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Matrix metalloproteinases: their potential role in the pathogenesis of diabetic nephropathy

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Abstract

Matrix metalloproteinases (MMPs), a family of proteinases including collagenases, gelatinases, stromely-sins, matrilysins, and membrane-type MMPs, affect the breakdown and turnover of extracellular matrix (ECM).Moreover, they are major physiologic determinants of ECM degradation and turnover in the glomerulus. Renal hypertrophy and abnormal ECM deposition are hallmarks of diabetic nephropathy (DN), suggesting that altered MMP expression or activation contributes to renal injury in DN. Herein, we review and summarize recent information supporting a role for MMPs in the pathogenesis of DN. Specifically, studies describing dysregulated activity of MMPs and/or their tissue inhibitors in various experimental models of diabetes, including animal models of type 1 or type 2 diabetes, clinical investigations of human type 1 or type 2 diabetes, and kidney cell culture studies are reviewed.

Keywords

Gelatinase; Podocyte; Extracellular matrix; Proteinases; Proteinuria

Diabetic nephropathy—general overview

Diabetic nephropathy is stated to be the most common cause of end-stage renal disease in the United States [1]. Between 20% and 30% of patients with type 1 diabetes mellitus (DM) or type 2 DM will develop nephropathy [1], and among patients with type 1 DM, diabetic nephropathy develops in 40-50% of patients with a 20-year history of disease [2]. Among those individuals who develop renal dysfunction, several risk factors for the development of renal disease have been identified, including duration of diabetes, age at diagnosis, race, systemic or glomerular hypertension, poor glycemic control, genetic predisposition to kidney disease, and dietary composition [1-3]. However, the precise pathogenic mechanisms involved in the initiation and progression of diabetic nephropathy remain incompletely understood.

The development of diabetic nephropathy has been described as a five-stage process, progressing from glomerular hyperfiltration and nephromegaly (Stage 1), to glomerular basement membrane thickening and mesangial expansion (Stage 2), to microalbuminuria and eventual decline in glomerular filtration rate (Stage 3), to frank proteinuria with severe hypertension and sequelae of moderate to severe renal insufficiency (Stage 4), to eventual end-stage renal disease (Stage 5) [4,5]. It has been hypothesized that the early changes in glomerular basement membrane thickness and content ultimately affect filtration properties of the

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glomerular basement membrane, leading first to increased urinary albumin excretion and eventually to proteinuria. Similar histological findings are also seen in many rodent models of diabetic nephropathy. Because enlargement of the kidney mesangium due to extracellular matrix over-accumulation is a major characteristic of diabetic nephropathy, and because MMPs produced by the mesangial cell account for up to 70% of extracellular matrix degradation and turnover in the kidney during normal matrix remodeling, a link between intrarenal dysregulation of MMP activity and the development of diabetic nephropathy has been hypothesized. The intent of this review is to summarize available information regarding the matrix metalloproteinases (MMPs) and their naturally occurring specific inhibitors (TIMPs), as related to a potential role for these enzymes in the pathogenesis of diabetic renal disease.

Matrix metalloproteinases—a general overview

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases, which together have the capacity to breakdown all components of the extracellular matrix. Currently, there are over 20 known mammalian MMPs, which can be subdivided into collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), matrilysins (MMP-7, -26), the membrane-type MMPs [MT-MMPs; MMP-14 (MT1-MMP), -15 (MT2-MMP), -16 (MT3-MMP), -17 (MT4-MMP), -24 (MT5-MMP), -25 (MT6-MMP)], and "other MMPs" (MMP-11, -12, -19, -20, -21, -23a, -23b, -27, and -28) [6-8]. Analysis of the structure of MMPs reveals that they are multidomain proteins, consisting typically of a prodomain, a catalytic domain, a hinge region, and in the case of collagenases, gelatinases, and MT-MMPs, a hemopexin domain. Most MMPs are secreted as inactive proMMPs that can be activated by cleavage of the prodomain by proteinases such as plasmin and MT-MMPs, or by oxidation of a reactive cysteine within the prodomain [6,7].

MT-MMPs are a unique class of MMPs anchored to the cell surface by transmembrane domains. MT-MMPs display a broad spectrum of activities, and because of their localization to the cell surface, they are thought to play a major role in controlling proteolytic events within the pericellular microenvironment [9]. MT1-MMP (MMP-14), the best studied MT-MMP, can degrade a variety of extracellular matrix proteins, is capable of activating both proMMP-2 and proMMP-13 and has been shown to function as a "sheddase" for a variety of surface proteins, such as CD44 and syndecan-1 [9].

MMPs are of significant biomedical interest because they have been implicated in many pathologic processes characterized by dysregulated turnover of connective tissue matrices, such as occurs in rheumatoid and osteoarthritis, periodontal disease, metastatic cancer, metabolic bone disease, aortic aneurysm and atherosclerosis, sterile corneal ulceration, dystrophic epidermolysis bullosa, lung damage associated with chronic obstructive pulmonary disease, and emphysema [10-13]. Because of their powerful degradative capacity, the activity of MMPs is tightly regulated by a family of tissue inhibitors of metalloproteinases (TIMPs 1-4), as well as other proteinase inhibitors, such as α2-macroglobulin and tissue factor pathway inhibitor-2 [6,7,14,15]. Numerous studies also suggest a role for proinflammatory cytokines (several of which have been associated with diabetic nephropathy [16,17]) in the regulation of MMP expression. Cytokines such as interleukin-1 β, and tumor necrosis factor-α stimulate MMP production, while factors such as transforming growth factor-β, interleukin-4, corticoid hormones, and insulin-like growth factors down-regulate MMP synthesis [18,19]. A role for glucose and for advanced glycation end-products [20] in the regulation of MMP expression has also been demonstrated in vitro [21-26].

Dysregulation of MMP activity has been implicated in the pathophysiology of several diabetic co-morbidities, including diabetic retinopathy and chronic non-healing diabetic ulcers [27-32]. Increased circulating concentrations of MMP-2 have also been noted in pediatric

patients with type 1 DM who developed microangiopathy over a 5-year interval [33]. Finally, hyperglycemia-induced upregulation of MMP-2 has been demonstrated in arterial vasculature in vivo [34], and in various vascular components in vitro, including endothelial cells [22], macrophages [22], and vascular smooth muscle cells [24]. Consistent with these data, increases in plasma MMP-2 and MMP-9 concentrations have been observed in patients with type 2 DM and peripheral arterial disease [35] or acute coronary syndrome [36,37].

