THE IMPORTANCE OF MT1-MMP DURING RENAL DEVELOPMENT

By

Karen S. Riggins

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Cancer Biology

December, 2010

Nashville, Tennessee

Approved:

Raymond Harris

Billy Hudson

Vito Quaranta

Ambra Pozzi

Roy Zent

ORIGINAL PUBLICATION

Riggins, K.S., Mernaugh, G., Su, Y., Quaranta, V., Seiki, M., Koshikawa, N., Pozzi, A., Zent, R. (2010) MT1-MMP-mediated basement membrane remodeling modulates renal development. <u>Experimental Cell Research</u>. *In press.*

DEDICATION

Dedicated to the ultimate source of my Strength and Covering. May my life bring you Glory and Honor.

ACKNOWLEDGEMENTS

To the entire Zent lab, thank you for creating such a warm and pleasant environment. I would like to especially thank Dr. Glenda Mernaugh, Dr. Nada Bulus, Dr. Dong Chen, Dr. Xi Zhang, and Dr. Leslie Gewin; your friendship through the years has been priceless. I owe you all so much; you have been a network of support, both personally and professionally. I acknowledge you for your graciousness.

To my IGP sisters, Sydika McKissic, Kimberly Mulligan, Christina Williams, and Robin Bairley, thank you for requiring that I find a balance between research and recreation. You are phenomenal women and you all deserve the very best that life has to offer. Your genuine concern and unprovoked compassion has imprinted an everlasting memory. I acknowledge you for your support.

To my Mount Zion church family, I thank you for your prayers and constant words of encouragement. I want to especially thank my Minister-In-Training colleagues. You have helped me develop in my purpose and calling. I acknowledge you for your encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

LIST OF ABBREVIATIONS

MT1-MMP: Membrane Type 1-Matrix Metalloproteinase

MMP: matrix metalloproteinase

UB: ureteric bud

IMCD: innermedullary collecting duct

MDCK: Madin-Darby Canine Kidney

ADPKD: Autosomal Dominant Polycystic Kidney Disease

MET: mesenchymal-to-epithelial transition

TIMP: tissue inhibitor of matrix metalloproteinase

WT: wildtype

BM: basement membrane

ECM: extracellular matrix

GBM: glomerular basement membrane

Ln: Laminin

Ln-332: Laminin-332; formerly Ln-5

Col: Collagen

PG: Proteoglycan

HSPG: heparan sulfate proteoglycans

GAG: Glycosaminoglycan

GDNF: glial-derived neurotrophic factor

RET: rearranged during transfection protooncogene

HGF: hepatocyte growth factor

c-MET: mesenchymal-epithelial transition factor

MAPK: mitogen-activated protein kinase

ERK: extracellular-regulated kinase

H-JEB: Herlitz junctional epidermolysis bullosa

siRNA: small interfering ribonucleic acid

EDTA: disodium ethylenediamine tetra-acetate

H&E: hematoxylin and eosin

HRP: horseradish peroxidase

mg: microgram

μl: microliter

ml: milliliter

μm: micrometer

mM: millimolar

CHAPTER I

INTRODUCTION

Overview

This introductory chapter of my thesis discusses the processes involved in kidney development and describes the influence of growth factors, basement membrane composition, and basement membrane remodeling in renal development. This chapter will conclude with a detailed focus on membrane type 1 matrix metalloproteinase (MT1-MMP) and its speculated role in kidney development. Chapter II is the manuscript which describes our *in vivo* and *in vitro* findings which show that "MT1-MMP-MEDIATED extracellular matrix (ECM) REMODELING REGULATES NORMAL RENAL DEVELOPMENT." Chapter II will conclude with a discussion of the future directions proposed for this body of work.

Background and Motivation

The renal system is involved in a large proportion of childhood congenital abnormalities. Abnormalities of kidney and urinary tract development are the most common cause of renal failure in childhood in the United States, comprising 31% of children with end-stage kidney disease (Reidy K 2009). Our recent work on matrix metalloproteinases in mouse models has shown that these molecules may be involved in

the development and resolution of some of these kidney diseases, in particular renal dysplasia (abnormal tissue development) or hypoplasia (inadequate tissue development) (Koshikawa 2004).

Matrix metalloproteinases are a family of more than 20 zinc-dependent proteins that exist in the extracellular matrix of all tissues. Based on sequence homology and substrate specificities, the MMPs fall into several subgroups including collagenases, gelatinases, stromelysins, matrilysins and the membrane-type metalloproteinases. There is considerable overlap in substrate specificities, and the MMPs play a an important role in the degradation of most ECM components, including laminins, collagens and fibronectin. The MMPs also affect the release and turnover of cytokines and cell surface receptors of adjacent cells (Somerville RP 2003).

Cells secrete most MMPs as soluble enzymes into the extracellular milieu; however, some MMPs are membrane-bound and called the membrane-type metalloproteinases (MT-MMP). Despite *in vitro* evidence that some soluble MMPs play an important role in renal development, no mice lacking this class of MMPs have shown significant renal phenotypes. There is still significant disagreement in the community concerning the experimental and physiological relevance of MMPs in kidney development. The global focus of my research seeks to address this discrepancy by assessing the importance MT1-MMP-mediated extracellular matrix turnover on renal development.

Kidney Development

The kidneys are an essential part of the urinary system that function to regulate electrolytes and blood pressure, maintain acid-base balance, and excrete toxins from the body. Each kidney is comprised of two regions; the outer most region of the cortex and the inner medulla. Nephrons are the functional filtering units of the kidney and a largely contained within the cortex while the collecting duct system which carries the urine from the kidney through the ureter and into the bladder is housed in the medulla **(Figure 1)**. This complex structure derives from two distinct embryonic cell types. This part of the thesis will discuss the process of kidney development and some of the molecules and programs that are critical for mammalian renal development that forms the basis of my work.

Kidney development is a highly regulated process that involves the concerted action of growth factors, integrins, extracellular matrix components, and matrix metalloproteinases. In 1987, Lauri Saxén wrote a comprehensive review on kidney development that has become one of the best-known and most-cited works in the field (Davies J 2002). Strikingly, it contained no mention of the role of metalloproteinases (MMPs) and matrix turnover during renal development. Even today, the role of MMPs in development is poorly acknowledged because it is not well understood.

