proinflammatory properties

Decorin, another CS/DS-containing proteoglycan belonging to class I SLRP, shares ~60% homology with biglycan (Iozzo and Murdoch, 1996). In normal kidneys, decorin is primarily expressed by renal fibroblasts with only trace amounts in mesangial cells (Bianco et al., 1990, Schaefer et al., 1998, Schaefer et al., 2000, Thomas et al., 1991). In several experimental and human nephropathies, decorin accumulates in areas of tubulointerstitial fibrosis (De Heer et al., 2000, Mogyorosi and Ziyadeh, 1999, Schaefer et al., 2000, Stokes et al., 2001, Vleming et al., 1995), in glomeruli of IgA nephropathy patients (Ebefors et al., 2011), and in the skin of patients with nephrogenic systemic fibrosis (Gambichler et al., 2009). Notably, in primary glomerular diseases, such as minimal change disease, IgA nephropathy and membranous nephropathy, urinary decorin levels are elevated and negatively correlate with creatinine clearance (Kuroda et al., 2004).

6.1.1. Decorin mediates TGF- β activity in renal fibrosis—Through its LRR domains present in its protein core decorin can interact with several receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) (Iozzo et al., 1999), insulin-like growth factor receptor I (IGF-IR) (Fiedler et al., 2008, Fowlkes et al., 1995, Iozzo et al., 2011, Morrione et al., 2013, Schonherr et al., 2005), Met (Goldoni et al., 2009), VEGF receptor 2 (VEGFR2) (Buraschi et al., 2013, Khan et al., 2011), platelet derived growth factor receptor (Baghy et al., 2013) as well as the LRP (Brandan et al., 2006) and profibrotic

cytokine TGF- β (Ruoslahti and Yamaguchi, 1991, Yamaguchi et al., 1990). This direct interaction with TGF- β , the indirect influence on TGF- β signaling cascades, and/or the TGF- β modulators including fibrillin-1 and myostatin, have nominated decorin as a neutralizing factor of TGF- β (Merline et al., 2009b, Schaefer et al., 2004, Schaefer et al., 2007, Yokoyama and Deckert, 1996, Zhu et al., 2007). Additionally, decorin can regulate fibrogenesis through inhibition of connective tissue growth factor (Vial et al., 2011), by influencing on ECM synthesis and turnover as well as by modulating cell adhesion (Brandan et al., 2008, Dunkman et al., 2013, Dunkman et al., 2014, Merline et al., 2009b, Morcavallo et al., 2014, Schaefer, 2011). Administration of recombinant decorin to rats subjected to ATS-induced glomerulonephritis attenuates TGF- β -mediated fibronectin deposition (Border et al., 1992), thus inhibiting scarring during experimental renal diseases. Such antifibrotic properties have been further illustrated in a variety of organs (Al Haj Zen et al., 2006, Giri et al., 1997, Kolb et al., 2001a, Kolb et al., 2001b, Shi et al., 2006). Although reduced collagen-rich scars formed when adenovirus-derived decorin was transferred to the mice suffering from bleomycin-induced pulmonary fibrosis (Kolb et al., 2001a), this treatment also led to patchy infiltration on day 7 but later decreased compared to administering adenoviral vector alone. These findings suggest that decorin-mediated proinflammatory signaling might reduce its potency during antifibrotic therapy. Besides mediating fibrosis, the binding ability of decorin to TGF- β can also modulate various cellular processes such as reversing the repressive effect of TGF- β on macrophage activation (Comalada et al., 2003), suppressing TGF- β -dependent apoptosis in bone marrow stroma cells (Bi et al., 2005) and even sequestering cytokines in the ECM (Markmann et al., 2000, Schaefer et al., 2001). However, decorin/TGF- β interaction has been reported as a negative, selective regulator of TGF- β activity (Takeuchi et al., 1994) or even as not having any effect (Hausser et al., 1994), suggesting that decorin plays a complex role in various signaling processes.

A better understanding of the complex role of decorin in renal fibrosis is provided by studies utilizing UUO model. In this case, decorin mRNA and protein expression are upregulated in the renal cortex of the obstructed kidney (Diamond et al., 1997). However, decorin depletion aggravates renal fibrosis by triggering massive apoptosis of tubular epithelial cells (Schaefer et al., 2002) through interacting with IGF-IR, followed by phosphorylation and activation of the Akt/PKB signaling cascade (Schaefer et al., 2007, Schonherr et al., 2005). In addition, deposition of collagen type I, an established binding partner of decorin, is abolished in decorin-null mice although synthesis is elevated (Geng et al., 2006, Schaefer et al., 2002). Similar effects of decorin deficiency on apoptosis and renal pathological outcomes have been reported in STZ-induced diabetic model (Merline et al., 2009a, Schaefer et al., 2007, Williams et al., 2007). Collectively, both matrix-bound and soluble decorin constrain fibrogenesis in both TGF- β -dependent and independent signaling pathways; these critical regulations may explain why disruption of TGF- β synthesis or activity is not sufficient in antifibrotic therapies (Cohen-Naftaly and Friedman, 2011).

