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Matrix metalloproteinases and their role in the renal epithelial mesenchymal transition

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Summary. Tubular cell epithelial-mesenchymal transition (EMT) is a fundamental contributor to renal fibrosis. The aim of this study was to investigate the activity of different matrix metalloproteinases by immunohistochemistry and gel-zymography in a model of chronic canine kidney disease. Immunohistochemistry for antibodies against MMP-9, MMP-2, MMP-13, MMP-14 and TIMP-2 was performed on 28 renal biopsy specimens. Selected cases were chosen for gelatin zymography. In moderate and severe tubulo-interstitial damage, increased expression of MMP-2 was noted. A peculiar staining pattern for MMP-2 in variable-sized vesicles, corresponding to the area of basement membrane splitting, was observed. The immuno-expression of MMP-9 and TIMP-2 was reduced in the same cases, compared to control dogs. The splitting of the membrane suggests an active role of this gelatinase in the disruption of type-IV collagen, the main basement membrane component, confirmed by MMP2 gelatinolytic activity by gel-zymography. These data could provide the basis for clinical trials examining the potential benefits of selective MMP-2 inhibitors in dogs with chronic kidney disease.

Key words: Fibrosis, MMP2, Splitting, Zymography

Introduction

Progressive fibrosis is a common process promoted by epithelial remodelling (EMT), inflammation, fibroblast activation, and reorganisation of cellular interactions with the extracellular matrix (ECM) (Kaissling and Le Hir, 2008; Hewitson, 2009). This process has been suggested to be a consequence of disequilibrium between increased ECM synthesis and decreased matrix metalloproteinase (MMP)-mediated degradation (Lelongt et al., 2001; Inkinen et al., 2005). Matrix metalloproteinases (MMPs) are enzymes with metal ion-dependent activity that degrade extracellular matrix (ECM) glycoproteins. MMPs play a vital role in various normal and pathological processes, including embryogenesis, tissue remodelling, angiogenesis, wound healing, and metastasis (Visse and Nagase, 2003; Mott and Werb, 2004). MMPs are also important in the tissue remodelling and repair that occurs in various renal diseases, such as glomerulonephritis and progressive renal fibrosis. In particular, MMP-2 and -9 have been studied extensively in the kidneys and in renal transplantation models of acute allograft rejection and chronic allograft nephropathy. Notably, results from recent clinical studies of renal allografts suggest that MMPs and TIMPs could play a role in the development of glomerular sclerosis and interstitial fibrosis (Johnson et al., 2002).

The current view of EMT postulates that it involves a mechanism in which tubular epithelial cells become activated by exogenous stimuli and subsequently lose contact with neighbouring cells and the basement membrane. After initiation of EMT, cells move through the basement membrane into the interstitial matrix where they become detectable as fibroblasts/myofibroblasts. Thus, in this hypothetical model of EMT, the epithelial phenotype is clearly associated with the TBM microenvironment, whereas the mesenchymal phenotype is associated with the interstitial microenvironment. Both in vitro and in vivo studies have provided evidence suggesting that tubular cells themselves may transdifferentiate, acquire fibroblast-like properties, and secrete matrix proteins, including types-I, -III, -IV and -V collagen, into the interstitial space (El-Nahas, 2003;

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Cheng et al., 2006). We hypothesised that MMPs may disrupt the tubular basement membrane (TBM), leading to EMT. To test this hypothesis, we investigated the role of MMP-2, MMP-9, MMP-13, MMP-14 and TIMP-2 during tubular interstitial damage (TID). In addition, the activities of MMP-2 and MMP-9 were evaluated by gelatine zymography.

Materials and methods

Twenty-eight dogs, 17 males and 11 females, were selected from a population of dogs presenting a positive serology for leishmaniasis during a survey study on Italian territory from 2004 to 2009. Portions of renal cortex were collected immediately after euthanasia performed when clinical conditions of the dogs were considered irreversible. Renal tissue was formalin-fixed, paraffin-embedded and stained for microscopic examination. For immunohistochemical studies, we used a commercial kit (Dako LSAB K680; Dako, Burlingame, CA, USA) containing peroxidase-conjugated streptavidin and a mixture of biotinylated goat anti-rabbit and anti-mouse immunoglobulins as the link antibody. Antibodies against MMP-2, MMP-9, MMP-14, MMP-13 and TIMP-2 were used (Table 1). Tissue sections cut at 3 µm were de-waxed, rehydrated and rinsed in phosphate-buffered saline (PBS), pH 7.4. Endogenous peroxidase activity was blocked with hydrogen peroxide (0.3%) in absolute methanol for 30 min. The primary antibodies were applied to the sections, which were then incubated at 4°C overnight. The reaction was developed with 3,3'-diaminobenzidine tetra-hydrochloride (Sigma, St Louis, MO, USA) containing hydrogen peroxide (0.03%) and the sections were counterstained with Periodic Acid Schiff (PAS).