The original studies of kidney development involved descriptions of the morphologic changes, including seminal work by Edith Potter on human fetal kidneys (Holliday 1994). From this and more recent studies, we know that there are 2 embryonic

kidney transitory precursors that are vestiges of the nephrogenic cord: the pronephros and

mesonephros (Kanwar YS 2004).
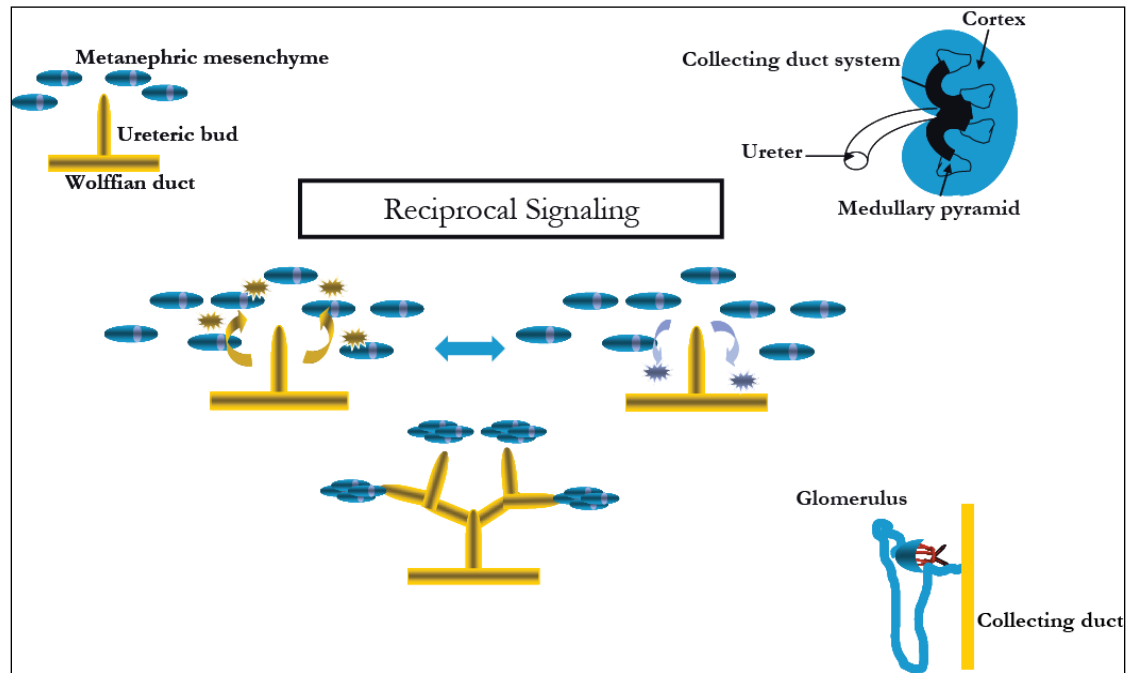
Figure 1: Reciprocal Signaling during Renal Development
Adapted from Gomez 1999

The pronephros consists of simple tubules that empty into a pronephric duct. As

the pronephros begins to regress, the mesonephros arises at embryonic days 10-11 in the

mouse and at about week 5 of gestation in humans (Saxėn 1987). The mesonephros will

fuse with the cloaca near the end of the development and contributes to formation of the

urinary bladder.

The last embryonic kidney, the metanephros, forms as the ureteric bud branches

out of the caudal end of the Wolffian duct. During meta-nephrogenesis, the ureteric bud

develops as an epithelial-lined tubular out branch of the Wolffian duct. Reciprocal

interactions occur between the ureteric bud and the metanephric mesenchyme. The undifferentiated mesenchymal tissue condenses to form nephrons in response to signals from the tip of each successive branch of the ureteric bud (Hartman HA 2007).

During reciprocal signaling, subsets of cells within the mesenchyme form condensates and develop an epithelial phenotype (known as a mesenchymal-epithelial transition or MET) **(Figure 2)**. These cells mature into well-developed nephrons with vascular glomeruli connected to proximal and distal tubules that drain into ureteric bud. As the cells mature through MET, they undergo a sequence of morphologic changes, starting as a sphere of cells called the vesicle, becoming a comma-shaped body, and then an S-shaped body. Three segments of the S-shaped body emerge, oriented with the distal segment adjacent to the ureteric bud tips: the proximal segment differentiates into the glomerular epithelial cell (podocytes), the midsection forms the proximal tubule and loop of Henle, and the distal segment becomes the distal tubule and joins with the ureteric bud branches. Vascular development in the kidney occurs concurrent with glomerular development (Sariola 1985; Eremina 2007).

The ureteric bud undergoes arborization where each branch subsequently induces the production of one nephron. The induction of nephrons in mice continues until ~1 week after birth and then ceases once the full complement of nephrons has formed (Saxėn 1987). Mice typically reach kidney maturity having 10,000-100,000 nephrons. These UB branches will eventually form the collecting system, including collecting ducts, renal pelvis, ureter, and bladder trigone in the adult kidney. While mammalian kidney morphogenesis is well understood, the molecules involved continue to be the focus of our group.