6.1.2. Soluble decorin as a signaling molecule in renal inflammation—Similar to biglycan, decorin is an endogenous ligand of TLR2/4 (Merline et al., 2011). This binding can rapidly activate mitogen-activated protein kinases (MAPK) and NF- κ B pathways thus enhancing the synthesis of the proinflammatory cytokines TNF- α and IL-12 in

macrophages. By reducing oncogenic microRNA (miR)-21 and the activity of TGF- β 1, decorin suppresses translational repression of programmed cell death 4 (PDCD4), a unique regulator of tumorigenesis and inflammation, leading to PDCD4 abundance in a TLR2/4-independent manner. This induction can subsequently lower protein levels of anti-inflammatory cytokine IL-10, and therefore contributes to a more proinflammatory environment. Moreover, decorin is involved in the recruitment of mononuclear cells to the site of injury by stimulating CCL2 production (Köninger et al., 2006), thereby sustaining the inflammatory state.

Similar to biglycan, soluble decorin released from its binding partners in the ECM, including collagen type-I, can enter the blood circulation (Schaefer et al, 1998, Schaefer et al, 2000) and thus act as a signaling molecule. In the UUO model, soluble decorin-driven inflammation was examined by overexpressing human decorin (hDCN) employing the pLIVE (Liver *in vivo* Expression) vector (for details see Zeng-Brouwers et al., 2014). To this end we injected wild-type C57BL/6 mice with vehicle, pLIVE, pLIVE-hDCN and pLIVE-hDCN^{E180Q} vectors. Our results reveal for the first time that overexpression of soluble decorin enhances the recruitment of F4/80+ macrophages to the tubulointerstitium of the obstructed kidney as well as in the contralateral kidney vis-à-vis vehicle and pLIVE injected animals (Fig. 2). This induction is even more elevated in both contralateral and obstructed kidneys when pLIVE-hDCN^{E180Q} is administered, a construct that encodes a decorin mutant with much lower binding affinity for collagen type-I (Kresse et al., 1997, Nareyeck et al., 2004), compared to pLIVE-hDCN-injected mice. These effects are not due to different expression levels of decorin and its mutant in the liver of the mice after overexpression of the respective plasmids (data not shown), but it is rather a consequence of an increase in the amount of circulating decorin caused by less efficient retention in the ECM. These observations strengthen the role of soluble decorin as a critical regulator in pro-inflammatory signaling. In this light, modification of decorin to disable its binding to TLR2/4 may serve as a more promising avenue in antifibrotic therapy.

6.2. Lumican and fibromodulin in renal diseases

Lumican and fibromodulin are keratan sulfate (KS)-containing proteoglycans, belonging to the class II SLRPs (Nikitovic et al., 2008, Nikitovic et al., 2014, Schaefer, 2011). Similar to biglycan and decorin, in normal kidney, lumican and fibromodulin are preferentially expressed in the tubulointerstitium and are weakly detected in the mesangial matrix (Schaefer et al., 2000, Schaefer, 2011).

In a UUO model in rats, lumican mRNA is up-regulated in kidneys together with proinflammatory *Ccl-2*, *Il-1 β* , *interferon regulating factor-1* (*Irf-1*) (Silverstein et al., 2003). In diabetic nephropathy, both lumican and fibromodulin are overexpressed at mRNA level in the renal cortex, in analogy to other SLRPs (Schaefer et al., 2001). Notably, while all SLRPs are overproduced in the glomerular and tubulointerstitial compartments at all stages of disease, glomerular protein accumulation does not occur unless the patients have advanced diabetic glomerulosclerosis (Schaefer et al., 2001). In these patients only lumican alone accumulates mainly within nodular formations and in the glomerular basement membrane (Schaefer et al., 2001). The absence of these proteoglycans in the diseased

glomeruli, even when there is clear upregulation of their mRNA, suggest that lumican and fibromodulin can be released in the bloodstream or urine as soluble forms (Schaefer et al., 2001).