Kidney fibrosis in renal biopsies was determined on paraffin-embedded sections (4 µm) stained with Masson's trichrome. The amount of fibrotic tissue was assessed by point -counting using a 25-point grid randomly laid on the micrographs. The tubulo-interstitial damage grade (TID grade) was graded as follows: grade 0= no lesions or less than 5% of tubular lesions; grade 1= tubular lesions of less than 5%; fibrosis of less than 20% 5%>x<20%; grade 2= tubular lesions of less than 20%; fibrosis of less than 50% between 20%>x<50%, and grade 3= diffuse and severe tubular atrophy and fibrosis of greater than 50%, x> 50%. The tissue immunoreactivity of MMP-2, MMP-9, MMP-13, MMP-14 and TIMP-2 was assessed using a grading system based on the percentage of tubular epithelial cells with positive staining in the cytoplasm or cell membrane in the cortical area. The immunohistochemical staining results were divided into the following 4 categories based on the total percentage of tubular epithelial cells that stained positively; negative (-), = non-stained epithelial tubular cells or up to <20% of epithelial tubular epithelial cells with weak staining; weakly positive (+), ≥20%- to <40% of epithelial tubular epithelial cells with weak staining; moderately positive (++), ≥40% to <60% of epithelial tubular epithelial cells with intense staining; intensely positive (+++), greater than ≥60% of epithelial tubular epithelial cells with intense staining.

Table 1. Details of antibodies.

Antigen	Source	Clone	Dilution	Manufacturer
MMP-9	Human	MAB 3309	1:1000	Chemicon (Millipore)
MMP-2	Human	Ab-7	1:400	Neomarkers, Fremont, USA
TIMP-2	Human	MAB 3317	1:1000	Chemicon (Millipore)
MMP-13	Human	VIIIA2	1:100	Millipore Co., Billerica, USA
MMP-14	Human	-	1:200	Millipore Co., Billerica, USA

Immunostaining was scored by two independent observers, and discordant scores were re-evaluated by the investigators and the consensus score was used for further analysis.

For gelatine zymography, samples from renal cortex were stored at –20°C until use. MMP activity was studied by zymography, which reveals the gelatinase activity of latent pro-enzymes (zymogens) in addition to that of mature MMPs. Renal tissue was subjected to electrophoresis on 8% SDS-PAGE co-polymerised with 0.1% gelatine. Following electrophoresis, the gel was incubated for 1 h at room temperature in a 2.5% Triton X-100 solution, and incubated at 37°C for 16 h in Tris–HCl buffer, pH 7.4, containing 10 mM $CaCl_2$. The gels were stained with 0.1% Coomassie Brilliant Blue R-250, and then de-stained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue stained gelatine. Culture medium conditioned by A2058 melanoma cells was co-electrophoresed as a control to identify pro-MMP-9 gelatinolytic bands. Digestion bands were quantified using an image analyser system with GelDoc 2000 and Quantity One software (BioRad, Hercules, CA, USA). The Pearson and Spearman correlation coefficients have been used for measuring the strength of the linear relationship between three pair of variables (MMP-9 – Grade; TIMP-2 – Grade and MMP-2 – Grade).

Results

Four groups of seven dogs each covering all grades of tubulo-interstitial damage were selected for the study (Table 2).

The expression patterns of MMP-2, MMP-9, MMP-13, MMP-14 an(d TIMP-2 were investigated by immunohistochemistry to determine their localisation and staining intensity. In the normal tubular epithelial cells, a highly granular staining pattern of MMP-9 was detected primarily in the cytoplasm (Fig. 1a). In the fibrotic regions and in tubules with marked thickening of the tubular basement membrane, the diffuse loss of immunoreactivity for MMP-9 was evident (Fig. 1b,c). In addition, neutrophils and plasma cells exhibited positive