More recently, a role for MMPs in the pathophysiology of diabetic nephropathy is also emerging [38]. In the following sections, we review the literature supporting this hypothesis, first by presenting some pertinent concepts in regards to MMPs and the kidney. Next, we discuss available data regarding several individual MMP subclasses and the potential contribution of each subclass to the pathophysiology of diabetic nephropathy. Finally, details of those studies, which specifically provide evidence of intrarenal dysregulation of MMPs or TIMPs in various models of diabetes, are summarized in Table 1 .

MMPs and diabetic nephropathy

A number of MMP proteins have been identified throughout the kidney, and a detailed discussion of current knowledge regarding the expression of specific MMPs and TIMPs in the nephron of various species has been synthesized and presented elsewhere [39,40]. Data regarding the immunohistochemical analysis and sub-cellular localization of protein expression of MMP-2, -3, -9, -10, -11, -14 (MT1-MMP), -15 (MT2-MMP), TIMP-2, and TIMP-4 in human kidney tissues is also available at www.protein atlas.org [41,42].

MMPs are major determinants of extracellular matrix degradation and turnover in the glomerulus. Hence, changes in MMP expression or activity can influence intra-renal extracellular matrix composition [43,44]. Renal hypertrophy (i.e., an increase in kidney volume) is prognostic of the development of microalbuminuria in type 1 DM [45,46]. In fact, renal hypertrophy develops early in the course of type 1 DM, is predominant in those individuals who will develop diabetic nephropathy [45] and is associated with a poor renal prognosis [45,47,48]. Because abnormal extracellular matrix deposition is the hallmark of diabetic nephropathy, it is to be expected that altered MMP expression or activation also contributes to diabetic nephropathy, and specifically to the onset of this characteristic renal hypertrophy. Dysregulated activity of various growth factors and cytokines also contribute to the development of renal abnormalities characteristic of diabetic nephropathy [49]. Interestingly, MMPs, beyond their direct role in extracellular matrix turnover, have been shown to release or activate several growth factors that have been associated with renal hypertrophy, tubular cell proliferation, and renal scarring and fibrosis, such as pro-transforming growth factor-β, tumor necrosis factor-α, insulin like growth factors, and heparin-binding-epidermal growth factor [50-53].

Gelatinases (MMP-2 and MMP-9)

A role for intra-renal gelatinase dysregulation has been demonstrated for several renal disorders. Intra-renal MMP-2 expression or MMP-2 activity has been shown to be increased in AL-amyloidosis [54] and in tumor samples of human renal cell carcinoma [55]. Moreover, Cheng et al. [56] have demonstrated that MMP-2 is both absolutely necessary and sufficient for inducing the transformation of renal tubular epithelium to the myofibroblastic phenotype, a critical step heralding the development of renal interstitial fibrosis in many renal diseases, including diabetic nephropathy [56,57]. In fact, transgenic renal proximal tubular epithelial over-expression of MMP-2 is sufficient to generate the spectrum of pathologic changes characteristic of human chronic kidney disease [58].

Data suggesting a link between MMP-2 dysregulation, specifically, and diabetic nephropathy also exist but appear contradictory. Rodent models of diabetes reveal decreased expression and/or proteolytic activity of MMP-2 in renal tissues [59-61], as well as increased activity of the MMP-2 inhibitor, TIMP-2 [62]. However, both decreases and increases in MMP-2 secretion or enzyme activity have been demonstrated when rodent mesangial cells are cultured under high glucose culture conditions [23,25]. Human studies, on the other hand, suggest an increase in MMP-2 action. Active MMP-2 protein and MMP-2 enzyme activity were elevated (3.8-fold and 6-fold increases, respectively) in protein extracts from human diabetic kidney tissue samples [63]. An increase in urinary MMP-2 concentrations and/or MMP-2 activity has also been demonstrated in albuminuric patients with type 1 DM, compared to non-albuminuric patients or controls [38,64]. Reconciling differences between animal models of diabetes demonstrating decreased expression and/or proteolytic activity of MMP-2 in renal tissues [59-61] with findings in humans with diabetes [38,64] demonstrating increased circulating and excreted concentrations of MMP-2 might be explained by the confounding effects of diabetes treatment in humans. Specifically, insulin can induce MMP-2 activity in rat glomerular mesangial cells [65]. (For additional discussion, see *Reconciling differences between animal models and human disease*, below.)

In rodents, dysregulation of MMP-9 activity or expression has also been demonstrated in proteinuric renal diseases, including focal segmental glomerulosclerosis [66] and anti-glomerular basement membrane glomerulonephritis [67]. Among human proteinuria-associated renal dysfunction, over-expression of MMP-9 has been demonstrated in cortical renal biopsies from patients with HIV-associated nephropathy [68]. Kawata et al. [69] have demonstrated a strong correlation between the immunohistochemical expression of MMP-9 in renal cell carcinoma specimens and the severity of clinical symptoms.

With respect to diabetes, in the KKAy mouse (a rodent model of type 2 DM), expression of MMP-9 in the kidneys of mice that developed nephropathy was enhanced when compared to renal expression of this gelatinase in control animals [70]. In addition, increased urinary concentrations of MMP-9 have been detected in patients with type 2 DM and diabetic nephropathy, and the levels of MMP-9 increased in accordance with the degree of albuminuria (i.e., microalbuminuria < macroalbuminuria) [71,72].

Podocyte injury or apoptosis is recognized as a component of renal disease processes characterized by failure of the filtration barrier [73]. MMP-2 and MMP-9 are produced by cultured podocytes and podocyte MMP production in culture can be modified by numerous growth factors, cytokines, and by high ambient glucose levels [21,74,75]. Recent studies have demonstrated that hyperglycemia-mediated podocyte apoptosis and podocyte depletion occurs in mouse models of both type 1 and type 2 DM [76], and podocytopenia is present early in diabetic patients with both type 1 and type 2 DM [77]. Hypothetically, podocyte density could be diminished by glomerular basement membrane expansion, secondary to MMP-induced alterations of extracellular matrix turnover. When coupled with hyperglycemia-induced podocyte injury and increased podocyte apoptosis, a marked increase in membrane permeability would result, leading to diabetic albuminuria. Consistent with this hypothesis, Nakamura et al. [78] detected podocytes in the urinary sediment of diabetic patients with albuminuria, compared to the absence of podocytes in normoalbuminuric type 1 DM patients or healthy controls. Plasma concentrations of MMP-9 were significantly correlated with the number of urinary podocytes in these patients. Together, these observations suggest that diabetes-associated gelatinase dysregulation may also adversely affect podocyte integrity and glomerular basement membrane permeability.