Modulation of the ECM is critical for the normal growth and development of embryonic renal tissue. Recent work on matrix metalloproteinases (MMPs) suggest that these molecules may be involved in this ECM modulation. Our work focuses on the importance of the MMPs, particularly MT1-MMP during renal development. MMPs are a family of Zn^{2+} dependent proteases which collectively can cleave most of the components of the ECM and will be discussed in detail, later. The following discussion of kidney development is intended to focus on molecular programs relevant to this investigation.
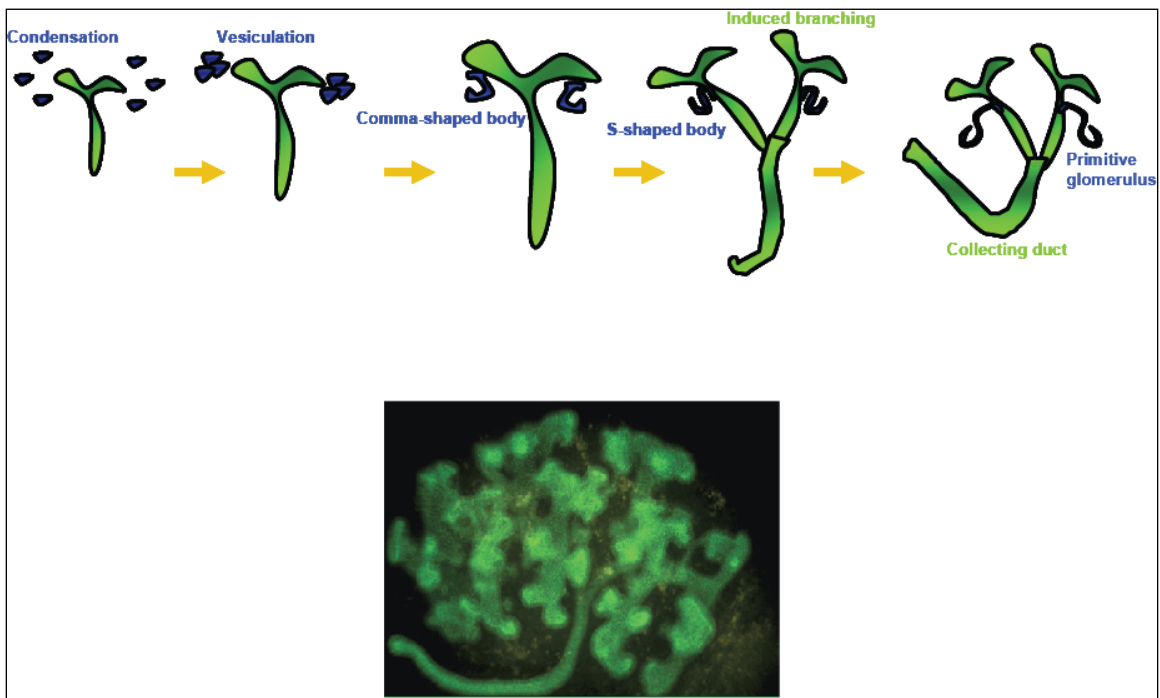
Figure 2: Development of the UB and mesenchyme
Adapted from Horster 1999

Molecular programs which regulate kidney development

Influence of Growth Factors

Numerous growth factors are significant during renal organogenesis, namely bone morphogenic proteins, fibroblast growth factor, glial cell–derived neurotrophic factor (GDNF), and hepatocyte growth factor (HGF) pathways. While I will not discuss every growth factor that has been ascribed a role, it is important to discuss certain growth factor programs activated during renal development that are relevant to my study. Recent evidence has even linked GDNF and HGF, two major growth factor receptors, to MT1-MMP activity.

GDNF

The metanephric mesenchyme cells produce GDNF, which is an important stimulant of ureteric bud branching. GDNF signals are important for ureteric bud cell proliferation, cell survival and branching of the epithelium. Although GDNF is a major trophic factor for bud branching, its effects are modulated by several other growth factors as well as inhibitors of bud branching (Reidy K 2009).

Renal cells in the Wolffian duct and later at the tips of the ureteric bud coexpress two receptors that GDNF acts through: the receptor tyrosine kinase RET (REarranged during Transfection protooncogene) and the coreceptor GDNF family receptor-α1. The ablation of GDNF or RET genes in mice results in the absence of the kidney, due to failure of ureteric bud outgrowth, or severe malformation, as a result of limited ureteric bud branching (Costantini 2006). In humans, RET mutations coincide with Hirschsprung

8

disease, cancer, and renal agenesis. For example, Skinner and colleagues, found RET

mutations in 7 of 19 stillborn human fetuses with bilateral renal agenesis (37%) and 2 of

10 fetuses (20%) with unilateral agenesis (Skinner MA 2008).

While the RET/GDNF pathway largely controls ureteric bud outgrowth and

branching morphogenesis; the exact mechanisms by which this occurs remain unknown.

GDNF regulates a number of genes, but none of them can account for effects of GDNF

on ureteric bud morphogenesis (Lu BC 2009). For this reason, Frank Costitini's group

conducted a genome-wide analysis of mRNA expression in isolated ureteric buds

cultured with or without GDNF. Among the genes identified were two transcription

factors, *Etv4* (*Pea3*) and *Etv5* (*Erm*). *Etv4* and *Etv5* are important in neuronal,

spermatogonial and limb development, but prior research had not investigated a role in

renal development. By analyzing the renal development of genetic crosses of mice

lacking three of the four *Etv4* and *Etv5* alleles, Costitini's group showed that reduced

ureteric bud branching caused moderate to severe defects in renal development.

Furthermore, they showed that mice lacking all four alleles do not develop kidneys. In

the same study, researchers found that in the hypoplastic kidneys of *Etv4$_{-/-}$*;

*Etv5$_{+/}$*compound mutants three genes showed greatly reduced expression: *Cxcr4*

(chemokine receptor), *Met* (HGF receptor), and *Mmp14*(MT1-MMP). The reduced

expression of these genes indicates that they are direct or indirect targets *Etv4* and *Etv5*

(Lu BC 2009).   These studies establish the first link between MT1-MMP and GDNF and

allude to the importance for MT1-MMP in early renal organogenesis.

<u>HGF</u>

The interaction of mesenchyme-derived HGF with its receptor tyrosine kinase c-MET (mesenchymal-epithelial transition factor) are required for ureteric bud elongation in renal development. The current literature holds that this HGF/c-MET system plays an essential role in cell growth, cell differentiation, organ regeneration, embryogenesis, and tumorogenesis (Liu 2002). Treating cultured embryonic kidneys with anti-HGF antibodies or inhibiting their endogenous HGF activator causes their UB development to stop through the inhibition of mesenchymal differentiation, increased cell death and perturbation of UB branching (Woolf 1995).

Additionally, embedding Madin Darby Canine Kidney (MDCK) epithelial cells in collagen matrix allows them to form branching tubules under HGF stimulation. However, inhibiting MT1-MMP expression in MDCK cells in otherwise identical conditions eliminates the formation of these branching tubules (Kadono Y 1998).