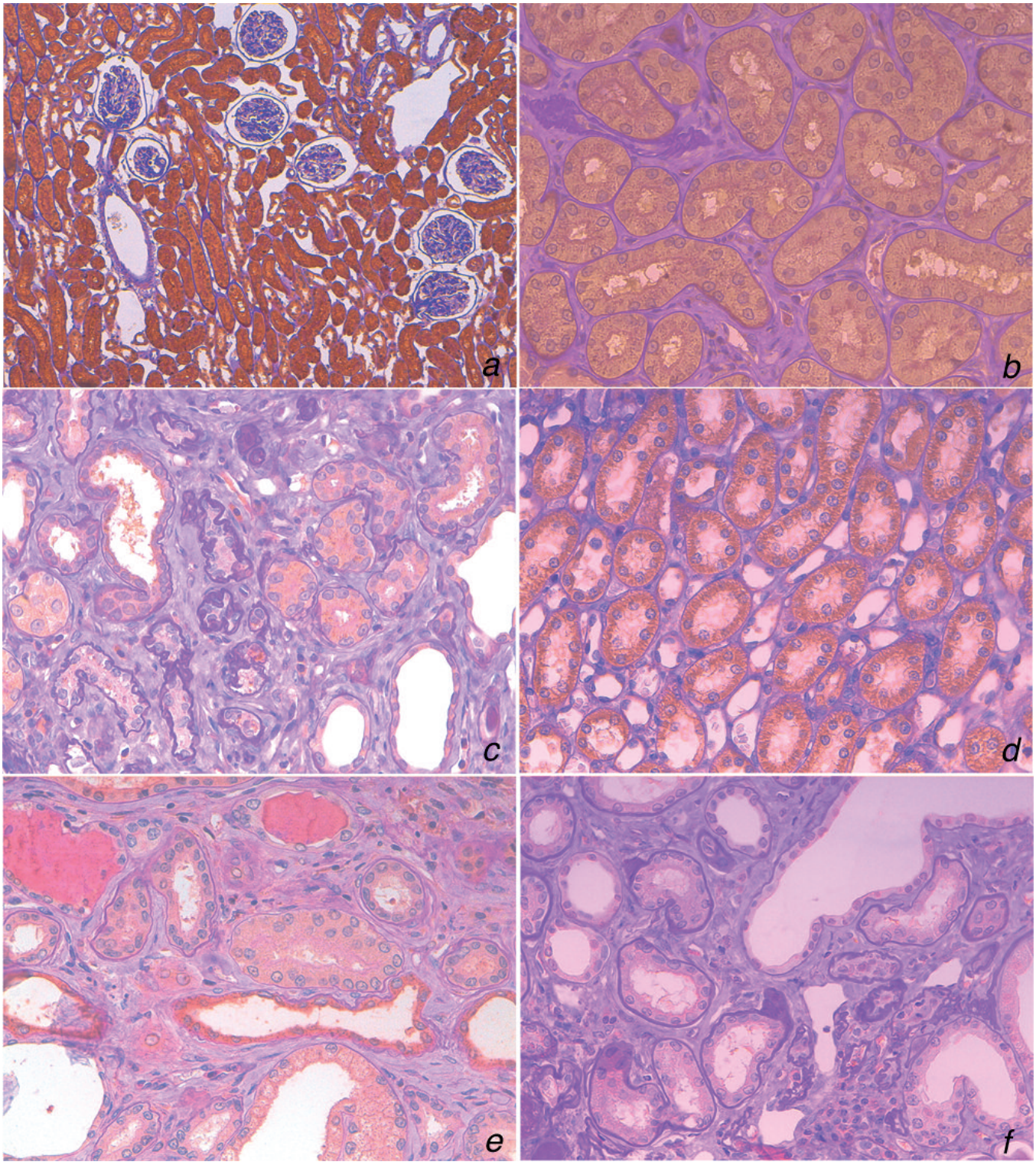

Fig. 1. Kidney sections of dogs with different TID grades. **a.** Control dog with strong immunopositive reaction (+++) to MMP-9. **b.** Immunolabelling for MMP-9 (++) in TID grade 1. **c.** Immunoreactivity for MMP-9 (+) in a fibrotic area and tubules with marked thickening of the tubular basement membrane in TID grade 2. **d.** Control dog with strong immunopositive reaction (+++) to TIMP-2. **e.** Immunolabelling for TIMP-2 (+) in TID grade 2. **f.** Loss of immunoexpression of TIMP-2 in tubules in area of fibrosis in TID grade 3. Immunohistochemestry PAS- counterstained. a, x 200; b-f, x 400

Table 2. Immunohistochemical results of MMP-9, MMP-2 and TIMP-2.