Matrilysin (MMP-7)

Several studies suggest that dysregulation of MMP-7 is associated with renal pathology. Using immunohisto-chemical analysis, matrilysin protein expression is *not* detected in healthy human renal tubular epithelium [79]. However, MMP-7 expression is up-regulated in autosomal dominant polycystic kidney disease [79,80], renal biopsy samples from hydronephrotic patients with progressive disease requiring hemodialysis [81] and canine X-linked Alport syndrome [82]. In addition, MMP-7 appears to play a role in the identification and development or progression of renal carcinoma [83,84]. Moreover, when examining renal tissue gene expression association with aging, MMP-7 was among a group of genes significantly up-regulated in very old kidneys with higher rates of histopathological changes including glomerulosclerosis, interstitial fibrosis, and tubular atrophy [85]. Together, these studies imply that either acute or chronic loss of renal function may be related to MMP-7-induced alterations in extracellular matrix turnover [86].

As noted, glomerular and renal hypertrophy, glomerular basement membrane thickening and mesangial expansion secondary to overproduction or accumulation of extracellular matrix proteins are major morphologic features of diabetic nephropathy [17]. In vitro, prolonged hyperglycemic culture conditions modify the proteolytic activity of MMP-7 secreted by a variety of cell types [87], including human mesangial cells [26]. MMP-7 is known to degrade Type IV collagen, small proteoglycans, fibronectin, laminin and entactin, all components of the glomerular extracellular matrix [86]. Thus, hyperglycemia-induced, dysregulated turnover of the extracellular matrix by MMP-7 could alter the normal micro-architecture of the glomerular apparatus. In addition, MMP-7 has been shown to enhance growth factor action. For instance, rodent and human studies have demonstrated that aberrant renal growth is preceded by a marked increase in intra-renal insulin-like growth factor (IGF)-I protein content, trafficked into the kidney by changes in IGF-binding protein (IGFBP) expression within renal tissues, rather than from enhanced local IGF-I production [88]. Specifically, induction of IGFBP-1, -3 and -5 expression has been demonstrated in early renal hypertrophy in streptozotocin-treated diabetic rats [89]. Consequently, MMP-7 overproduction within the kidney parenchyma may result in enhanced degradation of IGFBPs, thus releasing bioactive or “free” IGFs, leading to an increase in extracellular matrix and expansion of the mesangium. To date, MMP-7 has been shown to proteolytically degrade IGFBP-3 [90] and IGFBP-5 [91, 92], and cleavage of IGFBPs by MMP-7 has been shown to liberate IGF-II [92]. Transforming growth factor-β (TGF-β), a growth factor known to promote renal fibrosis [93,94] is made more bioavailable through MMP-7 cleavage of decorin, a small proteoglycan commonly present in the extracellular matrix and which binds TGF-β [95]. MMP-7 has also been shown to mediate ectodomain shedding of heparin-binding epidermal growth factor [96], a growth factor that is upregulated in the diabetic rodent kidney [97]. Together, these data suggest that MMP-7 activity may contribute, either directly or indirectly, to glomerular basement membrane thickening and mesangial expansion, events, which precede the onset of diabetic nephropathy.

Membrane-type MMPs (MT-MMPs)

Only limited data is available on the role of MT-MMPs in the development of renal pathology. MMP-14 (MT1-MMP) expression has been assessed in the Goto-Kakizaki rat, a rodent model of type 2 DM [98], and its expression is enhanced prior to the observation of glomerulosclerosis and fibrosis [99]. In contrast, in a rat model of long-term diabetic renal disease, MMP-14 expression is decreased in glomerular cells [100]. In humans, renal MMP-24 (MT5-MMP) expression and production is upregulated in the diabetic kidney [63]. Moreover, MMP-14 (MT1-MMP) is present at a higher concentration in the urine from persons with diabetic nephropathy compared to a control population [72]. The presence of MMP-14 in urine and its

up-regulation in the urine of diabetics may reflect one or more processes. MMP-14 has been shown to exist as a membrane-bound MMP, as well as in multiple soluble forms, the latter being produced through auto-cleavage, or through other poorly understood mechanisms [101]. Therefore, increased MMP-14 in urine could reflect increased production or cleavage of soluble MMP-14 from cells within the nephron, or could reflect loss of cells within the nephron which express membrane bound MMP-14.

Collagenases and stromelysins

Rodent studies have provided some insight into the potential contribution of the collagenases or stromelysins to the development of diabetic nephropathy. Renal MMP-3 (stromelysin-1) mRNA expression is decreased in rats with streptozotocin-induced diabetes [102,103]. Similarly, using in situ hybridization analysis of renal tissues from patients with diabetic nephropathy to compare those with mild, moderate or severe mesangial expansion, MMP-3 mRNA expression was found to be the highest in those with the mildest histopathology [104].

Using gelatin microspheres containing the human MMP-1 gene in plasmid DNA, which allows for the localized transfection and expression of the MMP-1 gene, delivery of the MMP-1 gene into the renal capsule of mice at the time of streptozotocin injection impeded collagen accumulation in the kidneys, suggested a prophylactic potential for localized collagenase over-expression [105]. In contrast, however, immunoreactivity for MMP-8, another collagenase, was found to be significantly elevated in the urine of patients with diabetic nephropathy, compared to healthy control individuals, though the tissue origin of this excreted protein is not known [72].

Tissue inhibitors of metalloproteinases (TIMPs)

TIMP-1 and TIMP-2

In rodents, gene therapy with hepatocyte growth factor administered to rats with advanced diabetic nephropathy has been shown to induce regression, putatively by inhibiting TIMP-1 gene expression in renal tissues [106]. In humans, a decrease in circulating concentrations of both TIMP-1 and TIMP-2 have been demonstrated in patients with type 2 DM and diabetic nephropathy, compared to diabetes alone or non-diabetes chronic renal failure [107]. In another study, among 35 type 2 DM patients with diabetic nephropathy, both serum and urine TIMP-1 concentrations increased in association with worsening glomerular lesions [108]. In contrast, TIMP-1 or TIMP-2 concentrations among younger type 1 DM patients with normal renal function were unchanged compared with age-matched non-diabetic subjects [38]. Differences in disease severity or duration, or differences in the pathophysiology of type 1 DM versus type 2 DM may explain these opposing clinical findings.