Research has shown that HGF can also induce several intracellular signaling pathways during branching. Kadono and colleagues showed that MT1-MMP was involved in one of these pathways and suggested a role for the protease in kidney tubulogenesis (Kadono Y 1998). Studies have confirmed similar analyses in cultured endometrial carcinomas (Park 2003) and mesothelioma cell lines(Harvey P 2000).

The literature has just begun to suggest a possible link between the role of MT1-MMP and growth factors during renal development **(Figure 3)**. It is possible that MT1-MMP could act synergistically with growth factors but research is lacking to support a definite link. As the role of GDNF and HGF have been defined by several groups, a

better understanding of the role of MT1-MMP during renal development is necessary in order to support the hypothesis of MT1-MMP and growth factor associations.
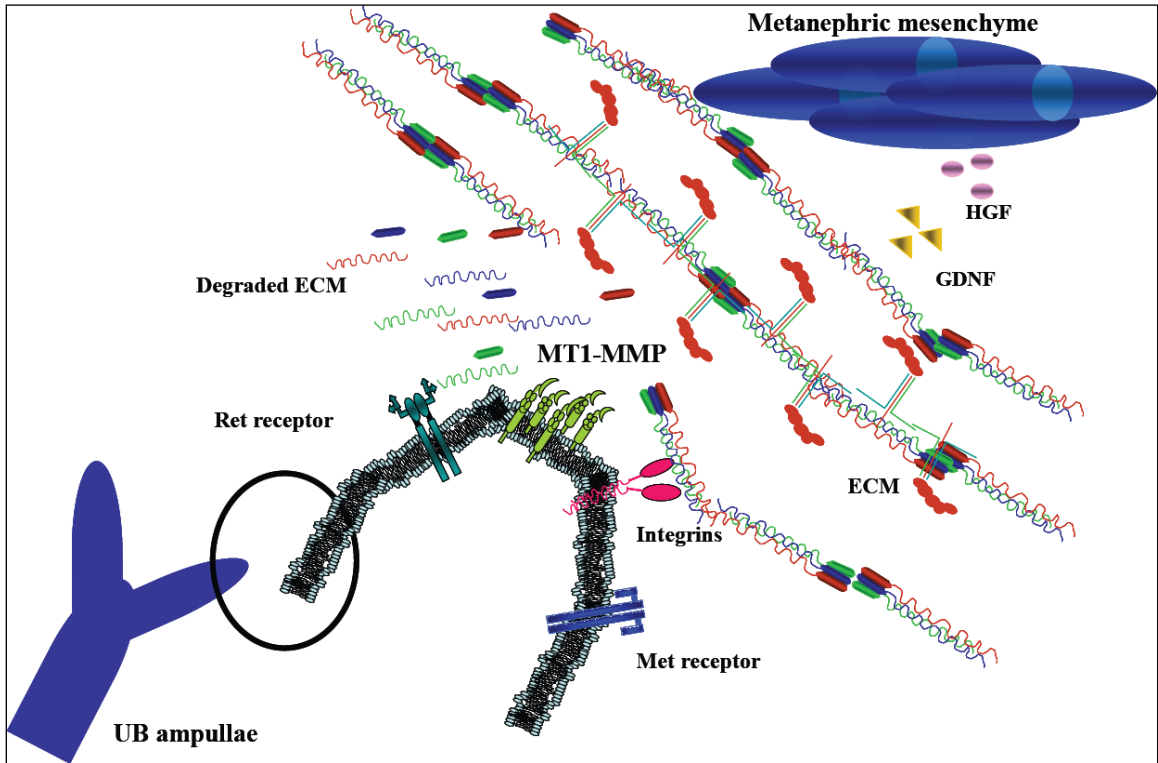
Figure 3: Cell-ECM Interaction in Renal Development

Influence of Basement Membrane

As early as 1955, Clifford Grobstein proposed that one tissue might induce another to develop through the presence of ECM (Grobstein 1955). He was able to show that ECM, alone, could induce mouse salivary gland tissue to differentiate (Powell 2005). This study and the work of others provide strong evidence supporting the idea that the ECM may provide many of the cues for cells to alter cellular function in organogenesis.

The ureteric bud lies proximal to a milieu of ECM components, some made by the bud and some by the surrounding mesenchyme. Immunohistochemistry can detect changes in the composition of the ECM of the mesenchyme, which shows that the ECM is dynamic (Ekblom 1980; Ekblom 1981). Specifically, an enhanced synthesis of a set of epithelial-type proteins, (namely collagen type IV, laminin, heparan sulphate proteoglycan, and entactin/nidogen) replace the interstitial proteins fibronectin, collagen type I, and collagen type III as they are removed from the condensed areas (Saxėn 1987).

The diverse composition of the ECM allows it to have uses well beyond its static function as the physical barrier that segregates adjacent tissues. Not only does the literature show that the ECM provides support and anchorage for cells and regulates intercellular communications, several studies have provided evidence to suggest that the ECM also influences a variety of epithelial cell behaviors including proliferation, differentiation, and morphogenesis—all of which are important processes during renal development.

Basement Membrane Composition

Basement membranes are specialized extracellular matrices 50-100 nm in thickness and found throughout the body. They play particularly important roles in the kidney, as demonstrated by the fact that defects in renal basement membranes are often concurrent with kidney malfunction. The kidney exhibits four different types of continuous basement membrane (BM) encasing every nephron. These types are tubular, glomerular, and vascular BMs as well as the BM surrounding the Bowmen's capsule. This regional heterogeneity makes the kidney an ideal model system in which to

investigate the importance of BM composition. In general, basement membranes contain

four main components: type IV collagen, laminin, nidogen, and the proteoglycan perlecan

(Breitkrentz D 2009). I will briefly describe each BM component and explain its relative

significance according to the current literature.

Collagen IV

As with all collagens, type IV collagen consists of three α chains coiled around

one another to form a triple helical molecule or protomer. In vertebrates, the genes

COL4A1-COL4A6 encode six distinct versions of these α(IV) chains, allowing for 56

possible protomer combinations. Despite all of these possible associations, there are only

three known protomers α1.α1.α2(IV), α3.α4.α5(IV), and α5.α5.α6(IV).   Collagen

protomers associate with each other outside of cells to form a two-dimensional meshwork.

