EXPERIMENTAL CELL RESEARCH 316 (2010) 2993–3005

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Research Article

MT1-MMP-mediated basement membrane remodeling modulates renal development

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ARTICLE INFORMATION

Article Chronology:
Received 6 August 2010
Accepted 9 August 2010
Available online 19 August 2010

Keywords:
Branching morphogenesis
Basement membrane
Matrix metalloproteinases
Kidney

ABSTRACT

Extracellular matrix (ECM) remodeling regulates multiple cellular functions required for normal development and tissue repair. Matrix metalloproteinases (MMPs) are key mediators of this process and membrane targeted MMPs (MT-MMPs) in particular have been shown to be important in normal development of specific organs. In this study we investigated the role of MT1-MMP in kidney development. We demonstrate that loss of MT1-MMP leads to a renal phenotype characterized by a moderate decrease in ureteric bud branching morphogenesis and a severe proliferation defect. The kidneys of MT1-MMP-null mice have increased deposition of collagen IV, laminins, perlecan, and nidogen and the phenotype is independent of the MT-1MMP target, MMP-2. Utilizing *in vitro* systems we demonstrated that MTI-MMP proteolytic activity is required for renal tubule cells to proliferate in three dimensional matrices and to migrate on collagen IV and laminins. Together these data suggest an important role for MT1-MMP in kidney development, which is mediated by its ability to regulate cell proliferation and migration by proteolytically cleaving kidney basement membrane components.

Introduction

The kidney is composed of multiple filtering units known as nephrons that connect to collecting ducts which ultimately join together to form the ureter. The nephron, which consists of a glomerulus and highly differentiated tubules, is derived from the metanephric mesenchyme (MM), while the collecting system is derived from the ureteric bud (UB). Kidney development begins when the UB invades the MM which condenses and transforms into epithelial cells that ultimately form the nephrons. Simultaneously, the MM signals to the UB inducing it to undergo numerous iterations of branching morphogenesis [1].

The key function of the kidney is to maintain fluid and electrolyte homeostasis and to excrete toxins from the body, which it does by selectively filtering and reabsorbing solutes of different sizes and charges in the glomerulus as well as different nephron segments. Due to the complexity of its functions, the kidney has developed some of the most specialized basement membranes (BMs) in the body whose formation and turnover are tightly controlled both spatially and temporally. The major constituents of these BMs are the

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0014-4827/$ – see front matter
doi:10.1016/j.yexcr.2010.08.003

ECM proteins collagen IV, laminins and heparan sulphate proteoglycans [2]. A tight balance between extracellular matrix (ECM) synthesis and degradation is important for normal kidney development. Matrix metalloproteinases (MMP) are matrix degrading enzymes that play an important role for the normal development and turnover of these BMs.

MMPs are a family of zinc-dependent endopeptidases that are either secreted and diffuse to their sites of action or are bound to the cell surface where they mediate pericellular proteolysis [3]. Although numerous MMPs are expressed in the kidney, the most extensively studied are the gelatinases, MMP-2 and MMP-9, due to their ability to degrade collagens and laminins, which are major kidney ECM components. MMP-2 and MMP-9 are expressed in both the MM and the UB by day E11 in mice [4]. Blocking MMP-9, but not MMP-2, synthesis or activity inhibits UB branching in organotypic cultures [4], however when deoxynucleotides inhibited MMP-2 synthesis in E13 rat kidney cultures, decreased UB branching morphogenesis was observed [5]. Despite these *in vitro* findings, mice harboring targeted null mutations for MMP-2 [6], MMP-9 [7] or MMMP-2/MMP-9 [8] had no obvious renal abnormalities. Although MMP-9 was demonstrated to preserve vessel structure and alleviate blood pressure increases in a disease model of angiotensin-II induced hypertension [9], progression of anti-glomerular basement disease was not affected in either MMP-2 or MMP-9 null mice [10]. These minor or lack of effect on renal development or following renal injury suggest that, in addition to gelatinases, other MMP family members might modulate ECM turnover in the kidney.

