Matrix Metalloproteinases in Renal Development and Disease

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Remodeling of extracellular matrix (ECM) is an important physiologic feature of normal growth and development. Many diseases have been associated with an imbalance of ECM synthesis and degradation, which may result in an accumulation of ECM molecules (1). In the kidney, these processes can lead to interstitial fibrosis, which has been reviewed elsewhere (2–4), and glomerulosclerosis (5). Progressive glomerulosclerosis leading to end-stage renal failure is a complication of a variety of diseases, such as diabetes mellitus, glomerulonephritis, focal sclerosis, chronic transplant rejection, or obstruction (6–11). Although many studies have tried to elucidate the pathogenesis, the exact underlying mechanisms are still not completely understood. While increased synthesis of ECM certainly plays an important role, recent studies have focused on the role of degradative systems. The major physiologic regulators of ECM degradation in the glomerulus are matrix metalloproteinases (MMP) (12). Both glomerular disease and normal renal development are characterized by a high rate of ECM turnover. Since the underlying mechanisms may follow similar patterns, we will discuss the role of MMP in chronic glomerular disease and normal renal development, and we will highlight implications of experimental findings for human renal disease.

Structure and Function of MMP

MMP are a large family of zinc-dependent matrix-degrading enzymes, which include the interstitial collagenases, stromelysins, gelatinases, elastases, and secreted, as well as membrane-type, RXKR containing MMP (13–18) (Table 1). They have been implicated in invasive cell behavior (18,19), embryonic development (20–22), interstitial fibrosis (23), and glomerulosclerosis (24,25). MMP share several structural and functional properties, which include pre/pro-peptide (26), hinge, hemopexin-like (except MMP-7), and catalytic zinc-binding domains (27,28) (Figure 1). They synergistically degrade a broad range of ECM compounds. Of the currently known MMP, MMP-1, MMP-13, MMP-3, MMP-2, MMP-9, and MT-1-MMP have been extensively studied in the kidney. Four members in this group of collagenases, MMP-1, MMP-2, MMP-8 and MMP-13, share the ability to degrade fibrillar collagens (29–31). The resulting fragments denature spontaneously under physiologic temperatures, which then can be further degraded by other proteases.

There are two gelatinases, MMP-2 and MMP-9. While sharing the ability to degrade both basement membrane collagens and gelatins, their substrate specificity is not identical. MMP-2 degrades fibronectin and laminin, and has significantly less activity against types IV and V collagen than MMP-9 (32–34). They also vary greatly in their promoter structure, and thus in their expression pattern (35). MMP-2 and MMP-9 are also unique in that they form proenzyme complexes with their endogenous inhibitors tissue inhibitor of metalloproteinase-2 (TIMP-2) and TIMP-1, respectively (36–39), which have been shown to be expressed in human glomeruli (40). Although the roles of these complex formations in ECM turnover have not been completely elucidated, they may at least in part account for the selective inhibition of MMP-2 by TIMP-2. Other possible roles of complex formation include regulation of cell surface localization of MMP via integrin receptors such as αvβ3 (41) (vide infra), mediation of the cell surface activation of MMP by MT-1-MMP (26,42,43), as well as regulation of MMP (44) and/or TIMP (45,46) cell growth-modulating activity. However, the growth-regulating activity of MMP and TIMP in renal development and disease has not been directly demonstrated.

Stromelysin-1 and stromelysin-2 share almost identical substrate specificity, but, like the gelatinases, are differentially regulated. With the exception of human T lymphocytes, stromelysin-2 is constitutively expressed at low levels or is absent, while stromelysin-1 can be induced by the cytokines interleukin-1β and tumor necrosis factor-α, the growth factors epidermal growth factor and platelet-derived growth factor, and phorbol esters, and inhibited by transforming growth factor-β (TGF-β) and retinoic acid (35).

Members of the RXKR group share the recognition motif that consists of the highly basic sequence of amino acid residues arginine,arginine/glutamine,lysine,arginine. This sequence of residues is recognized by the Golgi-associated proteinase furin, which has been implicated in the activation process of these MMP (14,43). The membrane-type MMP contain a transmembrane COOH-terminal end, and they have been shown to play a role...
in the regulation of proenzyme processing of the pro-MMP-2/TIMP-2 complex (42,43).

