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Matrix metalloproteinase-9 deficiency attenuates diabetic nephropathy by modulation of podocyte functions and dedifferentiation

Szu-Yuan Li[1], Po-Hsun Huang[2], An-Hang Yang[3], Der-Cherng Tarng[4], Wu-Chang Yang[5], Chih-Ching Lin[5], Jaw-Wen Chen[6], Geert Schmid-Schönbein[7] and Shing-Jong Lin[8]

[1]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital and Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; [2]Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital and Institute of Clinical Medicine, and Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; [3]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital and Institute of Anatomy and Cell Biology, National Yang-Ming University, Taipei, Taiwan; [4]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital and Institute of Physiology, National Yang-Ming University, Taipei, Taiwan; [5]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital and School of Medicine, National Yang-Ming University, Taipei, Taiwan; [6]Department of Medical Research and Education, Taipei Veterans General Hospital, Institute and Department of Pharmacology, and Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; [7]Department of Bioengineering, The Institute of Engineering in Medicine, University of California, San Diego, La Jolla, California, USA and [8]Department of Medical Research and Education, Taipei Veterans General Hospital, Institute of Clinical Medicine, and Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan

Diabetic nephropathy is characterized by excessive deposition of extracellular matrix protein and disruption of the glomerular filtration barrier. Matrix metalloproteinases (MMPs) affect the breakdown and turnover of extracellular matrix protein, suggesting that altered expression of MMPs may contribute to diabetic nephropathy. Here we used an MMP-9 gene knockout mouse model, with *in vitro* experiments and clinical samples, to determine the possible role of MMP-9 in diabetic nephropathy. After 6 months of streptozotocin-induced diabetes, mice developed markedly increased albuminuria, glomerular and kidney hypertrophy, and thickening of the glomerular basement membrane. Gelatin zymographic analysis and western blotting showed that there was enhanced MMP-9 protein production and activity in the glomeruli. However, MMP-9 knockout in diabetic mice significantly attenuated these nephropathy changes. In cultured podocytes, various cytokines related to diabetic nephropathy including TGF-β1, TNF-α, and VEGF stimulated MMP-9 secretion. Overexpression of endogenous MMP-9 induced podocyte dedifferentiation. MMP-9 also interrupted podocyte cell integrity, promoted podocyte monolayer permeability to albumin, and extracellular matrix protein synthesis. In diabetic patients, the upregulation of urinary MMP-9 concentrations occurred earlier than the onset of microalbuminuria. Thus, MMP-9 seems to play a role in the development of diabetic nephropathy.

Kidney International (2014) **86,** 358–369; doi:10.1038/ki.2014.67; published online 26 March 2014

KEYWORDS: diabetes mellitus; diabetic nephropathy; matrix metalloproteinases; podocyte

Correspondence: *Po-Hsun Huang, Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital and Institute of Clinical Medicine, and Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan. E-mail: huangbs@vghtpe.gov.tw or Shing-Jong Lin, Department of Medical Research and Education, Taipei Veterans General Hospital, Institute of Clinical Medicine, and Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan. E-mail: sjlin@vghtpe.gov.tw*

Received 1 June 2013; revised 29 January 2014; accepted 30 January 2014; published online 26 March 2014

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD), and it affects 10–40% of diabetic patients.[1,2] Hyperglycemia, hypertension, and genetic predisposition are the main risk factors for the development of DN. However, glycemic control along with currently available pharmacotherapies may delay, but do not stop, the progression of DN toward ESRD.[3,4] Therefore, identifying the key signaling culprits of DN in order to explore novel therapeutic agent demands immediate attention.

Glomerular basement membrane (GBM) thickening and glomerular extracellular matrix (ECM) accumulation–induced Kimmelstiel–Wilson nodules are pathological hallmarks of DN.[5] Matrix metalloproteinases (MMPs) affect the breakdown and turnover of ECM, suggesting that altered MMP expression may contribute to DN. Among various MMPs, MMP-9 digests collagen IV of the basement membrane, and it has been documented as a central corpus in diabetic retinopathy[6,7] and tissue remodeling.[8–10] It is thus important to define whether and how MMP-9 could contribute to DN. It has long been recognized that tubulointerstitial lesions have an important role in the progression of DN,[11–13] and renal tubular cell dedifferentiation has been considered a critical

step in tubulointerstitial damage. Recent studies have indicated that podocytes also undergo dedifferentiation in DN, which causes foot process effacement, albuminuria, and ultimately results in glomerular sclerosis and kidney fibrosis.[14–17] Furthermore, it is known that a complex network of molecular signals is involved in cell dedifferentiation, and MMP-9 is able to induce renal tubular cell dedifferentiation in *vitro*.[18,19] Accordingly, in this study, we showed the influence of the targeted deletion of the MMP-9 gene in an animal model of DN; we used podocyte culture to reveal the potential stimulators of MMP-9 and investigated the effects of MMP-9 on podocyte cell functions. Finally, we tested the hypothesis that DN patients have higher urinary MMP-9 concentrations than non-DN patients, and that the upregulation of MMP-9 occurs earlier than the onset of microalbuminuria in DN patients.

RESULTS

Upregulation of intraglomerular MMP-9 activity in a DN mouse model

To clarify the expression pattern of MMP-9 in DN, we created a DN mouse model. As shown in Figure 1, there was a greater MMP-9 production and activity in glomeruli in diabetic mice and MMP-9 is co-stained with nephrin, a podocyte marker. As expected, no MMP-9 protein production or activity could be detected in glomeruli in MMP-9$^{-/-}$ mice. These findings indicated that induction of diabetes can stimulate MMP-9 activation and increase protein production in kidney glomeruli.