MMP14, also referred to as MT1-MMP, which is the prototype membrane type (MT) MMP, has been studied in the context of renal development. This enzyme has intrinsic proteolytic capabilities and can also induce its effects by activating MMP-2 and MMP-13 [11]. Numerous ECM components, including collagens I, II and III, fibronectin, vitronectin, laminins 111 and 332, fibrin and proteoglycans are substrates for MT1-MMP [12]. In addition, MT1-MMP can cleave other cell surface proteins such as CD44 [13], transglutaminase [14], low-density lipoprotein receptor related protein [15], the integrin αv subunit [16], and syndecan-1 [17]. These highly divergent substrates for MT1-MMP make this enzyme a critical regulator of the pericellular environment and allow it to regulate multiple cellular functions. The physiological importance of MT1-MMP was demonstrated by the multiple abnormalities observed in the MT1-MMP null mice, which die shortly after birth with severe musculoskeletal abnormalities characterized by decreased chondrocyte proliferation and decreased collagenolytic activity [18,19]. More recent investigations on the musculoskeletal system have shown that reconstitution of MT1-MMP activity in the type II collagen-expressing cells of the skeleton in MT1-MMP null mice rescues the diminished chondrocyte proliferation in these mice and ameliorates the severe skeletal dysplasia by enhancing bone formation [20]. In addition, these null mice have submandibular gland branching morphogenesis abnormalities [21] as well as defects in lung development [21,22], angiogenesis [23] and myeloid cell fusion [24]. These deficiencies are ascribed to a lack of MT1-MMP catalytic ability, alterations in downstream pro-MMP-2 activation and alterations in cell functions regulated by the MT1-MMP cytoplasmic tail.

MT1-MMP is widely expressed in the kidney and is found in the UB at E11 and the MM at E12 [25]. Like the gelatinases, MT1-MMP function was shown to be required for UB branching morphogenesis in kidney organ cultures, where it induced its affects, at least in part, by activating MMP-2 [5]. In contrast to the gelatinase null mice, we previously described subtle, but distinct renal abnormalities in 10-week-old out-bred MT1-MMP mice, which were characterized by a proportional decrease in both cortical and medullary mass [26]. Both the glomeruli and the tubules were slightly dysmorphic and these renal abnormalities correlated with an increase in laminin 332 deposition, suggesting that lack of laminin 332 cleavage by MT1-MMP accounted for these abnormalities [26].

Although these data defined a role for MT1-MMP in renal development and suggested its role was the cleavage of at least one ECM component in renal BMs, the mechanisms whereby the renal abnormalities occur is unclear. We therefore explored the role of MT1-MMP in renal development in more detail and demonstrate that when MT1-MMP null mice are bred onto a pure C57/B6 background, they die at P14 with small kidneys due to a severe proliferative defect and a moderate UB branching abnormality. We show that MT1-MMP does not activate MMP-2 in the kidney *in vivo* and the proteolytic activity of MT1-MMP is required for normal UB branching in *in vitro* organ culture models. We further demonstrate increased deposition of laminins, collagen IV, nidogen and perlecan in MT-MMP-null kidneys. Utilizing MT1-MMP deficient renal tubular epithelial cells we show that MT1-MMP proteolytic activity is required for normal cell migration on ECM components and proliferation in 3 dimensional gels. Thus our results suggest that pericellular cleavage of multiple BM components by MT1-MMP is important for cell proliferation and migration and plays a critical role in normal kidney development.

Fig. 1 – Kidneys from MT1-MMP-null mice are dysmorphic and dysgenic. (A–F) Microscopy of hematoxylin and eosin stained kidney slides taken from P10 mice show decreased size of the MT1-MMP null (Null) relative to wild type (WT) mice (100×) (A–B). Loosely packed, dilated and dysmorphic tubules were present in the papilla (400×) (C–D) and the cortico-medullary junction is poorly delineated and fewer glomeruli were evident in the MT1-MMP null kidneys (400×) (E–F). (G) The number of glomeruli in null and WT animals were determined as stated in the methods and expressed as the average±the standard deviation. A significant difference in the number of glomeruli between 5 mice from each genotype was present ($p<0.05$). (H–K) Hematoxilin and eosin stained slides of E13.5 (100×) (H-I) and E17.5 (100×) (J–K) shows that the MT1-MMP kidneys were smaller with a moderate UB branching defect. (L–N) E12.5 MT1-MMP and wild type kidneys were isolated, grown on transwells and stained for E-cadherin as described in Materials and methods (L–M). The number of branches in 10 kidneys of MT1-MMP null and WT mice were counted and expressed as the average± the standard deviation. There was a significant difference in branch number between genotypes ($p<0.05$). (O–P) Cell proliferation in newborn kidneys from MT1-MMP null and WT mice was defined by Ki67 staining. The number of cells in 10 high powered fields in 4 mice per genotype was counted. The average and standard deviation is presented. * denotes a significant difference in proliferating cell number between genotypes ($p<0.05$) (P).