**MMP in Glomerular Disease**

**Mechanisms of Action**

The major physiologic regulators of ECM degradation in the glomerulus are MMP (12). A balance between ECM synthesis and degradation is a prerequisite for maintaining the structural and functional integrity of the glomerulus. Thus, changes in MMP expression or activity will directly translate into altered ECM turnover, which may lead to glomerular scarring and a decline in renal function.

Many forms of glomerular disease are characterized by a change in cellularity, which in turn may affect ECM composition and turnover. MMP have been shown to influence the behavior of glomerular cells either directly or via the generation of ECM cleavage products (44,47).

MMP may also indirectly influence ECM turnover via the regulation of certain growth factors. Recently, regulation of growth factor activity by MMP has been observed in MMP-9-deficient mice, which exhibit abnormal growth plate vascularization and ossification (48), and binding proteins for insulin-like growth factor-1 have been identified as substrates of MMP (49).

Finally, in diseases with altered matrix composition, such as Alport disease, an increased susceptibility of the basement membrane to degradation by MMP has been postulated to cause glomerular damage (50). These findings illustrate that MMP are involved at several levels of ECM turnover, and thus play a crucial role in maintaining the balance between ECM synthesis and degradation.

**MMP in Noninflammatory Glomerular Disease**

The role of MMP and their endogenous inhibitors in the development of glomerular damage has been studied in a variety of experimental conditions (Figure 2). In general terms, a downregulation of MMP has been associated with progression in noninflammatory diseases such as hypertensive glomerulosclerosis (51,52), growth hormone excess (25,53), hydronephrosis (54–56), hypercholesterolemia (57), heroin nephropathy (58), cyclosporine nephrotoxicity (59), or sex-related changes in the aging kidney (60). In this review, we chose diabetic nephropathy as one example to illustrate the link between progressive glomerulosclerosis and MMP expression.

Diabetic nephropathy has been studied extensively, as it is the leading cause of end-stage renal disease in the United States, Japan, and Europe (61–64). Histologically, glomerular hypertrophy, glomerular basement membrane thickening, and mesangial expansion characterize diabetic glomerulosclerosis (51,52), growth hormone excess (25,53), hypercholesterolemia (54–56), hypercholesterolemia (57), heroin nephropathy (58), cyclosporine nephrotoxicity (59), or sex-related changes in the aging kidney (60). In this review, we chose diabetic nephropathy as one example to illustrate the link between progressive glomerulosclerosis and MMP expression.

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diabetic nephropathy showed an inverse correlation between MMP-3/TIMP-1 mRNA levels and matrix accumulation (74). In addition, a marked decrease in MMP-2 mRNA expression was detected in glomeruli of diabetic patients (75). Taken together, these findings suggest that matrix accumulation in diabetic nephropathy is due to increased matrix synthesis and decreased degradation, and that MMP may play a pivotal role.

The mechanisms underlying this process have not yet been completely elucidated. Both circulating and/or locally synthesized growth factors and cytokines modulate MMP expression in diabetic nephropathy. We have shown that insulin-like growth factor-1 decreases MMP expression, and thus down-regulates collagen degradation in mesangial cells derived from diabetic NOD mice (76,77).

The role of TGF-β in the pathogenesis of ECM accumulation remains controversial. TGF-β has been shown to upregulate MMP-2 (78). In a recent study in db/db mice, TGF-β1 levels in diabetic kidney cortex have been shown to be decreased (79). It is not clear how this finding relates to previous observations of increased TGF-β levels in diabetic nephropathy (80,81). The role of TGF-β1 and TGF-β-neutralizing proteoglycans (82) in the pathogenesis of diabetic nephropathy may have to be reevaluated (83).

Oxidative stress (84) contributes to matrix accumulation either directly due to inhibition of MMP-2 (85), or by inducing a cytokine response (86,87).

