Role of matrix metalloproteinases in renal pathophysiology

J. M. Catania, G. Chen, and A. R. Parrish

Department of Systems Biology and Translational Medicine, College of Medicine, Texas A&M Health Science Center, College Station, Texas

MATRIX METALLOPROTEINASES (MMPs) are zinc-containing endopeptidases that are involved in remodeling the extracellular matrix (ECM) and are crucial for tissue development and homeostasis. All MMPs are multidomain enzymes, generally consisting of a prodomain, a catalytic domain, a hinge region, and a hemopexin-like domain. Most MMPs are secreted as proMMPs; activation usually requires cleavage of the prodomain by plasmin or other MMPs (88). The propeptide sequence contains a conserved cysteine switch (PRCGXP) to stabilize and inhibit the catalytic zinc ion, which is bound in a conserved HEXGHXXGXXH motif (53). The hemopexin-like domain is characterized by a “propeller” of four twisted β-sheets, which are thought to confer substrate recognition (53). Together, the hinge region and the hemopexin-like domain unravel substrate proteins for subsequent degradation. Originally thought to cleave only ECM proteins, this family of enzymes is now known to cleave a number of non-ECM substrates as well. For example, cell adhesion molecules (cadherins and integrins) and both growth factors and their receptors (TGF-β, FGF-R1) are targeted by MMPs (74).

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous, specific inhibitors that bind and inhibit MMPs. Four TIMPs (TIMP-1–4) have been identified in vertebrates; these proteins share a similar structure which fits into the active site of the MMP catalytic domain. TIMPs inhibit all MMPs, except TIMP-1, which does not inhibit MMP-14, -16, or -24 (88). A number of other MMP inhibitors have been identified, including RECK (81), α2-macroglobulin (4), and tissue factor pathway inhibitor-2 (33).

MMPs are classified into six groups based on substrate and sequence homology: collagenases, gelatinases, stromelysins, matrilysins, membrane-type matrix metalloproteinases and “other MMPs” (Fig. 1). Collagenases [MMP-1, -8 (neutrophil collagenase), -13, and -18 (Xenopus laevis collagenase)] cleave collagens I, II, and III at a specific site, generating two fragments. Gelatinases [MMP-2 (gelatinase A) and -9 (gelatinase B)] cleave the denatured collagens (gelatin) and laminin, as well as some chemokines. MMP-2 can also activate MMP-1 and MMP-9 by cleaving their prodomains (85). Stromelysins [MMP-3 (stromelysin-1) and -10 (stromelysin-2)] degrade a variety of substrates, including collagen, fibronectin, laminin, gelatin, and casein, and can cleave the prodomain of a number of other MMPs (57). Matrilysins [MMP-7 (matrilysin-1) and -26 (matrilysin-2)] are characterized by the lack of a hemopexin domain (24). MMP-7 degrades not only ECM components but also a large number of cell-surface molecules, such as E-cadherin and pro-α-defensin (69). MMP-26 contains a cysteine switch that is unique among MMPs; this protein digests a number of ECM proteins and has a role in the activation of MMP-9 (91). Membrane-type MMPs (MT-MMPs; MMP-14, -15, -16, -17, -24, and -25, also called MT1-, MT2-, MT3-, MT4-, MT5-, and MT6-MMP, respectively) are structurally similar to the other classes of MMPs but are anchored to the exterior of the cell membrane by one of two mechanisms. MMP-14, -15, -16, and -24 contain a transmembrane domain and a short cytoplasmic tail, while MMP-17 and -25 are anchored to the cell membrane by a glycosylphosphatidylinositol residue (34). Unlike the soluble MMPs, MT-MMPs exhibit significant differences in inhibition by TIMPs; TIMP-1 does not inhibit MMP-14, -16, and -24 (9, 93). The remaining MMPs, which do not easily fit into any of the above categories, are typically expressed in a single tissue or cell type, or are expressed only during specific events (48, 88). This group, often referred to as “other MMPs,” includes MMP-11, -12, -19, -20, -21, -22, -23a, -23b, and -28.