	CONTROL	GRADE 1	GRADE 2	GRADE 3
MMP-9	n=7 (%)	n=7 (%)	n=7 (%)	n=7 (%)
-	0 (0)	0 (0)	2 (28.6)	6 (85.7)
+	0 (0)	1 (24.3)	5 (71.4)	1 (24.3)
++	0 (0)	6 (85.7)	0 (0)	0 (0)
+++	7 (100)	0 (0)	0 (0)	0 (0)
MMP-2	n=7 (%)	n=7 (%)	n=7 (%)	n=7 (%)
-	7 (100)	6 (85.7)	5 (71.4)	4 (57.4)
+	0(0)	1 (24.3)	2 (28.6)	3 (42.6)
++	0 (0)	0 (0)	0 (0)	0 (0)
+++	0 (0)	0 (0)	0 (0)	0 (0)
TIMP-2	n=7 (%)	n=7 (%)	n=7 (%)	n=7 (%)
-	0 (0)	0 (0)	1 (24.3)	2 (28.6)
+	0 (0)	0 (0)	5 (71.4)	5 (71.4)
++	0 (0)	2 (28.6)	1 (24.3)	0 (0)
+++	7 (100)	5 (71.4)	0 (0)	0 (0)

immunoreactivity for MMP-9 (positive control). MMP-2 was found only in the pathological kidney (Fig. 2b). No immunoreactivity was shown by TEC in control kidneys and in TID grade 1 dog a minimal immunolabelling was observed in one dog (Fig. 2a). MMP-2 was localised to the cell surface and cytoplasm of scattered tubular epithelial cells. Notably, we observed a peculiar staining pattern for MMP-2 in variable-sized vesicles, corresponding to the area of basement membrane splitting (Fig. 2c,d). Localisation of TIMP-2 was comparable to MMP-9 (Fig. 1b). Data for MMP-9, MMP-2 and TIMP-2 are summarised in Table 2. Immunoreactivity for MMP-14 was occasionally found in a granular distribution, localised to the cytoplasm of tubular epithelial cells, but also diffusely detected in the cytoplasm of newly formed vascular endothelial cells and fibroblasts. Nuclear staining was rarely identified in