TIMP-3

Data from Qi et al. [109] and data from our own laboratory (Thrailkill, et al. unpublished data) demonstrate that C57Bl/6 mice are nephropathy resistant, in that little renal pathology or urinary albumin excretion are evident in this inbred mouse model, even after 25 weeks of untreated diabetes. Because TIMP-3 is expressed at high levels in the normal mouse kidney [39], we queried the Animal Models of Diabetic Complications Consortium website (http://www.amdcc.org) to determine whether TIMP-3 expression affects the development of nephropathy in a streptozotocin-induced diabetic mouse model. In contrast to the lack of effect of diabetes on urinary albumin excretion in wild-type C57Bl/6 mice, C57Bl/6 mice lacking functional copies of the Timp3 gene showed elevated levels of urinary albumin excretion compared to non-diabetic Timp3 null controls, suggesting that elimination of this important inhibitor of all MMPs is sufficient to allow for diabetes-mediated renal complications in an

otherwise nephropathy-resistant mouse strain (albumin-to-creatinine ratio:non-diabetic Timp3 null C57Bl/6; 54.4 ± 9.1 μg/mg vs. diabetic Timp3 null C57Bl/6; 97.3 ± 14.9 μg/mg; $P = 0.02$. For comparison: albumin-to-creatinine ratios of non-diabetic C57Bl/6, 36.4 ± 2.6 μg/mg; vs. diabetic C57Bl/6, 39.4 ± 7.0, $P = 0.7$). Consistent with a role for TIMP-3 in diabetic nephropathy is the observation that in humans with type 1 DM, gene polymorphisms in Timp3 have been associated with diabetic nephropathy [110].

Reconciling differences between animal models and human disease

Examination of studies summarized in Table 1 highlights potential controversy within this field. Specifically, in many instances comparison of animal models of diabetes with clinical investigations in humans provides opposing evidence of increased vs. decreased expression of a particular subclass of MMPs. Several possible explanations can be offered to reconcile these differences: (1) Most animal model studies examine MMP protein expression or activity in "generic" kidney tissues, rather than tissue fractions restricted to components of the glomerular versus tubular compartments: differential expression of a specific MMP subclass in these two compartments is plausible and may also explain seemingly contradictory cell culture data; (2) Animal models examining STZ-induced diabetes (a model for type 1 DM) compared with genetic models of type 2 DM often provide contradictory results: While diabetic nephropathy develops in both human disorders, *untreated* animal models essentially compare the effects of a systemic insulin deficiency state with a systemic insulin resistant state (i.e., hyperinsulinemia); (3) Due to limitations in renal biopsy specimens, most human studies have instead examined urinary excretion of MMPs/TIMPs as a biomarker of intrarenal pathology; however, increased urinary concentration of a protein could reflect enhanced intrarenal production of the protein, increased filtration of the protein, abnormal reabsorption of the protein, increased tubular shedding of the protein, or indirect measurement of membrane bound protein due to a loss of cells into the urine; and finally (4) Because clear strain-specific differences in both the susceptibility to diabetic nephropathy and the accompanying renal morphological changes have been delineated in rodents [23,109], differing mechanisms may contribute to the development of diabetic nephropathy in certain rodent models: Furthermore, how MMP activities are assessed in rodent models of renal disease (e.g. in situ zymography versus ex vivo zymography) may give contrasting results [111].

MMPs and diabetic nephropathy—Significance

A better understanding of the pathogenesis of diabetic nephropathy is necessary to allow for the development of more precise and earlier strategies to detect harmful alterations in renal parenchyma, and to develop and implement more target molecule-specific therapies. Using cumulative available animal and human data, Remuzzi and Bertani [112] have suggested a sequence of events that underlie the progression to irreversible renal parenchyma damage and end stage renal disease, which likely represents a final common pathway of chronic proteinuric nephropathies, including diabetic nephropathy. In this model, high glomerular capillary pressure early on in the course of renal damage augments glomerular permeability to proteins which are then filtered excessively, reaching the lumen of the proximal tubule. Persistent proteinuria can then result in renal interstitial injury through multiple mechanisms, including, induction of chemokine expression and intrarenal activation of the complement cascade, resulting in inflammatory cell infiltration, or induction of tubular cell apoptosis [113]. These events culminate in a sustained interstitial inflammatory reaction that predates end-stage renal scarring and correlates significantly with declining renal function. The cycle of injury, remodeling, and in the end, overproduction and expansion of the extracellular matrix and basement membranes results in scarring and abnormal healing of the kidney. Intra-renal abnormalities in MMP production and/or activity in the diabetic kidney may instigate disruption of the filtration integrity of the glomerular basement membrane. In support of this

hypothesis: (1) several MMPs and TIMPs have been shown to play a role in renal pathology; (2) enlargement of the kidney mesangium due to extracellular matrix over-accumulation is a major characteristic of diabetic nephropathy, preceding the onset of albuminuria; (3) MMPs produced by the mesangial cell are the major regulators of extracellular matrix turnover in the kidney during normal extracellular matrix remodeling; and (4) hyperglycemia-mediated alterations in MMP secretion or activation contribute to the pathophysiology of other diabetic co-morbidities. The impact and contribution of MMPs or TIMPs to the onset and progression of diabetic nephropathy may be most critical in the earlier phases of the disease process, at a time in which enhanced matrix turnover, release of pro-fibrotic growth factors and altered cell motility may damage the glomerular apparatus and tubular architecture.

A greater understanding of the spatio-temporal contribution of MMPs to the pathogenesis of diabetic nephropathy will be critical if MMPs are to be targeted therapeutically to prevent and/or reverse diabetic renal disease. This consideration is addressed in a pivotal study by Zeisberg et al. [114]. Using a rodent model of hereditary kidney disease, it was demonstrated that genetic ablation of any one of three key MMPs (MMP-2, MMP-3, or MMP-9) involved in the progression of renal dysfunction led to the compensatory upregulation of other MMPs, suggesting some redundancy and compensation within this family of proteinases. However, broad-spectrum pharmacological ablation of MMP enzymatic activity before the onset of proteinuria was “associated with delayed proteinuria and a marked extension in survival” [114]. This study also demonstrated that the preservation of glomerular basement membrane or extracellular matrix integrity *before* the onset of proteinuria lead to disease protection, whereas MMP-inhibition in later stages of disease resulted in accelerated glomerular and interstitial fibrosis [114]. If similar mechanisms are at play in the evolution of diabetic nephropathy, then very early intervention (prior to the onset of microalbuminuria) or even prophylaxis with anti-MMP therapies in genetically and metabolically susceptible individuals with diabetes could prevent or significantly retard the development of diabetic nephropathy in at-risk individuals.