Notably, lumican together with vaserin and retinol binding protein-4 have been identified in the plasma of patients with diabetic nephropathy as a biomarker (Ahn et al., 2010). Soluble lumican protein core binds lipopolysaccharide (LPS) and cluster of differentiation 14 (CD14) and potentiates the LPS-mediated proinflammatory effects in macrophages (Wu et al., 2007a). Lumican alone is not able to induce TNF- α in macrophages (Wu et al., 2007a); it is, however, conceivable that soluble lumican might have a potentiating role in biglycan-mediated renal disease progression. Fibromodulin has been reported to have therapeutic effects and decrease albuminuria in STZ-induced diabetic rats when a recombinant adenovirus expression vector containing fibromodulin gene was transfected (Maleki et al., 2014). Collectively, these studies suggest the potential coordinate role of several members of SLRP gene family during the progression of glomerulopathies and other fibrotic renal disorders.

7. Future perspectives

The past two decades of active research on the equilibrium between SLRP synthesis and degradation have demonstrated how these proteoglycans contribute to disease progression and how partially-processed fragments of SLRP protein cores can contribute to immunomodulation and fibrosis via interaction with several receptor species. Soluble, matrix-unbound biglycan can aggravate renal inflammatory diseases via TLR2/4 (Moreth et al., 2010, Moreth et al., 2014, Zeng-Brouwers et al., 2014). Therefore, blockade of soluble biglycan-mediated signaling through TLR2/4 could represent a novel strategy aim at ameliorating renal inflammation. On the other hand, the protease-released forms could represent end-products of ECM remodeling, and could thus serve as biochemical markers in disease progression (Karsdal et al., 2013). The advantage of this approach is that several SLRPs are produced in large amount and accumulate early in the disease process, rendering them amenable to early detection. For example, using the protein fingerprint approach (Karsdal et al., 2011), biglycan fragments can be detected by antibodies specifically raised to recognize neo-epitopes in several biological fluids including plasma (Genovese et al., 2013, Genovese et al., 2014). Thus, biglycan could serve as an indicator that directly reflects the renal physiological outcome. In conclusion, emerging studies strongly support the hypothesis that soluble biglycan is an initiator and regulator of inflammation in renal diseases, therefore underlining its dual role in both therapeutic development and in clinical diagnosis.

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Abbreviations

5/6-Nx	5/6-nephrectomized Sprague-Dawley rats
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
ALK	Activin like kinase
ATS	hymocyte serum
BGN (Bgn)	biglycan
BDL	bile duct ligation
BMP	bone morphogenic protein
CCL	chemokine (C-C motif) ligand
CCR	chemokine (C-C motif) receptors
CD	cluster of differentiation
CS	chondroitin sulfate
CsA	chronic cyclosporine
CXCL	chemokine (C-X-C motif) ligand
DAMP	damage-associated molecular pattern
DCN	decorin
DS	dermatan sulfate
ECM	extracellular matrix

ELISA	enzyme-linked immunosorbent assay
Erk	extracellular signal-regulated kinase
Fbn1	fibrillin-1
GAG	glycosaminoglycan
GrB	Granzyme B
HPF	high-power fields
HSP90	heat shock protein 90
Ig	immunoglobulin
IL-1β	interleukin-1 β
IL-6	interleukin-1-6
Irf-1	interferon regulating factor-1
IRI	ischemia-reperfusion injury
KS	keratan sulfate
LDL	low-density lipoprotein
LPS	lipopolysaccharide
LRP	low-density lipoprotein receptor-related protein
LRR	leucine-rich repeat
MMP	matrix metalloproteinase
MNS	Milan normotensive strain
Mpv17	mitochondrial inner membrane protein
MRL	Murphy Roths Large
MyD88	myeloid differentiation factor 88
NF-κB	nuclear factor- κ B
NLR	Nod-like receptor
NLRP3	NLR pyrin domain containing 3
NO	nitric oxide
PAI-1	plasminogen activator inhibitor-1
PDGF	platelet-derived growth factor
pLIVE	liver <i>in vivo</i> expression vector
RA	rheumatoid arthritis
ROS	reactive oxygen species
SLE	systemic lupus erythematosus

SLRP	small leucine-rich proteoglycan
SR-A	class A scavenger receptor
STZ	streptozotocin
TGF	transforming growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
TRIF	TIR-domain-containing adaptor-inducing interferon beta
UO	unilateral ureteral obstruction
VEGF	vascular endothelial growth factor
WISP-1	Wnt-1-induced secreted protein-1