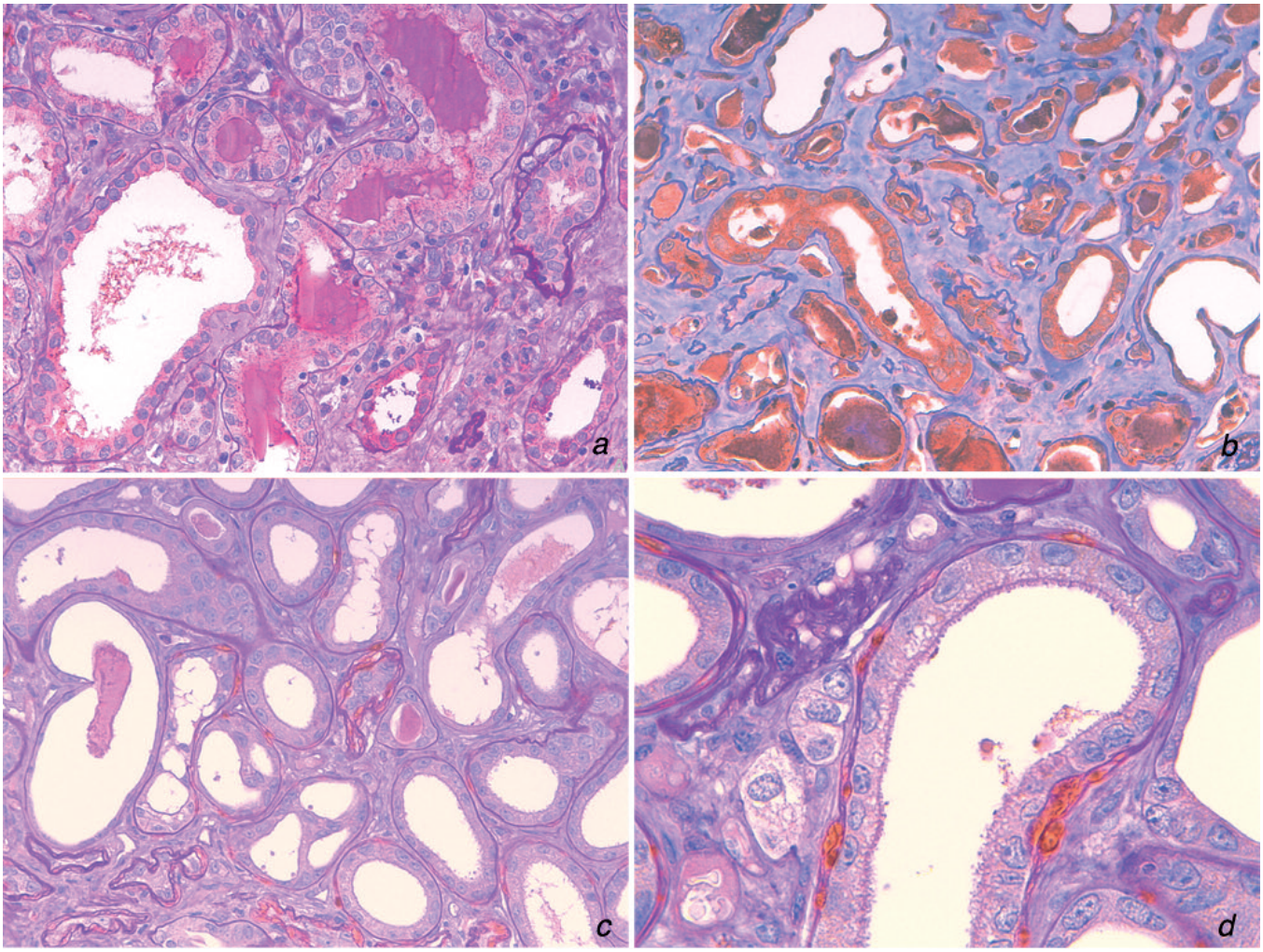

Fig. 2. Kidney sections of dogs with different TID grades. **a.** MMP-2 minimal immunolabelling (+) in TID grade 1. **b.** MMP-2 strong immunolabelling (+++) in TID grade 3. **c.** Localization of MMP-2 in variable-sized vesicles, corresponding to the area of basement membrane splitting in TID grade 2. **d.** Vesicles of MMP-2 in the tubular basement membrane in TID grade 2. Immunohistochemestry PAS- counterstained. a-c, x 400; d, x 600

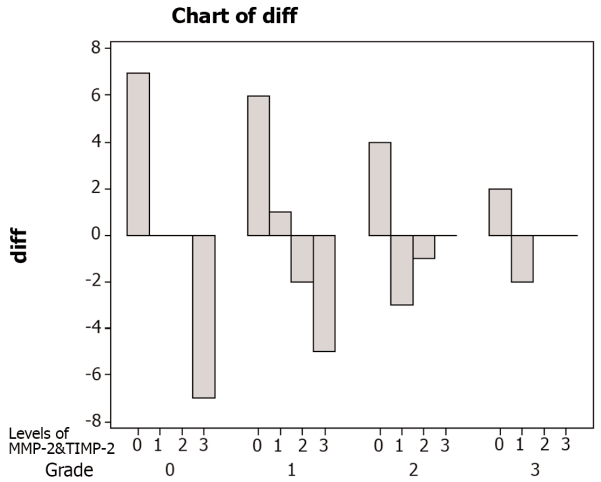

Fig. 3. Bar diagram of the differences of immunohistochemical results for MMP-2 and TIMP-2 according to the TID grade (immunohistochemical results for MMP-2 and TIMP-2 are represented numerically).

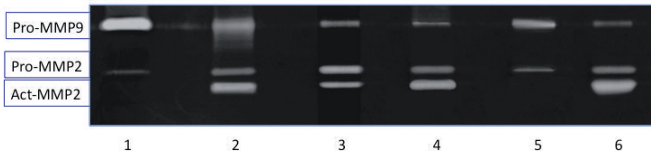

Fig. 4. Zymographic assay of gelatinase activity. Lanes 1 and 5: control dogs. Lanes 2, 3, 4, 6: dogs TID grade 3.

normal epithelium. In contrast to MMP-2 and MMP-9, MMP-13 showed no immunoreactivity in the tubular epithelial cells in all grades of damage. Statistical results for MMP-9-Grade are: Pearson's r -0.946638 and Spearman's rho -0.943821. Since both the indices are approximately -1, the relationship might be considered significantly linear, i.e. immunohistochemical expression for MMP-9 decreases compared to TID grade (Fig. 5a). Similar results are evident for TIMP-2-Grade (Pearson's r -0.877058 and Spearman's rho -0.879648). While results for MMP-2-Grade do not exhibit evidence of linear relationship (Pearson's r 0.389249 and Spearman's rho 0.389249) (Fig. 5b). We also plotted the differences of immunohistochemical results for MMP-2 and TIMP-2 (Fig. 3).