The detrimental role of hyperglycemia itself has been established by the observation that glycemic control can prevent or at least slow progression of diabetic nephropathy in a number of patients (88). This may be explained in part by the observation that decreased gelatinase activity in STZ-treated rats can be restored by insulin treatment (89). Glucose levels also may indirectly influence matrix turnover, since glycated fetal bovine serum was shown to decrease matrix turnover by glomerular epithelial cells (90), and glycated type IV collagen has been shown to be less degradable by MMP-3 and MMP-9 (91). The need for tight glycemic control in diabetes is emphasized by a recent study showing that pancreas transplantation can reverse glomerular lesions in patients with diabetic nephropathy (92), even though other intrinsic factors that have not yet been identified may play a role in this setting.

In addition, the genetic background may have a strong influence on the incidence of renal complications in diabetes (93), and the level of MMP expression may be one of the determinants. STZ-treated Sprague Dawley rats developed diabetic nephropathy and exhibited decreased metalloproteinase levels. These nephropathy changes and altered MMP expression may have to be reevaluated (83).

In summary, progressive glomerulosclerosis in a variety of diseases, such as diabetic nephropathy, is characterized by a profound shift in ECM turnover toward increased matrix accumulation, leading to mesangial matrix expansion, decrease in filtration area, and ultimately end-stage renal failure. Glomerular MMP levels could determine the slope of progression and the degree of glomerular scarring. Experimental data suggest

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**Figure 1.** Domain structure of the matrix metalloproteinases (MMP). (A) Simplified schematic of the minimal domain structure of the MMP. Most MMP have the same basic domain structure consisting of signal (pre-), pro-, and catalytic domains. The prodomain contains a highly conserved sequence (shown in single letter amino acid code) that is responsible for maintaining the latency of the proenzyme. (B) Additional domains observed in various members of the MMP family. The F denotes location of the furin RXKR cleavage site of MMP-11 and the membrane-type MMP. The FBN represents the type II repeats of fibronectin found in the gelatinases. C represents a type V collagen-like sequence found in MMP-9. H denotes the flexible hinge region that usually separates the catalytic and hemopxin-like (PEX) domains. TM represents the transmembrane domain characteristic of the membrane-type (MT)-MMP.

**Figure 2.** Schematic overview of glomerulosclerosis disease progression. This diagram compares and contrasts the disease process associated with both inflammatory mechanisms and noninflammatory mechanisms that can result in glomerulosclerosis.
that this is a general theme in a variety of primarily noninflammatory diseases associated with glomerulosclerosis.

MMP in Glomerulonephritis

In inflammatory glomerular diseases, the findings are quite different with regard to MMP expression. Increased levels of MMP are generally associated with disease activity and influx of inflammatory cells, and both the level and the duration of MMP elevation determine the extent of glomerular damage.

In mice with experimental lupus nephritis, MMP-1, -2, and -3 were shown to be increased (94), while an elevation of MMP-2 and MMP-9 was observed in both anti-Thy1.1 nephritic rats (78,95,96) and rats with passive Heymann nephritis (97). Increased MMP expression in Thy1.1 nephritis may be partly mediated by growth factors released by invading activated neutrophils and macrophages (98,99). Another possibility is a permanent change in the phenotype of mesangial cells due to the inflammatory process, since mesangial cells isolated from nephritic animals maintain their altered phenotype, partly mediated by growth factors released by invading activated neutrophils and macrophages (98,99). Another possibility is a permanent change in the phenotype of mesangial cells due to the inflammatory process, since mesangial cells isolated from nephritic animals maintain their altered phenotype in vitro (100). Data from human studies also show that elevated MMP levels are associated with a highly active inflammatory response associated with glomerular damage. Elevated levels of MMP were found in various forms of glomerulonephritis in Japanese patients (101,102).

Taken together, experimental data and findings from human studies suggest that elevated glomerular MMP expression contributes to the disease process, and that increased MMP activity correlates with structural glomerular damage. Unlike experimental Thy1.1 nephritis in rats, which resolves spontaneously when only one dose is given, many forms of glomerulonephritis in humans progress to end-stage renal failure. In experimental nephritis, both dietary measures (103) and MMP inhibition attenuate glomerular lesions (96), suggesting a role for MMP inhibitors in the treatment of acute glomerulonephritis (96,104,105). Thus, future therapeutic approaches may include targeting selected members of the MMP family and/or their endogenous inhibitors. Additional studies will be needed, however, to establish the efficacy of this measure in preventing progressive renal failure.