Figure 1. Fate of biglycan in renal injury

Biglycan is synthesized at mRNA level in renal diseases by macrophages and renal resident cells in response to IL-1 β , IL-6, TNF- α and TGF- β , PDGF, respectively. The resulting biglycan binds to the ECM. During disease progression, the ECM might become saturated and unable to sequester the excess of biglycan. Consequently, synthesized biglycan is then released in the intercellular space in a soluble form. At the same time, proteinases can cleave the ECM-bound biglycan and then release soluble biglycan. In turn, soluble biglycan signals in both macrophages and renal resident cells. In macrophages, biglycan binds TLR2 and TLR4 and induces the production of TNF- α and pro-IL-1 β cytokines. By interacting with P2X₇ biglycan induces the assembly of the NLRP3 inflammasome and activation of Caspase-1, which, in turn, leads to maturation of IL-1 β . Biglycan induces as well the production of chemokines and recruitment of the respective immune cells via either the TLR2/TLR4/MyD88 or TLR4/TRIF pathways. In renal resident cells, biglycan induces the synthesis of the *Fbn1* gene and thus production of fibrillin-1.

At the same time, soluble biglycan and fragments are released in the bloodstream and might be directed to other organs or might be brought to the kidney via the circulation.

Abbreviations: *Bgn*, biglycan; *Fbn1*, fibrillin-1; IL-6, interleukin 6; IL-1 β , interleukin 1 β ; MyD88, myeloid differentiation primary response 88; NLRP3, NLR family, pyrin domain-containing 3; PDGF, platelet-derived growth factor; TGF- β 1, transforming growth factor- β 1;

TLR, toll-like receptor; TNF- α , tumor necrosis factor beta; TRIF, TIR-domain-containing adaptor-inducing interferon beta.

Figure 2. Effects of soluble decorin overexpression and its mutant pLIVE-DC^{E180Q} on renal interstitial influx of F4/80+ macrophages following 7 days UUO in C57BL/6 mice

(A) Representative immunohistochemical staining of F4/80+ macrophages (brown, some depicted by arrow) and (B) the respective quantification in obstructed and contralateral (control) kidneys from 8-week-old C57BL/6 mice transfected with vehicle, empty pLIVE vector, pLIVE-hDCN and pLIVE-hDCN^{E180Q} 3 days before UUO. Injection of pLIVE was performed as previously described (Zeng-Brouwers et al., 2014). UUO was performed as previously described (Babelova et al., 2009). Infiltrating F4/80+ cells were counted in 10 randomly selected non-overlapping high-power fields (HPF) (HPF, x200) of renal sections. Bar indicates 100 μ m. Sections were examined by two blinded observers. Data are given as mean $\pm$ standard deviation. Asterisks indicate significance between defined groups and asterisks above bars indicate significance versus respective control, * p < 0.05.

Abbreviations: DCN, decorin; HPF, high-power fields; pLIVE, liver *in vivo* expression vector; UUO: unilateral ureteral obstruction

Table 1
Biglycan abundance in the kidney and plasma in experimental and human renal diseases

Experimental model	Pathology	mRNA	Protein (kidney)	Protein (plasma)	References
<i>In vitro</i>					
Isolated glomeruli from Anti rat Thy-1.1 IgG-injected rats	Acute mesangioproliferative glomerulonephritis	+	/	/	(Schaefer et al., 1998)
<i>Animal models</i>					
Anti rat Thy-1.1 IgG-injected rats	Acute mesangioproliferative glomerulonephritis	+	+	/	(Keteler et al., 2000, Schaefer et al., 2003, Schaefer et al., 1998)
Renal transplantation	Chronic allograft dysfunction	+	+	/	(Bedke et al., 2007, Kiss et al., 2010, Wang et al., 2010)
LDL receptor-deficient mice	Diabetic nephropathy	+	+	/	(Thompson et al., 2011)
STZ-injected mice					
MNS rats	Focal glomerulosclerosis	/	+	/	(Schaefer et al., 1998)
5/6-Nx rats	Interstitial fibrosis	/	+	/	(Schaefer et al., 1998)
MRL/lpr	Glomerulohypertrophy, Glomerulosclerosis	+	+	+	(Moreth et al., 2010)
Han:SPRD rat	Lupus nephritis	/	+	/	(Schaefer et al., 1998)
IRI	Polycystic kidney disease	+	/	+	(Moreth et al., 2014, Wu et al., 2007)
CsA-induced nephrotoxicity	Renal ischemia	+	/	/	(Shihab et al., 2002, Shihab et al., 2000)
UUO mouse model	Tubulointerstitial fibrosis	+	+	/	(Leemans et al., 2009, Merline et al., 2009, Schaefer et al., 2002, Schaefer et al., 2004)
<i>Human</i>					
Acute renal allograft rejection		/	+	+	(Moreth et al., 2010)
Amyloidosis		/	+	/	(Stokes et al., 2000)
Crescentic glomerulonephritis		+	+	/	(Stokes et al., 2001)
Diabetic nephropathy		+	+	+	(Moreth et al., 2010, Schaefer et al., 2001, Stokes et al., 2000)
Fibrillary glomerulonephritis		+	/	/	(Stokes et al., 2000)
Idiopathic mesangial sclerosis		+	/	/	(Stokes et al., 2000)
IgA nephropathy		+	+	/	(Ebefors et al., 2011, Kuroda et al., 2004)
Immunotactoid glomerulopathy		+	/	/	(Stokes et al., 2000)