Gelatine zymography identifies and separates the gelatinases, MMP-2 and MMP-9, in both latent and active forms. Figure 4 shows the representative patterns of the gelatine zymogram of various tissue samples. Gelatinolytic activity of MMP-9 was near the detection limit in the three control kidneys. In the five kidneys examined with TID grade 3, we found mostly latent MMP-9 (95 kDa) and only minor amounts of active MMP-9 migrating at 82 kDa. MMP-2 specific activity was increased in pathological kidneys, while totally absent in controls. Indeed, pathological kidney extracts contained the latent form of MMP-2 at about 72 kDa, and considerable amounts of active MMP-2 were detectable.

Discussion

In the epithelial to mesenchymal transition, tubular epithelial cell injury results in the progressive loss of defined epithelial features, including disassembly of E-cadherin junctional complexes and ß-catenin, and

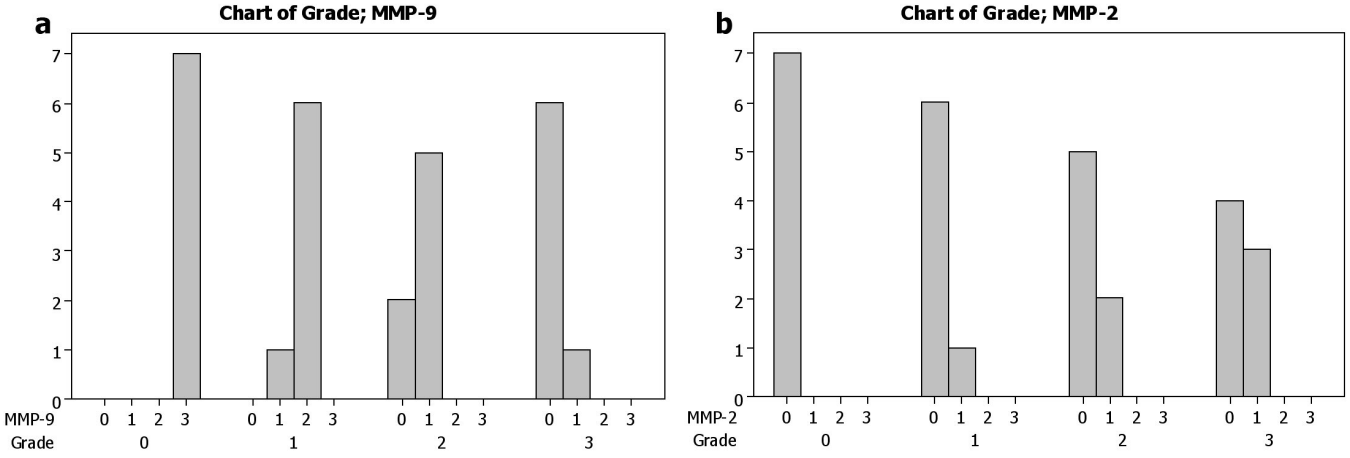

Fig. 5. Bar diagram of the immunohistochemical results for MMP-9 and TID grade **(a)** and MMP-2 and TID grade **(b)** according to the TID grade (immunohistochemical results for MMP-2 and TIMP-2 are represented numerically).

diminished cytokeratin expression. The loss of epithelial features is accompanied by the acquisition of a mesenchymal phenotype, including expression of vimentin (Kriz et al., 1998; Hewitson, 2009; Yabuki et al., 2009). Recent research has focused on the potential role of MMPs as key mediators of the dissolution of the tubular basement membrane, which in turn promotes tubular EMT, tubular atrophy, and progressive renal fibrosis (Zeisberg et al., 2001). Imbalances between the synthesis and degradation of glomerular extracellular matrix (ECM) proteins are thought to play important roles in the progression of renal fibrosis. The MMP family consists of about 18 members. MMP-1 and -13 are interstitial collagenases that degrade type-I and -III collagens, whereas MMP-2 and -9 are gelatinases that act on type-IV collagens (fibronectin and laminin are amongst their preferred substrates). The kidney also expresses 3 inhibitors of MMPs, TIMP-1, -2 and -3. TIMP-2 has preferential action on MMP-2 whereas TIMP-1 and -3 act on most MMPs. TIMP-1 and -2 directly inhibit the action of MMPs by binding to their free catalytic sites (McCawley and Matrisan, 2001; Mott and Werb, 2004).