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References

1. American Diabetes Association. Diabetes care 2003;26(90001):S94–S98. [PubMed: 12502629]
2. DeFronzo, RA. Diabetic nephropathy: diagnostic and therapeutic approach. In: DeFronzo, RA., editor. Current Therapy of Diabetes Mellitus. Mosby-Year Book, Inc.; Saint Louis: 1998.
3. Boright AP, Paterson AD, Mirea L, Bull SB, Mowjoodi A, Scherer SW, Zinman B. Diabetes 2005;54 (4):1238–1244. [PubMed: 15793268]
4. Feld, LG. Diabetic nephropathy. In: Barratt, TM.; Avner, ED., editors. Pediatric Nephrology. Lippincott, Williams and Wilkins; Baltimore: 1999.
5. Maxwell P. Practitioner 2005;249(1666):9–10. [PubMed: 15686256]
6. Visse R, Nagase H. Circ. Res 2003;92(8):827–839. [PubMed: 12730128]
7. Nagase H, Visse R, Murphy G. Cardiovasc. Res 2006;69(3):562–573. [PubMed: 16405877]
8. Werb Z. Cell 1997;91(4):439–442. [PubMed: 9390552]
9. Itoh Y, Seiki M. J. Cell. Physiol 2006;206(1):1–8. [PubMed: 15920734]
10. Okada Y, Takeuchi N, Tomita K, Nakanishi I, Nagase H. Ann. Rheum. Dis 1989;48(8):645–653. [PubMed: 2675782]
11. Nagase, H. Matrix Metalloproteinases. Taylor and Francis; London: 1996.
12. Parks WC, Shapiro SD. Respir. Res 2001;2(1):10–19. [PubMed: 11686860]

13. Silva JA, Lorencini M, Peroni LA, De La Hoz CL, Carvalho HF, Stach-Machado DR. J. Periodontal Res 2008;43(1):48–54. [PubMed: 18230106]
14. Barrett AJ. Methods Enzymol 1981;80(Pt C):737–754. [PubMed: 6176834]
15. Herman MP, Sukhova GK, Kisiel W, Foster D, Kehry MR, Libby P, Schonbeck U. J. Clin. Invest 2001;107(9):1117–1126. [PubMed: 11342575]
16. Sharma K, McGowan TA. Cytokine Growth Factor Rev 2000;11(12):115–123. [PubMed: 10708959]
17. Schrijvers BF, De Vriese AS, Flyvbjerg A. Endocr. Rev 2004;25(6):971–1010. [PubMed: 15583025]
18. Van Noort JM, Amor S. Int. Rev. Cytol 1998;178:127–206. [PubMed: 9348670]
19. Nee L, Tuite N, Ryan MP, McMorrow T. Nephron 2007;107(2):e73–e86. [PubMed: 17890880]
20. McLennan SV, Kelly DJ, Schache M, Waltham M, Dy V, Langham RG, Yue DK, Gilbert RE. Kidney Int 2007;72(4):481–488. [PubMed: 17554258]
21. Bai Y, Wang L, Li Y, Liu S, Li J, Wang H, Huang H. Cell Physiol. Biochem 2006;17(12):57–68. [PubMed: 16543722]
22. Death AK, Fisher EJ, McGrath KC, Yue DK. Atherosclerosis 2003;168(2):263–269. [PubMed: 12801609]
23. Fornoni A, Striker LJ, Zheng F, Striker GE. Diabetes 2002;51(2):499–505. [PubMed: 11812761]
24. Hao F, Yu JD. Acta Pharmacol. Sin 2003;24(6):534–538. [PubMed: 12791179]
25. Singh R, Song RH, Alavi N, Pegoraro AA, Singh AK, Leehey DJ. Exp. Nephrol 2001;9(4):249–257. [PubMed: 11423724]
26. Abdel Wahab N, Mason RM. Biochem. J 1996;320(Pt 3):777–783. [PubMed: 9003362]
27. Ishizaki E, Takai S, Ueki M, Maeno T, Maruichi M, Sugiyama T, Oku H, Ikeda T, Miyazaki M. Am. J. Ophthalmol 2006;141(1):129–134. [PubMed: 16386986]
28. Noda K, Ishida S, Inoue M, Obata K, Oguchi Y, Okada Y, Ikeda E. Invest. Ophthalmol. Vis. Sci 2003;44(5):2163–2170. [PubMed: 12714657]
29. Giebel SJ, Menicucci G, McGuire PG, Das A. Lab. Invest 2005;85(5):597–607. [PubMed: 15711567]
30. Medina A, Scott PG, Ghahary A, Tredget EE. J. Burn Care Rehabil 2005;26(4):306–319. [PubMed: 16006837]
31. Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Diabetologia 2002;45 (7):1011–1016. [PubMed: 12136400]
32. Jacqueminet S, Ben Abdesselam O, Chapman MJ, Nicolay N, Foglietti MJ, Grimaldi A, Beaudeux JL. Clin. Chim. Acta 2006;367(12):103–107. [PubMed: 16426593]
33. Derosa G, Avanzini MA, Geroldi D, Fogari R, Lorini R, De Silvestri A, Tinelli C, Rondini G, d'Annunzio G. Diabetes Res. Clin. Pract 2005;70(2):119–125. [PubMed: 16188574]
34. Chung AW, Hsiang YN, Matzke LA, McManus BM, van Breemen C, Okon EB. Circ. Res 2006;99 (2):140–148. [PubMed: 16778129]
35. Signorelli SS, Malaponte G, Libra M, Di Pino L, Celotta G, Bevelacqua V, Petrina M, Nicotra GS, Indelicato M, Navolanic PM, Pennisi G, Mazzarino MC. Vasc. Med 2005;10(1):1–6. [PubMed: 15920993]
36. Derosa G, Cicero AF, Scalise F, Avanzini MA, Tinelli C, Piccinni MN, Peros E, Geroldi D, Fogari E, D'Angelo A. Endothelium 2007;14(1):45–51. [PubMed: 17364896]
37. Derosa G, D'Angelo A, Scalise F, Avanzini MA, Tinelli C, Peros E, Fogari E, Cicero AF. Heart Vessels 2007;22(6):361–370. [PubMed: 18043992]
38. Thrailkill KM, Bunn RC, Moreau CS, Cockrell GE, Simpson PM, Coleman HN, Frindik JP, Kemp SF, Fowlkes JL. Diabetes care 2007;30(9):2321–2326. [PubMed: 17563344]
39. Catania JM, Chen G, Parrish AR. Am. J. Physiol 2007;292(3):F905–F911.
40. Mason RM, Wahab NA. J. Am. Soc. Nephrol 2003;14(5):1358–1373. [PubMed: 12707406]
41. Barbe L, Lundberg E, Oksvold P, Stenius A, Lewin E, Bjorling E, Asplund A, Ponten F, Brismar H, Uhlen M, Andersson-Svahn H. Mol. Cell. Proteomics 2008;7(3):499–508. [PubMed: 18029348]
42. Hober S, Uhlen M. Curr. Opin. Biotechnol 2008;19(1):30–35. [PubMed: 18187316]
43. Lelongt B, Legallicier B, Piedagnel R, Ronco PM. Curr. Opin. Nephrol. Hypertens 2001;10(1):7–12. [PubMed: 11195055]