Deficiency of MMP-9 attenuates diabetic kidney injury

To determine the potential pathological role of MMP-9 in DN *in vivo*, we examined the severity of kidney injury in MMP-9$^{-/-}$ and MMP-9$^{+/+}$ mice after the development of diabetes. The blood sugar and hemoglobin A1c levels were comparable between the two diabetic groups (Supplementary Figure online). There was no difference in the 24-h urine albumin levels between nondiabetic MMP-9$^{-/-}$ and MMP-9$^{+/+}$ mice throughout the 6 months of the study. Urinary albumin was significantly elevated in diabetic MMP-9$^{+/+}$ mice starting from the second month of diabetes ($P<0.05$; Figure 2a), and the extent of urinary albumin progressively increased in diabetic mice through the fourth and sixth month ($P<0.01$). However, as shown in Figure 2a, the diabetic MMP-9$^{-/-}$ mice had significantly less 24-h urinary albumin than diabetic WT mice ($P<0.05$). After killing the mice at the sixth month, kidney glomerular volume was calculated. As shown in Figure 2b and c, diabetic mice had increased glomerular volume compared with nondiabetic mice ($P<0.01$, separately). However, diabetic

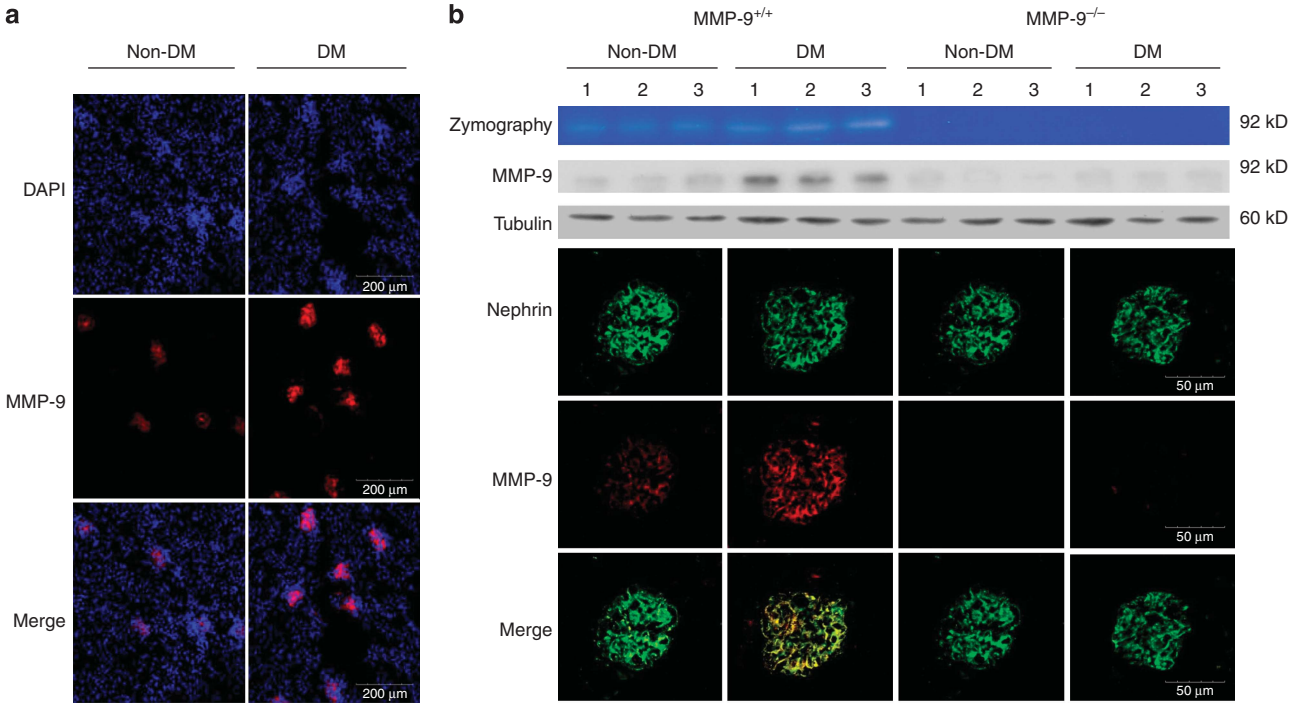

Figure 1 | Increased glomerular matrix metalloproteinase-9 (MMP-9) protein expression and catalytic activity in diabetic mice. (**a**) Immunostaining of MMP-9 in control and diabetic kidneys. MMP-9 was expressed in glomeruli and upregulated in diabetic nephropathy; 4,6-diamidino-2-phenylindole (DAPI) is used for nuclear counterstaining. (**b**) Zymography and western blot analysis from the sieved glomeruli lysate showed that both MMP-9 activity and protein expression were upregulated in MMP-9$^{+/+}$ mice after induction of diabetes (15 μg protein per well, $n=12$ each group, $P<0.01$). As expected, no MMP-9 enzymatic activity or protein expression was detected in MMP-9$^{-/-}$ mice with or without diabetes. Intraglomerular MMP-9 is costained with podocyte marker nephrin in wild-type (MMP-9$^{+/+}$) and MMP-9$^{-/-}$ mice. DM, diabetes mellitus.