We previously demonstrated that EMT in canine renal tissue correlated with the decreased expression of the cadherin-catenin system (Aresu et al., 2008). However, it remains unclear whether the loss of E-cadherin is a consequence or a cause of EMT. In previous studies, EMT was not found to be associated with interstitial invasion of TEC and most epithelial cells acquired mesenchymal phenotypes and remained within tubular structures. The aim of this work was to describe the role of the TBM in this transition with particular attention to localisation of MMPs. The crucial observation seems to be the localisation of MMP-2 to the tubular basement membrane splitting. The splitting of the membrane suggests an active role of this gelatinase in the disruption of type-IV collagen, the main basement membrane component. Observations in vitro emphasised the importance of basement membrane organisation in the development of EMT and that it can be induced in tubular epithelial cells after disruption of the tubular basement membrane by MMP-2 (Zeisberg et al., 2002; Cheng and Lovett, 2003). MMP-2 proteolytically cleaves denatured collagen (gelatine) and has some ability to cleave native type-IV collagen, the key structural component of the basement membrane. Type-IV collagen is the most abundant protein in basement membranes and acts as a scaffold to provide structural and functional integrity. By degrading type-IV collagen, gelatinases may also aggravate disease progression through EMT, and migration of phenotypically changed tubular cells through the damaged basement membrane (Endo et al., 2006). Enzymatic disruption of cell-cell or cell-matrix attachments has been proposed in different studies, but only as an alternative mechanism for induction of epithelial-mesenchymal transformation. The TBM provides a tightly regulated microenvironment for normal function of tubular epithelial cells and facilitates numerous cell-matrix interactions that are pivotal for the maintenance of epithelial phenotypes (Visse and Nagase, 2003). MMP-2 also facilitates the production of active TGF-1 protein, in a kind of vicious cycle that exists within most pathways involved in progressive kidney disease (Rao et al., 2005).

Results from gelatine zymography demonstrate that the active form of MMP-2 was particularly abundant in grade 3 fibrosis. This suggests a large conversion from latent to active form, which could be due to increased proteolytic or oxidative activation of this MMP.

Immunohistochemical expression of MMP-9 confirms that the reduction in activity of this specific gelatinase is associated with the progression of renal scarring and fibrosis. Several studies have reported predominantly intracellular MMP-9 staining in the tubular epithelium with a similar pattern as we describe here in dogs (Ahmed et al., 2007; Catania et al., 2007). Obviously, MMP-9 must be synthesised within the cell and be exported through the classical endoplasmic reticulum, Golgi apparatus, and secretary vessel route (Johnson et al., 2002). Unfortunately, the use of immunohisto-chemistry rather than higher resolution immuno-fluorescence makes it difficult to localise MMP-9 to these structures, which would suggest "storage before requirement" (Rao et al., 2003). However the diffuse cytoplasmic staining and the absence of extracellular staining raises the possibility that MMP-9 may have an additional role in the regulation of tubular epithelium and ECM. Nonetheless, one cannot exclude a role in the breakdown of immature ECM proteins before release. There are reports suggesting that MMP-9 may act as an anti-apoptotic factor (Lelongt et al., 2001; Laplante et al., 2003). MMPs and TIMPs may also influence cell proliferation and turnover. Various MMPs have been implicated in intracellular signalling, though the precise mechanism for these functions remains unclear. Therefore, more detailed investigation of the functions of these molecules in renal tubular cells is required. On the other hand, decreased MMP-9 production or activity, correlated with a reduction in immunohistochemical expression of MMP-9, can lead to decreased collagen degradation with a net accumulation of ECM proteins in the tissue. MMP-13, together with other metalloproteases, seems to particularly contribute to endochondral ossification, cell enlargement during chondrocyte hypertrophy and matrix calcification, and thus may be assumed to play a role in skeletal development, tissue remodelling and wound healing (Visse and Nagase, 2003). No significant data are present on the role of MMP-13 in fibrosis. In this study the immunostaining for MMP-13 was completely negative, excluding a possible role of this MMP with the other MMPs.

In conclusion, in this work we identified the role of TBM integrity in providing numerous cell-matrix

interactions that are pivotal for the maintenance of the epithelial phenotype. The data on MMP-2 could provide the basis for clinical trials examining the potential benefits of selective MMP-2 inhibitors in dogs with chronic kidney disease.

References

Ahmed A.K., Haylor J.L., El Nahas A.M. and Johnson T.S. (2007). Localization of matrix metalloproteinases and their inhibitors in experimental progressive kidney scarring. Kidney Int. 71, 755-763.

Aresu L., Rastaldi M.P., Pregel P., Valenza F., Radaelli E., Scanziani E. and Castagnaro M. (2008). Dog as model for down-expression of E-cadherin and beta-catenin in tubular epithelial cells in renal fibrosis. Virchows Arch. 453, 617-625.