44. Lenz O, Elliot SJ, Stetler-Stevensen WG. J. Am. Soc. Nephrol 2000;11:574–581. [PubMed: 10703682]
45. Zerbini G, Bonfanti R, Meschi F, Bognetti E, Paesano PL, Gianolli L, Querques M, Maestroni A, Calori G, Del Maschio A, Fazio F, Luzi L, Chiumello G. Diabetes 2006;55(9):2620–2625. [PubMed: 16936212]
46. Frazer FL, Palmer LJ, Clarey A, Thonell S, Byrne GC. J. Pediatr. Endocrinol. Metab 2001;14(7): 875–881. [PubMed: 11515728]
47. Baumgartl HJ, Sigl G, Banholzer P, Haslbeck M, Standl E. Nephrol. Dial. Transplant 1998;13(3): 630–634. [PubMed: 9550638]
48. Lawson ML, Sochett EB, Chait PG, Balfe JW, Daneman D. Diabetes 1996;45(1):51–55. [PubMed: 8522059]
49. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M. Kidney Int 1991;40 (6):1007–1012. [PubMed: 1762301]
50. Fowlkes JL, Thrailkill KM, Serra DM, Suzuki K, Nagase H. Progress in growth factor research 1995;6 (24):255–263. [PubMed: 8817668]
51. Ii M, Yamamoto H, Adachi Y, Maruyama Y, Shinomura Y. Exp. Biol. Med. (Maywood) 2006;231 (1):20–27. [PubMed: 16380641]
52. Fowlkes JL, Serra DM, Nagase H, Thrailkill KM. Ann. N. Y. Acad. Sci 1999;878:696–699. [PubMed: 10415811]
53. Zhuang S, Kinsey GR, Rasbach K, Schnellmann RG. Am. J. Physiol 2008;294:F459–F468.
54. Keeling J, Herrera GA. Kidney Int 2005;68(4):1590–1603. [PubMed: 16164636]
55. Bhuvarahamurthy V, Kristiansen GO, Johannsen M, Loening SA, Schnorr D, Jung K, Staack A. Oncol. Rep 2006;15(5):1379–1384. [PubMed: 16596214]
56. Cheng S, Lovett DH. Am. J. Pathol 2003;162(6):1937–1949. [PubMed: 12759250]
57. Essawy M, Soylemezoglu O, Muchaneta-Kubara EC, Shortland J, Brown CB, el Nahas AM. Nephrol. Dial. Transplant 1997;12(1):43–50. [PubMed: 9027772]
58. Cheng S, Pollock AS, Mahimkar R, Olson JL, Lovett DH. FASEB J 2006;20(11):1898–1900. [PubMed: 16891619]
59. Zhang SX, Wang JJ, Lu K, Mott R, Longeras R, Ma JX. J. Am. Soc. Nephrol 2006;17(2):475–486. [PubMed: 16394111]
60. Inada A, Nagai K, Arai H, Miyazaki J, Nomura K, Kanamori H, Toyokuni S, Yamada Y, Bonner-Weir S, Weir GC, Fukatsu A, Seino Y. Am. J. Pathol 2005;167(2):327–336. [PubMed: 16049320]
61. Sun SZ, Wang Y, Li Q, Tian YJ, Liu MH, Yu YH. Chin. Med. J. Engl 2006;119(10):814–821. [PubMed: 16732983]
62. Han SY, Jee YH, Han KH, Kang YS, Kim HK, Han JY, Kim YS, Cha DR. Nephrol. Dial. Transplant 2006;21(9):2406–2416. [PubMed: 16728425]
63. Romanic AM, Burns-Kurtis CL, Ao Z, Arleth AJ, Ohlstein EH. Am. J. Physiol 2001;281(2):F309–F317.
64. Diamant M, Hanemaaijer R, Verheijen JH, Smit JW, Radder JK, Lemkes HH. Diabet. Med 2001;18 (5):423–424. [PubMed: 11472458]
65. Lee MP, Sweeney G. Diabetes Obes. Metab 2006;8(3):281–288. [PubMed: 16634987]
66. Liu S, Li Y, Zhao H, Chen D, Huang Q, Wang S, Zou W, Zhang Y, Li X, Huang H. Mol. Cell. Biochem 2006;284(12):9–17. [PubMed: 16477388]
67. Kuroda T, Yoshida Y, Kamiie J, Kovalenko P, Nameta M, Fujinaka H, Yaoita E, Endo T, Ishizuka S, Nakabayashi K, Yamada A, Nagasawa T, Yamamoto T. Clin. Exp. Nephrol 2004;8(3):206–215. [PubMed: 15480897]
68. Ahuja TS, Gopalani A, Davies P, Ahuja H. Nephron Clin. Pract 2003;95(3):c100–c104. [PubMed: 14646370]
69. Kawata N, Nagane Y, Igarashi T, Hirakata H, Ichinose T, Hachiya T, Takimoto Y, Takahashi S. Urology 2006;68(3):523–527. [PubMed: 16979746]
70. Qing-Hua G, Ju-Ming L, Chang-Yu P, Zhao-Hui L, Xiao-Man Z, Yi-Ming M. J. Diabetes Complicat 2007;22:408–412. [PubMed: 18413157]