This provides a scaffold for assembly of the basement membrane.

The α1.α1.α2(IV)–α1.α1.α2(IV) network is ubiquitous and expressed in all

basement membranes of immature nephrons during embryonic development. During

glomerular maturation, a switch in collagen IV networks occurs in the glomerular

basement membrane (GBM) wherein the α3.α4.α5(IV) trimer replaces the α1.α1.α2(IV)

trimer (Hudson 1993). The α1.α1.α2(IV)–α5.α5.α6(IV) network is prevalent in the

Bowman's capsule and in the collecting duct basement membrane (JH. 1998; Miner 1998;

Hudson BG 2003).

While BM formation does not require type IV collagen, many experts believe that

the type IV collagen network confers BMs with their structural integrity. α1.α1.α2(IV)-

null mice display a defective placental structure and embryonic hemorrhaging. They

subsequently die at E10.5-E11.5 (Pöschl E 2004). This study suggests that Collagen IV is

fundamental for the maintenance of integrity and function of basement membranes under conditions of increasing mechanical demands

Other studies have shown that mutations in type IV collagen genes cause hereditary glomerular diseases. Alport's syndrome results from the production of post-translational defects in α3(IV), α4(IV), or α5(IV) chains, which lead to rapid degradation of the protein. These mutations arrest the normal developmental switch and cause the persistence of α1.α1.α2(IV) networks in glomerular basement membrane (Hudson BG 2003). Furthermore, Lemmink and others have shown that a point mutation in the α4 chain gene is responsible for thin GBM disease, which many texts describe as autosomal dominant benign familial hematuria (Lemmink 1996).

Goodpasture syndrome (or anti-glomerular basement membrane disease) is an autoimmune disorder involving a deficiency in collagen IV. Individuals with this disease develop antibodies against the NC1 domain of α3 (Butkowski 1987; Saus 1988; Turner 1992; Kalluri 1996). As a major component of the GBM, attack on type IV collagen α3 leads to glomerulonepritis, which can result in renal failure and even death. As the absence of specific collagen IV chains promotes disease, it is likely that this component of the BM is more than a barrier, as it plays an actual role in kidney function.

As collagen IV provides the basement membrane with most of its structural integrity, the expression of type IV collagenase is critical for developing tissues that require migration through the BM. Most notably, soluble MMP-2 and MMP-9 are classified as type IV collagenases and their roles in renal development will be discussed later in this chapter. In a mouse model of Alport's syndrome, researchers found that inhibition of MMPs early in the disease, before the buildup of proteins in the urine,

reduced symptoms (Schubert 2006). Furthermore, the MMP-2 that degrades type IV collagen is also activated by MT1-MMP (Sato 1994) but there has been much debate over the capabilities of MT1-MMP to directly degrade collagen IV as studies have both suggested (Ohuchi 1997; Hotary 2006; Hotary K 2007) and denied (Itoh 2006) MT1-MMP type IV collagenase abilities. As it is clear that collagen IV is a major contributor to renal BM integrity, a study of collagen IV in reference to MT1-MMP activity is important for our study.

Laminins

Another key component for BM formation is the laminin (Ln) family. In mammals, there are at least 15 Lns derived from five α, three β, and three γ subunits (Li 2003). While immunohistochemical assays have found that the highest numbers of laminin chains are in the kidney, research has uncovered only a few laminins that affect renal development. These laminins are laminin-111 (Ln-111), laminin-511 (Ln-511), laminin-521 (Ln-521), and laminin-411 (Ln-411).

During development, a basement membrane containing Ln-111 and Ln-511 binds the ureteric bud. Meanwhile, an assembly of a nascent basement membrane containing primarily Ln-111, but also Ln-411, accompanies the MET of the nephron-bound cells (St John PL 2001). Finally, as the endothelial cells of the developing glomerulus invade the wall of the tissue to form capillaries adjacent to epithelial cells, Ln-521 replaces Ln-111 in a developmental transition. In addition to the complexity of the temporal laminins in the kidney, there is a difference in the spatial expression of laminin chain in the various segments of the kidney. Sophisticated studies using *in vitro* blocking antibodies as well

15

as targeted gene deletions in mice model system have uncovered a role for several of the laminin chains, namely lamα1, lamα4, lamα3, lamα5, lamβ2 and lamγ1 chains. As MT1-MMP can cleave Ln-111 (Pei 1996), Ln-332 (Koshikawa 2004), Ln-511(Bair 2005), I will briefly discuss the laminin chains that are relevant to our study.

Ln-111

The inactivation of the lamγ1 chain demonstrates the importance of laminins during development. This inactivation prevents the formation of 10 out of the 14 known laminin isoforms. Mice homozygous for the mutation lack basement membranes and die at 5.5 days *post coitum* through a failure of ectodermal and endodermal cell differentiation (Smyth 1999; Murray 2000). Classic experiments that use antibodies to lamα1 (of Ln-111 and Ln-121) to block either assembly of or cell interaction with Ln-111 in cultured metanephroi inhibit mesenchymal to epithelial transition through this technique (Klein G 1988).

Ln-332

Our lab has shown that laminin-332 is expressed within the developing UB and required for normal UB branching morphogenesis in whole embryonic kidney organ culture as well as isolated UB culture (Zent 2001). When a well-characterized functional blocking antibody directed against laminin-332 was used in normal UB development was inhibited in both whole-kidney and isolated UB culture. Comparatively, mice with targeted disruption of the lamα3 gene have a skin blistering defect that mimics the Herlitz junctional epidermolysis bullosa (H-JEB) phenotype in humans. These lamα3 null animals develop abnormalities in glomerulogenesis, similar to the recent post-mortem analysis done on an infant with H-JEB (Hata 2005).