Experimental model	Pathology	mRNA	Protein (kidney)	Protein (plasma)	References
Ischemia reperfusion injury in renal transplantation		+	/	/	(Kruger et al., 2009)
Light-chain deposition disease		+	+	/	(Stokes et al., 2000)
Lupus nephritis		/	+	+	(Moreth et al., 2010)
Membranous nephropathy		+	+	/	(Kuroda et al., 2004, Schaefer et al., 2000)
Nephrosclerosis		+	+	/	(Stokes et al., 2000)

+increased levels; /: no data available.

Abbreviations: 5/6-Nx, 5/6-nephrectomized Sprague-Dawley rats; CsA, cyclosporin; MNS, Milan normotensive strain; IRI, ischemia reperfusion injury; LDL, low-density lipoprotein; STZ, streptozotocin; UUO, unilateral ureteral obstruction

Table 2
Alteration of proteinases involved in the generation of biglycan fragments in association with renal diseases

Enzyme	Cleavage site on biglycan	Proteinases expression/ activity in renal pathologies	References
<i>Metalloproteinase</i>			
ADAMTS-4	LRNMN ¹⁸⁶ C ¹⁸⁷ IEMG	Protein level is detectable in plasma in chronic renal failure (not present in healthy individuals)	(Grgurevic et al., patent US8263072 B2)(Melching et al., 2006)
ADAMTS-5	LRNMN ¹⁸⁶ C ¹⁸⁷ IEMG	-	(Melching et al., 2006)
BMP-1	GPFMN ³⁷ D ³⁸ EEASGADT	Increased enzyme activity in plasma in renal fibrosis	(Grgurevic et al., 2011, Scott et al., 2000)
MMP-2	-	Increased expression in Alport Syndrome, focal segmental glomerulosclerosis, lupus nephritis and Thy1.1 nephritis Gene downregulation in diabetic nephropathy	(Binder et al., 1999, Del Prete et al., 1997, Nakamura et al., 1995, Rao et al., 2003, Reuter et al., 1998, Steinmann-Niggli et al., 1998, Weiher et al., 1990)
MMP-3	SE ¹¹⁴ L ¹¹⁵ RK	Increased expression in Alport Syndrome, diabetic nephropathy and lupus nephritis	(Nakamura et al., 1995, Rao et al., 2005, Stegemann et al., 2013, Suzuki et al., 1997)
MMP-8	-	Genetic depletion protects mice from IRI	(Vandenbroucke et al., 2012)
MMP-9	SE ¹¹⁴ L ¹¹⁵ RK	Increased expression in Alport Syndrome and membranous glomerulonephritis Inhibition attenuates Thy1.1 nephritis	(McMillan et al., 1996, Rao et al., 2003, Stegemann et al., 2013, Steinmann-Niggli et al., 1998)
MMP-12	-	Enhanced expression in Alport Syndrome	(Rao et al., 2006)
MMP-13	SE ¹¹⁴ L ¹¹⁵ RK RKVPKG ¹⁷⁷ V ¹⁷⁸ FSGLRN	Esculetin treatment upregulates MMP-13 mRNA synthesis thus attenuating progression of diabetic nephropathy	(Monfort et al., 2006, Stegemann et al., 2013, Surse et al., 2011)
MMP-14	-	Increased expression in Alport Syndrome Increased active form in IRI	(Covington et al., 2006, Rao et al., 2003)
<i>Other protease</i>			
GrB	ISPD ⁹¹ L-T ⁹² TLLDLQNN	GrB-expressing lymphocytes infiltrate the kidney allografts in acute renal graft rejection	(Boivin et al., 2012, Kummer et al., 1995)

Abbreviations: GrB: Granzyme B; IRI: ischemia reperfusion injury; MMP: matrix metalloproteinase; Mpv17: mitochondrial inner membrane protein