71. Tashiro K, Koyanagi I, Ohara I, Ito T, Saitoh A, Horikoshi S, Tomino Y. J. Clin. Lab. Anal 2004;18 (3):206–210. [PubMed: 15103687]
72. Lauhio A, Sorsa T, Srinivas R, Stenman M, Tervahartiala T, Stenman UH, Gronhagen-Riska C, Honkanen E. Ann. Med 2008;40(4):312–320. [PubMed: 18428024]
73. Pavenstadt H, Kriz W, Kretzler M. Physiol. Rev 2003;83(1):253–307. [PubMed: 12506131]
74. Asanuma K, Shirato I, Ishidoh K, Kominami E, Tomino Y. Kidney Int 2002;62(3):822–831. [PubMed: 12164864]
75. Liu S, Liang Y, Huang H, Wang L, Li Y, Li J, Li X, Wang H. Cell Physiol. Biochem 2005;16(46): 207–216. [PubMed: 16301820]
76. Susztak K, Raff AC, Schiffer M, Bottinger EP. Diabetes 2006;55(1):225–233. [PubMed: 16380497]
77. Wolf G, Chen S, Ziyadeh FN. Diabetes 2005;54(6):1626–1634. [PubMed: 15919782]
78. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, Koide H. Nephrol. Dial. Transplant 2000;15(9):1379–1383. [PubMed: 10978394]
79. Surendran K, Simon TC, Liapis H, McGuire JK. Kidney Int 2004;65(6):2212–2222. [PubMed: 15149334]
80. Riera M, Burtey S, Fontes M. Kidney Int 2006;69(9):1558–1563. [PubMed: 16541020]
81. Henger A, Kretzler M, Doran P, Bonrouhi M, Schmid H, Kiss E, Cohen CD, Madden S, Porubsky S, Grone EF, Schlondorff D, Nelson PJ, Grone HJ. Kidney Int 2004;65(3):904–917. [PubMed: 14871410]
82. Rao VH, Lees GE, Kashtan CE, Delimont DC, Singh R, Meehan DT, Bhattacharya G, Berridge BR, Cosgrove D. Pediatr. Nephrol 2005;20(6):732–739. [PubMed: 15782307]
83. Sarkissian G, Fergelot P, Lamy PJ, Patard JJ, Culine S, Jouin P, Rioux-Leclercq N, Darbouret B. Clin. Chem 2008;54(3):574–581. [PubMed: 18202161]
84. Patraki E, Cardillo MR. Int. J. Immunopathol. Pharmacol 2007;20(4):697–705. [PubMed: 18179742]
85. Melk A, Mansfield ES, Hsieh SC, Hernandez-Boussard T, Grimm P, Rayner DC, Halloran PF, Sarwal MM. Kidney Int 2005;68(6):2667–2679. [PubMed: 16316342]
86. Woessner, JF.; Nagase, H., editors. Protein Substrates of the MMPs. Vol. 1st edn.. Oxford University Press; New York: 2000. Matrix Metalloproteinases and TIMPs.
87. Maldonado A, He L, Game BA, Nareika A, Sanders JJ, London SD, Lopes-Virella MF, Huang Y. J. Periodont. Res 2004;39(6):415–423. [PubMed: 15491346]
88. Flyvbjerg A. Diabetologia 2000;43(10):1205–1223. [PubMed: 11079738]
89. Park IS, Kiyomoto H, Alvarez F, Xu YC, Abboud HE, Abboud SL. Am. J. Kidney Dis 1998;32(6): 1000–1010. [PubMed: 9856516]
90. Miyamoto S, Yano K, Sugimoto S, Ishii G, Hasebe T, Endoh Y, Kodama K, Goya M, Chiba T, Ochiai A. Cancer Res 2004;64(2):665–671. [PubMed: 14744783]
91. Hemers E, Duval C, McCaig C, Handley M, Dockray GJ, Varro A. Cancer Res 2005;65(16):7363–7369. [PubMed: 16103088]
92. McCaig C, Duval C, Hemers E, Steele I, Pritchard DM, Przemeck S, Dimaline R, Ahmed S, Bodger K, Kerrigan DD, Wang TC, Dockray GJ, Varro A. Gastroenterology 2006;130(6):1754–1763. [PubMed: 16697739]
93. Liu Y. Kidney Int 2006;69(2):213–217. [PubMed: 16408108]
94. Bottinger EP, Bitzer M. J. Am. Soc. Nephrol 2002;13(10):2600–2610. [PubMed: 12239251]
95. Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y. Biochem. J 1997;322(Pt 3):809–814. [PubMed: 9148753]
96. Yu WH, Woessner JF Jr. McNeish JD, Stamenkovic I. Genes Dev 2002;16(3):307–323. [PubMed: 11825873]
97. Lee YJ, Shin SJ, Lin SR, Tan MS, Tsai JH. Biochem. Biophys. Res. Commun 1995;207(1):216–222. [PubMed: 7857268]
98. Movassat J, Saulnier C, Serradas P, Portha B. Diabetologia 1997;40(8):916–925. [PubMed: 9267986]
99. Portik-Dobos V, Harris AK, Song W, Hutchinson J, Johnson MH, Imig JD, Pollock DM, Ergul A. Am. J. Physiol. Regul. Integr. Comp. Physiol 2006;290(2):R435–R441. [PubMed: 16239374]

100. Boucher E, Mayer G, Londono I, Bendayan M. Kidney Int 2006;69(9):1570–1577. [PubMed: 16541018]
101. Osenkowski P, Toth M, Fridman R. J. Cell. Physiol 2004;200(1):2–10. [PubMed: 15137052]
102. Yang Q, Xie RJ, Yang T, Fang L, Han B, Zhang GZ, Cheng ML. Chin. Med. Sci. J 2007;22(4):243–249. [PubMed: 18246672]
103. Nakamura T, Fukui M, Ebihara I, Osada S, Tomino Y, Koide H. Ren. Physiol. Biochem 1994;17(6):316–325. [PubMed: 7533311]
104. Suzuki D, Miyazaki M, Jinde K, Koji T, Yagame M, Endoh M, Nomoto Y, Sakai H. Kidney Int 1997;52(1):111–119. [PubMed: 9211353]
105. Aoyama T, Yamamoto S, Kanematsu A, Ogawa O, Tabata Y. Tissue Eng 2003;9(6):1289–1299. [PubMed: 14674437]
106. Cruzado JM, Lloberas N, Torras J, Riera M, Fillat C, Herrero-Fresneda I, Aran JM, Alperovich G, Vidal A, Grinyo JM. Diabetes 2004;53(4):1119–1127. [PubMed: 15047630]
107. Rysz J, Banach M, Stolarek RA, Pasnik J, Cialkowska-Rysz A, Koktysz R, Piechota M, Baj Z. J. Nephrol 2007;20(4):444–452. [PubMed: 17879211]
108. Kanauchi M, Nishioka H, Nakashima Y, Hashimoto T, Dohi K. Nippon Jinzo Gakkai Shi 1996;38(3):124–128. [PubMed: 8721333]
109. Qi Z, Fujita H, Jin J, Davis LS, Wang Y, Fogo AB, Breyer MD. Diabetes 2005;54(9):2628–2637. [PubMed: 16123351]
110. Ewens KG, George RA, Sharma K, Ziyadeh FN, Spielman RS. Diabetes 2005;54(11):3305–3318. [PubMed: 16249459]
111. Ahmed AK, Haylor JL, El Nahas AM, Johnson TS. Kidney Int 2007;71(8):755–763. [PubMed: 17290295]
112. Remuzzi G, Bertani T. N. Engl. J. Med 1998;339(20):1448–1456. [PubMed: 9811921]
113. Abbate M, Zoja C, Remuzzi G. J. Am. Soc. Nephrol 2006;17(11):2974–2984. [PubMed: 17035611]
114. Zeisberg M, Khurana M, Rao VH, Cosgrove D, Rougier JP, Werner MC, Shield CF 3rd, Werb Z, Kalluri R. PLoS Med 2006;3(4):e100. [PubMed: 16509766]
115. McLennan SV, Kelly DJ, Cox AJ, Cao Z, Lyons JG, Yue DK, Gilbert RE. Diabetologia 2002;45(2):268–275. [PubMed: 11935159]
116. Song RH, Singh AK, Leehey DJ. Am. J. Nephrol 1999;19(3):441–446. [PubMed: 10393385]
117. McLennan SV, Martell SY, Yue DK. Diabetologia 2000;43(5):642–648. [PubMed: 10855539]