16

Ln-521

In vivo studies in mice show that blocking the laminin transition through the mutation the α5 component of laminin 511/521(or lamα5) results in breakdown of the GBM, disorganization of glomerular cells, and failed glomerular vascularization (Miner JH 2000). Furthermore, about 20% of Lamα5 null embryos lack either one or both kidneys. Studies have not yet determined the exact mechanism for this defect, but the fact that Ln-511 is normally present in the ureteric bud basement membrane, and that cultured Lamα5 null metanephroi exhibit attenuated ureteric bud branching, suggests that agenesis results from a primary ureteric bud defect (Kanwar YS 1997). Comparatively, in the absence of laminin β2 (of Ln-521), the GBM does not develop correctly resulting in proteinuria in adult mice (Jarad G 2006).

From these observations, it is evident that laminin networks are important for renal development through their direct contribution to BM integrity thus relevant to our discussion. Furthermore, our novel observation that the dysplastic dysgenic phenotype of MT1-MMP null mice was associated with decreased cleavage of Ln-332 (Koshikawa 2004) has both established a link between MT1-MP and Ln-332 is kidney development and been the foundation of this study. Therefore, exploring other laminins involved in development is critical to our work.

Nidogen

Nidogen (or entactin) is a ubiquitous BM glycoprotein that consists of two amino domains (G1, G2) and one carboxyl globular domain (G3). A rod domain consisting primarily of EGF repeats connects these two domains. There are two closely related nidogen genes in mammals denoted as nidogen-1 and nidogen-2. While nidogens-1 and -

2 show a divergent expression pattern in certain adult tissues, both have a similar distribution during development (Bader B 2005). Specifically, nidogen-1 and -2 are present at sites of epithelial-mesenchymal interactions in embryonic tissue in epithelial and endothelial cells of the adult mouse kidneys (Miosge N 2000). This localization makes nidogen of particular interest within our studies.

Biochemical studies have suggested that nidogen can mediate the formation of the ternary complexes between laminins and collagen IV (Fox 1991). However, some studies have shown that nidogen-1 also binds to the basement membrane protein perlecan (Timpl 1996). A study conducted by Ekblom used antibodies against the nidogen binding site on the laminin β2 chain to determine the relative importance of nidogen during kidney organogenesis. Interfering with nidogen-laminin interactions perturbs *in vitro* epithelial development in embryonic kidney and lung (Ekblom P 1994). These studies suggested that mesenchymal nidogen could be important for early stages of epithelial morphogenesis. Furthermore, the G3 domain binds with high affinity to the laminin γ1 (Pöschl E 1994), and γ3 (Gersdorff 2005) chain, while the G2 domain can bind perlecan and type IV collagen. The ability of nidogen to form a ternary complex with laminin and type IV collagen has led many to suggest nidogen as a BM protein linker (Kramer 2005).

Gene knockout studies in mice demonstrated that the loss of either isoform has no effect on basement membrane formation and organ development, suggesting compensatory functions. However, complete ablation of both nidogens results in perinatal lethality (Bader 2005). While nidogen-1 and -2 do not appear to be crucial in establishing tissue architecture during organ development, studies have implicated them

in ensuring the late stages of lung development and for maintaining the integrity of cardiac tissue (Miosge 2002). Despite the ubiquitous presence of nidogens in basement membranes, defects do not occur in all tissues or in all basement membranes, suggesting a varying spectrum of roles for nidogens in the basement membrane.

Nidogen-directed ECM remodeling could potentially effect the entire BM meshwork. While it is been shown that MT1-MMP cleaves nidogen, *in vitro* (d'Ortho 1997), this association has not been confirmed within any *in vivo* analysis. The cleavage of nidogen by MT1-MMP could be key in the disruption of basement membranes, as nidogen acts as a structural linker to bridge the laminin and collagen IV networks. Therefore, a study of nidogen is important for our study.

Proteoglycans

Proteoglycans (PGs) also play a significant role in the morphogenesis of several tissues. They consist of core peptides that are bound by O-glycosidic linked glycosaminoglycan (GAGs) chains. The GAG chains enable proteoglycans to interact with other matrix molecules, (such as laminin), and become a reservoir for various growth factors, (e.g., basic FGF). Such interactions allow PGs to influence morphogenesis by more than one mechanism.

The kidney expresses several different types of PGs, including perlecan and syndecans as heparan sulfate-proteoglycans (HSPG), chondroitin sulfate-proteoglycan (CSPG), decorin, and biglycan (Wallner EI 1998). At the time of induction, sulfated PGs concentrate densely at the tips of the ureteric bud branches, at the location of epithelial-mesenchymal interface. Inhibiting these sulfated PGs likely causes blunting of the tips of the ureteric bud branches and dysmorphogenesis of the kidney (Kanwar YS 2004).

19

Interestingly, ureteric buds developing in culture in the absence of GAGs will develop normally if HGF, GDNF, neurturin, persephin, or BMP-4 is present (Ekblom P 1994). Thus, it appears that GAG chains mediate the biological actions of PGs. Experiments with salivary glands showing that enzymatic deletion of GAGs inhibits tubulogenesis support this notion. Complementary to these findings, studies have shown that proteoglycans are necessary for branching of epithelial tissues, including the ureteric bud, in organ culture and in the isolated UB (Lelongt B 1988; Meyer TN 2004; Shah 2004; Steer 2004). However, in the absence of perlecan, mice do not reveal any defective renal phenotype. Instead, most of them die at midgestation due to bleeding diathesis. Those that survive appear to have normal BMs (Kanwar YS 2004).

In vitro proteolysis analysis has suggested that MT1-MMP cleaves perlecan at multiple sites within the molecule (d'Ortho 1997). This cleavage of perlecan could be of particular importance as this molecule is involved in growth-factor binding. As MT1-MMP is already association with GFNF and HGF during renal development, perlecan is of interest to our study.

The aforementioned described renal abnormalities suggest that the basement membrane is bioactive and capable of directing cellular events such as adhesion, migration, proliferation, differentiation, and survival. While there are no known direct associations between aberrant BM turnover and dysplactic/hypoplastic renal disease in humans, our recent work on MT1-MMP in a mouse model has shown that these BM molecules may be involved in the development and resolution of some of these kidney diseases., in particular renal dysplasia (abnormal tissue development) or hypoplasia (inadequate tissue development) (Koshikawa 2004).