Address for reprint requests and other correspondence: A. R. Parrish, Systems Biology and Translational Medicine, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114 (e-mail: parrish@medicine.tamhsc.edu).
Renal MMPs

The spatial expression of MMPs and TIMPs in the kidney is complex and has not been completely characterized. However, MMP-2, -3, -9, -13, -14, -24, -25, -27, -28 and TIMP-1, -2, and -3 are all expressed in the kidney (Fig. 2). MMP-2 is expressed in the collecting duct in the rabbit (62), the glomerulus and proximal tubules of rats (39, 71, 84), and in the proximal and distal tubules in the monkey (60). MMP-3 is not detected in the proximal tubules of rats (71) but is expressed in the proximal and distal tubules of the monkey (60), as well as in the glomerulus and some tubular epithelial cells in humans (79). The expression of MMP-9 appears to be mainly confined to the glomerulus (39, 46, 55, 71), although there are reports of expression in the proximal and distal tubules of the monkey (60) and in the rabbit collecting duct (62). MMP-13 and -14 are localized in the glomerulus in rats (84); however, these studies investigated mRNA expression in isolated glomeruli and did not investigate expression along the entire nephron. MMP-14 is also expressed in distal tubules in a mouse model of polycystic kidney disease (PKD) (59). MMP-7 is not detected in normal human renal tubular epithelium but is significantly upregulated in distal tubules in a number of pathological states in mice and humans (77). MMP-24 is also expressed in tubular epithelial cells along the nephron of human kidney (49, 68). mRNA for MMP-24, -25, -27, and -28 has also been detected in the rat kidney (7). In mouse kidney, TIMP-1 is expressed at low levels, TIMPs-2 and -3 at high levels, and TIMP-4 is not expressed (28). TIMP-1 is expressed in the glomerulus in rats (84) and humans (79, 80). In addition, TIMP-3 is detected in the rat kidney (89), and human glomeruli express TIMP-1 and -2 mRNA (15). Taken together, MMP and TIMP expression in the kidney is complex and may be species dependent; importantly, the localization of these proteins has not yet been completely elucidated.

MMPs in Acute Kidney Injury

Over a decade ago, Bonventre (11) postulated a role for proteolysis of tubular cells during acute ischemia-reperfusion injury in the kidney. Although a detailed analysis of proteinases during acute kidney injury is rather limited, a role for MMPs is emerging, particularly in ischemia-reperfusion injury (Fig. 3). Although 30 mg/kg of BB-94, a broad spectrum MMP inhibitor, did not attenuate increased serum creatinine associated with ischemia-reperfusion injury (92), the same dose significantly attenuated proteinuria in an experimental model of acute kidney allograft rejection (29). MMP-9 levels increased during rejection, while MMP-2 levels decreased (29); this is similar to other reports of increased proMMP-2 but decreased active MMP-2 during rejection (47). Interestingly, increased proMMP-1 was seen in a study of 30 human patients with acute transplant rejection (65). A role for MMPs in ischemia-reperfusion injury is supported by several studies, including the finding that MMP-2 and -9 were increased in renal tubules and the interstitium following 52 min of ischemia in rats (5). In rats, 30 min of ischemia and 60 min of reperfusion were associated with increased expression of MMP-2 and -9 and TIMP-2 in glomeruli, and a decrease in TIMP-1 (17). Degradation of the tight junction protein zonula occludens-1 in the glomerulus was linked to the increased activity of MMP-9. In an in vitro model of ischemic injury, our laboratory has shown that cadherin
cleavage in tubular epithelial cells is mediated by MMPs (21), specifically MMP-14 (22).

Recent data also suggest a role for MMPs in endothelial injury during ischemia-reperfusion injury. Following ischemia-reperfusion, increased MMP-9 activity was associated with degradation of occludin in endothelial cells, which was postulated to increase vascular permeability (16). This hypothesis is supported by the finding that minocycline, a tetracycline MMP inhibitor, and ABT-518, a specific inhibitor of MMP-2 and -9, attenuated increased microvascular permeability following ischemia-reperfusion (78). Taken together, these data support the hypothesis that MMPs mediate acute kidney injury and are involved in changes in the vascular endothelium, glomeruli, and tubular epithelial cells. Cell adhesion molecules have been identified as critical targets of MMP in several of these studies, consistent with the increased vascular and tubular permeability characteristic of acute kidney injury.