Table 1

Renal dysregulation of MMPs or TIMPs in models of diabetes

MMP/TIMP[a] (Reference)	In vivo/in vitro	Species or cell type	Tissue/cell type/biofluid	Condition studied	Outcome
Gelatinases					
MMP-2 [60]	In vivo	Mouse	Glomeruli	ICER Iγ transgenic mouse model of diabetic nephropathy	*Decreased* MMP-2 expression by IHC
MMP-2 [61]	In vivo	Rat	Kidney tissues/glomeruli	STZ-induced diabetes	*Decreased*: (a) MMP-2 expression by IHC; (b) Mmp-2 mRNA by RT-PCR
MMP-2 [99]	In vivo	Rat	Kidney tissues/cortical and medullary	Goto-Kakizaki rat model of type 2 DM	*Increased*: (a) MMP-2 activity by zymography; (b) MMP-2 expression by western blot
MMP-2 [25]	In vitro	Rat mesangial cells	N/A	30 mM Glucose	*Decreased*: (a) MMP-2 levels by ELISA; (b) MMP-2 activity by zymography
MMP-2 [23]	In vitro	Mouse mesangial cells	N/A	25 mM Glucose	*Increased*: (a) MMP-2 activity by zymography; (b) Mmp-2 mRNA by RT-PCR
MMP-2 [26]	In vitro	Human mesangial cells	N/A	30 mM Glucose	*Increased*: (a) MMP-2 activity by zymography; (b) MMP-2 expression by western blot; (c) Mmp-2 mRNA by RT-PCR
MMP-2 [63]	In vivo	Human	Kidney tissues/tubules	Type 1 and type 2 DM	*Increased*: MMP-2 expression by western blot; MMP-2 activity by activity-based ELISA
MMP-2 [38,64]	In vivo	Human	Urine	Type 1 DM	*Increased*: (a) MMP-2 levels by F-MAP; and/or (b) MMP-2 activity by activity-based ELISA
MMP-9 [70]	In vivo	Mouse	Kidney tissues/glomeruli	KKAy mouse model of Type 2 DM	*Increased*: (a) MMP-9 expression by IHC; (b) Mmp-9 mRNA by RT-PCR.
MMP-9 [115]	In vivo	Rat	Kidney tissues	STZ-induced diabetes	*Decreased*: (a) MMP-9 activity by zymography; (b) Mmp-9 mRNA by RT-PCR
MMP-2 and MMP-9 [116]	In vivo	Rat	Kidney tissues/glomeruli	STZ-induced diabetes	*Decreased* total gelatinase activity by zymography
MMP-9 [23]	In vitro	Mouse mesangial cells	N/A	25 mM Glucose	*Decreased*: (a) MMP-9 activity by zymography; (b) Mmp-9 mRNA by RT-PCR
MMP-9 [21]	In vitro	Mouse podocytes	N/A	25 mM Glucose	*Increased*: (a) MMP-9 activity by zymography; (b) Mmp-9 mRNA by RT-PCR
MMP-9 [71]	In vivo	Human	Urine	Type 2 DM with nephropathy	*Increased* MMP-9 levels by ELISA
MMP-9 [72]	In vivo	Human	Urine	Type 1 and type 2 DM	*Increased*: (a) MMP-9 activity by zymography; (b) MMP-9 immunoreactivity by western blot
Matrilysins					
MMP-7 [26]	In vitro	Human mesangial cells	N/A	30 mM glucose	*Decreased*: (a) MMP-7 activity by zymography; (b) Mmp-7 mRNA by RT-PCR.
MT-MMPs					
MT5-MMP [63]	In vivo	Human	Kidney tissues/tubules	Type 1 and type 2 DM	*Increased* MT5-MMP protein expression by western blot and IHC.
MT1-MMP [99]	In vivo	Rat	Kidney tissues/cortical and medullary	Goto-Kakizaki rat model of type 2 DM	*Increased* MT1-MMP protein expression by western blot
MT1-MMP [100]	In vivo	Rat	Kidney tissue/glomeruli	STZ-induced diabetes	*Decreased*: (a) MT1-MMP expression by IHC; (b) MT1-MMP protein expression by western blot
MT1-MMP [117]	In vitro	Human mesangial cells	N/A	25 mM Glucose	*Decreased* MT1-MMP mRNA by RT-PCR
MT1-MMP [72]	In vivo	Human	Urine	Type 1 and type 2 DM	*Increased* MT1-MMP protein expression by western blot

Studies describing dysregulation of MMP and/or TIMP activity in various experimental models of diabetes, including kidney cell cultures exposed to high glucose, animal models of type 1 or type 2 DM, and human type 1 or type 2 DM (kidney tissues or urine specimens) are summarized, according to MMP subclass.

MMP/TIMP[a] (Reference)	In vivo/in vitro	Species or cell type	Tissue/cell type/biofluid	Condition studied	Outcome
Stromelysins and Collagenases					
MMP-1 and MMP-3 [103]	In vivo	Rat	Kidney tissues/glomeruli	STZ-induced diabetes	*Decreased* MMP-1 and MMP-3 mRNA by RT-PCR
MMP-8 [72]	In vivo	Human	Urine	Type 1 and type 2 DM	*Increased* total and active MMP-8 protein expression by Western blot
TIMPS					
TIMP-1 [103,115]	In vivo	Rat	Kidney tissues	STZ-induced diabetes	*Increased*: TIMP-1 mRNA by RT-PCR
TIMP-1 [108]	In vivo	Human	Urine	Type 2 DM with diabetic nephropathy	*Increased* TIMP-1 by ELISA, in association with progression of severity of glomerular lesions
TIMP-2 [61]	In vivo	Rat	Kidney tissues/glomeruli	STZ-induced diabetes	*Increased*: (a) TIMP-2 expression by IHC; (b) Timp-2 gene expression by RT-PCR
TIMP-2 [25]	In vitro	Rat mesangial cells	N/A	30 mM Glucose	*Increased* TIMP-2 levels by ELISA

Studies describing dysregulation of MMP and/or TIMP activity in various experimental models of diabetes, including kidney cell cultures exposed to high glucose, animal models of type 1 or type 2 DM, and human type 1 or type 2 DM (kidney tissues or urine specimens) are summarized, according to MMP subclass.

[a] Abbreviations: DM, diabetes mellitus; IHC, immunohistochemistry; MMP, matrix metalloproteinases; STZ, streptozotocin; TIMP, tissue inhibitor of metalloproteinases