MMPs in Basement Membrane Remodeling

MMPs are a family of related yet structurally distinct proteases. The MMP family

has ~26 members that are classified as such because of the conserved pro-domain and

catalytic domain. The pro-domain of a typical MMP is ~80 amino acids and contains the

consensus sequence PRCXXPD, where X denotes any amino acid (the exception is

MMP-23) (Velasco 1999). With the exception of MMP-7, -23 and -26, MMPs have a

flexible proline-rich hinge region and a carboxy (C)-terminal hemopexin-like domain,

which functions in substrate recognition. Although MMPs are often subdivided into

groups based on differences in domain composition **(Figure 4)**, there is little consensus in

the field about how such subdivisions should be assigned as domain structure alone does

not predict function (Parks 2004). There is considerable overlap in substrate specificities,

and the MMPs play an important role in the degradation of most ECM components,

including laminins, collagens, and fibronectin. The MMPs also affect the release and

turnover of cytokines and cell surface receptors of adjacent cells (Somerville RP 2003).
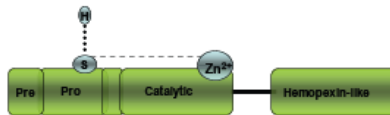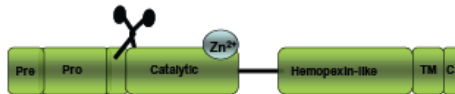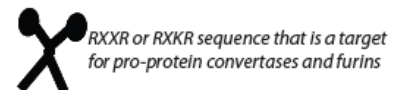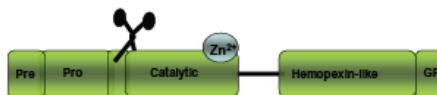
Figure 4: MMP Structure
Adapted from VanSaun 2006

MMP regulation is critical for balance between maintenance and disease.

Endogenous MMP regulation can occur at the level of gene transcription, enzyme

activation, and the balance between MMPs and their natural inhibitors known as TIMPs

(Matrisian 1990; Matrisian 1992). TIMPs or tissue inhibitors of metalloproteinases are endogenous inhibitors of these metalloproteinases and are consequently important regulators of ECM turnover, tissue remodeling, and cellular behavior. There have been 4 genes identified to encode TIMPs-1 to TIMP-4. All four TIMPs inhibit MMPs, but with varying affinities (Nagase 2008).

The expression patterns of TIMPs and MMPs suggest a role for MMPs during renal development. At embryonic days 11 and 12, TIMP-1, -2, and -3, MMP-2,-3, -9, and MT1-MMP are all expressed in the kidney groups (Reponen 1992; Apte 1994; Lelongt 1997; Ota 1998; Tanney 1998; Barasch 1999; Kanwar YS 1999). However, unlike MMP-2, -9, and MT1-MMP, MMP-3 expression is brief, rapidly decreases over time, and has not been localized to an embryonic cell type **(Table 1)** (Pohl 2000).

Table 1: MMPs in Early Renal Development

MMPs in the Kidney Development	
MMP	**ECM Substrates**
MMP-2 *Gelatinase A*	Collagen types I, IV, Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-3 *Stromelysin-1*	Collagen types II, IV, IX, X, and gelatin, Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican
MMP-9 *Gelatinase B*	Collagen types IV, V, VII, X, and XIV, Fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-14 *MT1-MMP*	Collagen types I, II, III, and gelatin, Aggrecan, dermatan sulphate proteoglycan, fibrin, fibronectin, laminin, nidogen (in vitro), perlecan (in virtro), tenascin, and vitronectin

Adapted from Lelongt 2001 and Pohl 2000

Researchers have thoroughly investigated the roles of the mesenchymal derived gelatinases, MMP-9 and MMP-2 in renal development. The gelatinases are a subfamily of MMP that share the ability to degrade type IV and V collagen in the basement membrane, as well as the denatured collagens aggrecan, elastin and gelatins (Vu TH 1998). The mesenchyme of 11-day mouse embryonic kidneys synthesizes both MMP-9 and MMP-2, whereas these enzymes are undetectable in the ureter bud (Lelongt 1997). In these systems, inhibition of MMP-9 appears to cause a more severe branching phenotype (Kanwar YS 1999). Anti-MMP-9 IgG with enzyme-blocking activity, impairs

the morphogenesis of embryonic day 11 mouse kidney, in a concentration-dependent

manner, by inhibiting T-shaped branching and further divisions of the ureter bud (Lelongt

1997). Although it inhibits enzyme activity, anti-MMP-2 IgG has no apparent effect on

kidney morphogenesis (Lelongt 1997). However, when embryonic day 13 rat kidneys

were treated with MMP-2 antisense oligodeoxynucleotides, UB abnormalities were

evident but mild (Kanwar YS 1999).

Lelongt and colleagues suggested that this discrepancy could be related to the

different stages of kidney development in which the scientists preformed the experiments

(embryonic days 11 versus 13). They hypothesized that MMP-9 and MMP-2 could act

sequentially in branching morphogenesis of the ureter bud and that MMP-2 only plays a

role at embryonic day 13 (Lelongt 2001). However, Kanwar and others have reasoned

that the discrepancies may be due to the fact that anti-MMP-2 antibodies may not be the

blocking type (Kanwar YS 2004).

Researchers have further analyzed the requirement for MMP-2 and MMP-9 in

kidney development for mice harboring a targeted null mutation in either of the genes

encoding these proteases. *In vivo* analyses show that MMP-2-deficient mice develop

normally, are fertile, and do not show obvious defects in branching morphogenesis.

However, the MMP-2-deficient mice have an approximately 15% slower growth rate than

control littermates (Itoh 1997). Similarly, MMP-9 null mice are viable without any

observed renal abnormalities, but show a 12% reduction in nephron numbers, which most

likely results from subtle defects in branching morphogenesis (Lelongt 1997).

More recently MMP14, also referred to as MT1-MMP, which is the prototype

membrane type (MT) MMP has been studied in this context. To understand the role of

MT1-MMP, it is important to dissect the protease at the molecular level. To do so, I will discuss MMP structure, activity, and the field's current understanding of about the role of MT1-MMP in developing tissue. This discussion is intended to speculate a role for the protease during renal development.