**MMPs in Chronic Kidney Disease**

Glomerulosclerosis/tubulointerstitial fibrosis. The unilateral ureteral obstruction model has provided insight into the molecular mechanisms involved in the development of renal fibrosis, including the role of MMPs and TIMPs (Fig. 3). In this model, there is an early increase in MMP-2 expression and activity (37, 72) and decreased MMP-1 and -9 activity (31). In addition, the model is characterized by an increase in TIMP expression (72), specifically TIMP-1 (26). Interestingly, TIMP-1−/− mice are not more susceptible to the development of fibrosis following unilateral ureteral obstruction, possibly due to a compensatory increase in TIMP-3 (43). A role for MMPs in glomerulosclerosis and tubulointerstitial fibrosis is also demonstrated in several other experimental models. Decreased MMP-9 expression was correlated with the development of tubulointerstitial fibrosis and glomerulosclerosis in rats (10, 52) and mice (86, 87). Eight weeks after ischemia-reperfusion injury in the rat, MMP-2 gene levels were increased, but fell at 16 and 24 wk, when increased TIMP-1 expression was observed (40). Increased TIMP-1 expression following ischemia-reperfusion injury was also observed by Basile et al. (6). These results suggest increased ECM turnover following injury, but reduced degradation favoring the development of tubulointerstitial fibrosis at later time points. In spontaneously hypertensive rats, levels of cortical MMP-2 and MMP-9 are decreased, while the activity of medullary MMP-7 and -9 is increased, suggesting a role for MMPs in hypertensive nephropathy (13). An exciting recent development is the production of a transgenic model in which renal proximal tubular cells overexpress MMP-2. These mice recapitulate human chronic kidney disease (CKD), including tubular atrophy, glomerulosclerosis, and tubulointerstitial fibrosis, providing compelling evidence for a role of MMP-2 in CKD (19).

These data are interesting as most work on CKD has focused on the relationship between decreased MMP activity and increased ECM deposition; however, these data suggest that overexpression of a single MMP may be a critical mediator of CKD, but the critical targets of MMP-2 mediating these effects remain undefined. The relationship between MMP activity and inflammation remains an important component of CKD that remains to be addressed.

Over a decade ago, increased TIMP-1 and -2 expression was found to be associated with glomerulosclerosis in humans (15). Urinary levels of TIMP-1 were increased in patients with CKD (n = 54) vs. controls (n = 176) (36). The expression of MMP-13 and -14 is elevated in chronic renal inflammation and/or fibrosis in the human kidney (32). Plasma samples from 60 CKD patients had elevated MMP-2 and decreased MMP-9 activity compared with 40 controls; increased serum creatinine concentrations correlated with MMP-2 expression and inversely correlated with MMP-9 expression (18). The relative contribution of the kidney, as well as other cellular/organ systems, to the altered MMP expression patterns in both the plasma and urine is, at this time, unclear.

**Chronic Allograft Nephropathy**

A rat model of chronic allograft nephropathy (CAN) showed decreased TIMP-3 and increased proMMP-2 and -9 and active MMP-2 (39) (Fig. 3). BAY 12–956, an inhibitor of MMP-2, -3, and -9, attenuated CAN in a rat model when administered early (first 10 days following transplantation); however, disease progression was exacerbated if administered late (weeks 12–20) (50). Therefore, attention must be paid to the temporal relationship between MMP inhibition and therapeutic outcome. In CAN patients, serum proMMP-2 and -3 are increased (65); however, in biopsies of CAN patients, MMP-2 was decreased (61).

**Diabetic Nephropathy**

A number of studies have demonstrated a link between aberrant MMP expression and the progression of diabetic nephropathy in animal models (Fig. 3). The expression of MMP-2 is decreased before overt structural changes in a streptozocin (STZ)-induced diabetes (type 1) model (90); however, increased expression of MMP-2, -9, and -14 was seen in Goto-Kakizaki rats (type 2) before the development of glomerulosclerosis and tubulointerstitial fibrosis (63). Diabetic ne-
phrophathy is also associated with decreased expression of MMP-2 (38, 54) and decreased expression and activity of MMP-9 (54), while a more recent study suggests an increase in TIMP-2 expression and activity (31a). These increases in MMP inhibition, as well as decreased MMP activity, are consistent with increased ECM deposition in diabetic nephropathy. However, in a rat model of lithium-induced diabetes insipidus, downregulation of TIMP-3 was observed (67). Glomerular MMP-14 expression is decreased in STZ-induced diabetes (12). A number of potential interventions to attenuate diabetic nephropathy involve increasing MMP activity. For example, in a STZ model of diabetic nephropathy, MMP-1 gene delivery via microspheres implanted under the renal capsule reduced collagen content and attenuated increased blood urea nitrogen levels (2). Hepatocyte growth factor attenuates diabetic nephropathy in rats, putatively via inhibition of TIMP-1 expression (23). Several interventions that attenuate diabetic nephropathy are related to increased MMP-2 activity, including the angiotensin-converting enzyme inhibitor benazepril (76), 17β-estradiol (51), and the peroxisome proliferator-activated receptor-γ agonist pioglitazone (66).