Catania J.M., Chen G. and Parrish A.R. (2007). Role of matrix metalloproteinases in renal pathophysiologies. Am. J. Physiol. Renal Physiol. 292, 905-911.

Cheng S. and Lovett D.H. (2003). Gelatinase A (MMP-2) is necessary and sufficient for renal tubular cell epithelial-mesenchymal transformation. Am. J. Pathol. 162, 1937-1949.

Cheng S., Pollock A.S., Mahimkar R., Olson J.L. and Lovett D.H. (2006). Matrix metalloproteinase 2 and basement membrane integrity: a unifying mechanism for progressive renal injury. FASEB J. 20, 1898-1900.

El-Nahas A.M. (2003). Plasticity of kidney cells: role in kidney remodeling and scarring. Kidney Int. 64, 1553-1563.

Endo T., Nakabayashi K., Sekiuchi M., Kuroda T., Soejima A. and Yamada A. (2006). Matrix metalloproteinase-2, matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 in the peripheral blood of patients with various glomerular diseases and their implication in pathogenetic lesions: study based on an enzyme-linked assay and immunohistochemical staining. Clin. Exp. Nephrol. 10, 253-261.

Hewitson T.D. (2009). Renal tubulointerstitial fibrosis: common but never simple. Am. J. Physiol. Renal Physiol. 296, 1239-1244.

Inkinen K.A., Soots A.P., Krogerus L.A., Lautenschlager I.T. and Ahonen J.P. (2005) Fibrosis and matrix metalloproteinases in rat renal allografts. Transpl. Int. 18, 506-512.

Johnson T.S., Haylor J.L., Thomas G.L., Fisher M. and El Nahas A.M. (2002). Matrix metalloproteinases and their inhibitions in experimental renal scarring. Exp. Nephrol. 10, 182-195.

Kaissling B. and Le Hir M. (2008). The renal cortical interstitium: morphological and functional aspects. Histochem. Cell. Biol. 130, 247-262.

Kriz W., Hosser H., Hähnel B., Gretz N. and Provoost A.P. (1998). From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. Nephrol. Dial. Transplant. 13, 2781-2798.

Laplante A., Liu D., Demeule M., Annabi B., Murphy G.F., Daloze P., Chen H. and Béliveau R. (2003). Modulation of matrix gelatinases and metalloproteinase-activating process in acute kidney rejection. Transpl. Int. 16, 262-269.

Lelongt B., Bengatta S., Delauche M., Lund L.R., Werb Z. and Ronco P.M. (2001). Matrix metalloproteinase 9 protects mice from anti-glomerular basement membrane nephritis through its fibrinolytic activity. J. Exp. Med. 193, 793-802.

McCawley L.J. and Matrisian L.M. (2001). Matrix metalloproteinases: they're not just for matrix anymore! Curr. Opin. Cell. Biol. 13, 534-540.

Mott J.D. and Werb Z. (2004). Regulation of matrix biology by matrix metalloproteinases. Curr. Opin. Cell. Biol. 16, 558-564.

Rao V.H., Lees G.E., Kashtan C.E., Delimont D.C., Singh R., Meehan D.T., Bhattacharya G., Berridge B.R. and Cosgrove D. (2005). Dysregulation of renal MMP-3 and MMP-7 in canine X-linked Alport syndrome. Pediatr. Nephrol. 20, 732-739.

Rao V.H., Lees G.E., Kashtan C.E., Nemori R., Singh R.K., Meehan D.T., Rodgers K., Berridge B.R., Bhattacharya G. and Cosgrove D. (2003). Increased expression of MMP-2, MMP-9 (type IV collagenases/gelatinases), and MT1-MMP in canine X-linked Alport syndrome (XLAS). Kidney Int. 63, 1736-1748.

Visse R. and Nagase H. (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ. Res. 92, 827-39.

Yabuki A., Mitani S., Fujiki M., Misumi K., Endo Y., Miyoshi N. and Yamato O. (2009). Comparative study of chronic kidney disease in dogs and cats: Induction of myofibroblasts. Res. Vet. Sci. 88, 294-299

Zeisberg M., Bonner G., Maeshima Y., Colorado P., Müller G.A., Strutz F. and Kalluri R. (2001). Renal fibrosis: collagen composition and assembly regulates epithelial-mesenchymal transdifferentiation. Am. J. Pathol. 159, 1313-1321.

Zeisberg M., Maeshima Y., Mosterman B. and Kalluri R. (2002). Renal fibrosis. Extracellular matrix microenvironment regulates migratory behavior of activated tubular epithelial cells. Am. J. Pathol. 160, 2001-2018.

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