The most common form of nephrotic syndrome in adults is membranous glomerulonephritis. A role for MMP in the alteration of basement membrane permeability has been postulated by an uncontrolled study in patients with membranous glomerulonephritis, which revealed elevated levels of TGF-β and collagenase activity in the urine (106). This notion is supported by the finding that MMP-9 expression by podocytes is increased in a model of membranous nephropathy (97). In a mouse model mimicking focal segmental glomerulosclerosis, characterized by the lack of Mpv17 gene expression leading to persistently high levels of MMP-2, the authors found foot process flattening, heavy proteinuria, and ultimately severe glomerulosclerosis (107–109). However, the glomerular expression pattern of MMP and TIMP has not yet been studied in detail. In addition, the overexpression of MMP-2 occurs from the first days of life on, which makes it difficult to correlate this model with human disease. Future approaches may use conditional knockouts or the conditional overexpression of MMP and/or TIMP to further define the role of MMP in glomerulonephritis.

MMP in Renal Development

Both renal disease and kidney development are characterized by a high rate of ECM turnover, which may indicate that similar mechanisms are at work. During renal development, a constant remodeling of ECM is required to allow invasion and branching of the ureteric bud in the metanephric mesenchyme, which suggests a role for matrix-degrading enzymes. Nephrogenesis is characterized by a complex interaction between epithelium and mesenchyme (21,110–112). Since a differential spatiotemporal expression of MMP and TIMP has been shown in the development of other branching organs (113), a similar role of MMP and TIMP in renal organogenesis has been proposed (114,115). In the developing kidney, MMP-2 mRNA expression is limited to the mesenchyme. MMP-2 protein, however, has been found in immature nephron structures underdetermined epithelial differentiation, where it colocalizes with MT-1-MMP, TIMP-2, and TIMP-3 (116,117). This has given rise to the hypothesis that the interaction between MMP-2, MT-1-MMP, and TIMP-2 may be crucial for the interaction between mesenchyme and epithelium, facilitating branching of the developing ureteric bud. This hypothesis is supported by the fact that treatment with MT-1-MMP antisense oligodeoxynucleotides inhibits the branching process (118), and that the expression of MMP-2 and MT-1-MMP is regulated in a temporal manner (117). A recent study showed that inhibition of MMP-2 by TIMP-2 strongly reduced the number of branches in the rat metanephros (119). It is unclear why only treatment with an anti-MMP-9 antibody prevented ureteric bud branching in 11-d mouse kidneys, while an anti-MMP-2 antibody did not have a deleterious effect (22). Analysis of the kidney morphology in MMP-2- and MMP-9-deficient mice may help to clarify the role of the two gelatinases in renal organogenesis (48,120). However, other MMP, such as MMP-1, have also been shown to play a role in the branching morphogenesis and may be modulated by growth factors such as TGF-β (121). Thus, MMP seem to play an important role in epithelium–mesenchyme interactions in renal development and the determination of nephron number. A decrease in the total number of nephrons has been postulated to play a role in the development of chronic renal disease later in life (122–124). Conditions known to reduce nephron number, such as malnutrition (125) or gentamicin nephrotoxicity (126), may be mediated by decreased fetal MMP expression.

In summary, the tight regulation of the MMP system is essential for normal renal development. Although it may be possible to counteract MMP dysregulation pharmacologically, additional studies will be needed to elucidate the regulation of MMP expression in the developing kidney under normal and pathologic conditions, so that the appropriate time and type of intervention can be determined.

Conclusion

Mounting evidence indicates that MMP play a pivotal role in the regulation of ECM turnover in the glomerulus. Various
forms of glomerular disease are characterized by a profound shift in the balance between matrix synthesis and degradation. While in the scarring process the balance is tilted toward increased synthesis, excess degradative activity promotes glomerular destruction in inflammatory diseases. Recent studies show that MMP are also intricately involved in renal organogenesis. Disturbances in kidney development may in part be linked to a dysregulation of MMP expression during embryogenesis and may have profound implications for renal disease in adult life. Future studies to better understand the underlying genetic and molecular regulation may lead to pharmacologic manipulations of MMP activity or expression to treat and/or prevent glomerular diseases.

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