A role for MMPs in diabetic nephropathy has also been demonstrated in several clinical studies. In a study of 35 diabetic patients, TIMP-1 expression was elevated in serum and urine; urinary TIMP-1 levels correlated with increased urine albumin (42). Glomerular MMP-2 expression was decreased in 16 patients with diabetic nephropathy compared with the 5 normal controls (25). Importantly, increased MMP-9 in the plasma precedes the development of microalbuminuria in patients without changes in either MMP-1 or TIMP-1 levels (27). This finding is supported by more recent data demonstrating that urinary levels of MMP-9 are elevated in type 2 diabetic patients, and correlated with albuminuria (83). In human diabetic patients, expression of MMP-24 correlated with tubular atrophy (68). In addition, polymorphisms in TIMP-3 have been associated with diabetic nephropathy in type 1 diabetes (30).

PKD

Several lines of evidence suggest alterations in MMPs/TIMPs may be involved in the pathogenesis of PKD (Fig. 3). Early studies demonstrated a decrease in tubular expression of MMP-2 in a rat model of PKD (71); however, elevated levels of MMP-2 and -9 are synthesized and secreted by cultured kidney tubules derived from polycystic C57BL/6J-CPK mouse model (64). MMP-14 is localized to cysts in a rat model of PKD (59). In patients with PKD (16 patients), serum levels of MMP-1 and -9 and TIMP-1 were increased compared with controls (20 patients) (58).

Renal Carcinoma

A link between renal cell carcinoma (RCC) and overexpression of MMPs was suggested about a decade ago when the ratio of MMP-2 and -9 to TIMP-1 and -2 was shown to be elevated in RCC (45) (Fig. 3). Since this finding, RCC has been linked to overexpression of MMP-14, -15, and -16 (43) and MMP-1, -2, -3, and -9 (8). Perhaps depending on the methodology of the assay, RCC has also been associated with increased MMP activity in the urine (73), although others saw no significant difference between urinary MMP activity in 15 cases of RCC and 47 healthy volunteers (14). While MMP overexpression is clearly associated with RCC, the use of urinary MMP activity as a marker remains to be fully defined.

MMP overexpression may be linked to tumor progression, as suggested by a number of recent studies. MMP-2 and TIMP-1 were simultaneously transfected into a mouse RCC line, and clones with various MMP-2:TIMP-1 ratios were selected (56). The metastasis potential of these cells in mice was higher in clones with a higher MMP-2:TIMP-1 ratio. Expression of MMP-2 (1, 41, 75, 82), MMP-7 (74), and MMP-9 (20) correlates with advanced stage and poor prognosis in RCC. Interestingly, several MMP polymorphisms have been linked to RCC. Polymorphisms in both MMP-1 and -3 are also associated with increased risk of developing RCC (35). A polymorphism in MMP-9 is not associated with increased risk of RCC, but is correlated with malignancy potential (3). Taken together, these data suggest overexpression of MMPs is associated with RCC and that MMPs may play a role in the progression of RCC.

Summary

Studies over the past decade have linked aberrant MMP and/or TIMP expression to a number of renal pathophysiological, both acute and chronic. While tremendous progress has been made in this area, a number of issues remain to be resolved. The precise localization of these proteins, including potential species differences, remains to be determined. In addition, the critical targets of MMPs in both acute kidney injury and chronic kidney disease remain to be identified, as well as the contribution of the kidney relative to other organs to the altered levels of both serum and urinary MMPs associated with renal pathophysiology. Little work at this time has focused on the cause-and-effect relationship between altered MMP activity and renal pathophysiology; while a number of studies have demonstrated a relationship between altered MMP expression and disease, very few have defined potential MMP targets, or shown a causal relationship between MMP activity and critical changes. Importantly, the potential for therapeutic intervention targeting MMPs and TIMPs in renal disease is suggested by a number of studies but has not been thoroughly investigated.

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