MT1-MMP

MT1-MMP or MMP14 was first discovered in 1994 by Seiki and colleagues as a gene product expressed on the cell surface of invasive tumor cells including lung and stomach carcinomas (Sato 1996). Originally, MT1-MMP was shown to induce specific activation of MMP-2 (Sato 1994; Cao 1995; Strongin 1995; Takino 1995; Yu 1995) *in vitro* and enhance cellular invasion of the reconstituted basement membrane (Sato 1994). Later in 1997, Apte and colleagues determined the structure of the Mmp14 gene encoding MT1-MMP. MT1-MMP has been mapped to chromosome 14 and shown to be encoded by ten exons. The novel C-terminal peptide domains of MMP-14 are encoded by a single large exon that also encodes the 3′-untranslated region (Apte 1997).

MT1-MMP shares a common domain structure with other MMP family members, including a pre/propeptide (M1–R111), a catalytic domain (Y112–G285), a hinge region (linker-1) (E286–I318), a hemopexin domain (C319–C508), a stalk (linker-2) region (P509–S538), a transmembrane domain (A539–F562), and a cytoplasmic tail (R563–V582) (Brinckerhoff 1991; Sato 1994; Itoh 2002; Seiki 2003). Each domain has been the point of interest for several groups. I will briefly take time to describe the importance of each domain.

Pro-domain

MT1-MMP is kept in a catalytically inactive state by the interaction between the thiol group of a pro-domain cysteine residue and the zinc ion of the catalytic site. Conversion to an active state by disruption of this interaction which is achieved by proteolysis of the pro-domain (VanSaun 2006). MT1-MMP has a basic amino acid motif of RRKR111 at the end of the propeptide which is cleaved by furin or related proprotein convertases (Sato 1996; Yana 2000). This sequence allows the proMT1-MMP (~64 kDa) to be converted to a catalytically active enzyme (~55 kDa) by the proteolytic cleavage of furin in the trans-Golgi network prior to its arrival at the plasma membrane (Yana 2000; Mazzone 2004); the process is similar for other MT-MMPs. However, for soluble MMPs, the activation takes place extracellularly (Mazzone 2004).

Hemopexin Domain

It is believed that the hemopexin domain determines MT1-MMP specificity for its substrates including ECM components: type-I, -II and -III (and possibly type-IV) collagen, gelatin, laminins-111 and -332, fibronectin, vitronectin, aggrecan and fibrin (Hotary 2006). Additionally, the hemopexin domain is required for protein dimerization as well as MT1-MMP-mediated invasion and growth in three-dimensional type I collagen (Itoh 2008). Purified MT1-MMP catalytic domain itself cannot cleave native type I collagen *in vitro*. Thus, it is inferred that the deletion of the hemopexin domain incapacitates MT1-MMP to bind, degrade substrates, and consequently impairs cell invasiveness (Hurst DR 2004). Additionally, this domain is believed to play a role in the activation of proMMP-2 **(Figure 5)** and pro-MMP-13 (Lehti 2002) however, studies have been conducted to refute these claims (Wang 2004).

Transmembrane Domain

The transmembrane domain is unique to MT1-, MT2-, MT3-, and MT5-MMPs. This stretch of 23 amino acid restricts MT1-MMP to the cell surface and enables the protease to modify the immediate pericellular environment while also providing an optimal position to function at the leading edge of migrating and invasive cells (Buccione 2004).

Catalytic Domain

The catalytic domain is responsible for the proteolytic activity of MT1-MMP. In addition to the aforementioned ECM, MT1-MMP cleaves several cell surface proteins such as CD44 (Kajita 2001), transglutaminase (Belkin 2001), low-density lipoprotein receptor related protein (Rozanov 2004), αv integrin (Deryugina 2002), and syndecan-1 (Endo 2003). The expression of MT1-MMP on the cell surface, together with the soluble MMPs that it activates allows for numerous modifications within much of the pericellular space which could ultimately result in modulation of cellular function. In the instance of cellular growth, Golubkov and colleagues have even suggested that MT1-MMP catalytic activity qualifies the protein as an oncogene that promotes malignant transformation of normal cells rather than just an enzyme that supports the growth of preexisting tumors (Golubkov 2006).

Cytoplasmic Domain

Increasing evidence suggests that the cytoplasmic tail of MT1-MMP may regulate its activity at the cell surface. It has been demonstrated that MT1-MMP is internalized from the cell surface and that this process requires the presence of the cytoplasmic

domain (Uekita 2001; Moss 2009). Cytoplasmic tail truncation inhibits internalization and restricts MT1-MMP to the cell surface (Uekita 2001; Moss 2009) but the underlying mechanism has yet to be determined. Interestingly, both invasion and migration are down-regulated in cells where MT1-MMP is restricted to the cell surface (Uekita 2001; Moss 2009). These data suggest a correlation between internalization and matrix turnover, where MT1-MMP activity is either abrogated or enhanced under appropriate stimuli.

The cytoplasmic domain of MT1-MMP has three potential phosphorylation sites: Thr567, Tyr573, and Ser577. Recent work by Nyalendo and others indicates that MT1-MMP is phosphorylated at tyrosine residue Tyr573, and that this modification influences cell migration (Nyalendo 2007). The Thr567 within the MT1-MMP cytoplasmic tail has homology with the consensus sequence for both protein kinase C and ERK1/2, suggesting the possibility that active MT1-MMP might also be regulated through phosphorylation of this cytoplasmic tail residue (Moss 2009).

Understanding of the functional domains of MT1-MMP, allude to the pleiotropic and potent nature of the protein. While MT1-MMP is involved in normal growth and tissue maintenance, unregulated enzymatic activity is key to acquiring a metastatic phenotype in a variety of tumor cells, including lung, colon, breast, and cervical carcinomas (Yana 2000; Sabeh 2004; Zhai 2005; Itoh 2006). Stringent cellular regulation of MT1-MMP enzymatic activity is necessary and accomplished through gene transcription, enzyme activation, and TIMP-2, -3, and -4 activity (Matrisian 1990; Matrisian 1992; Nagase 2008).
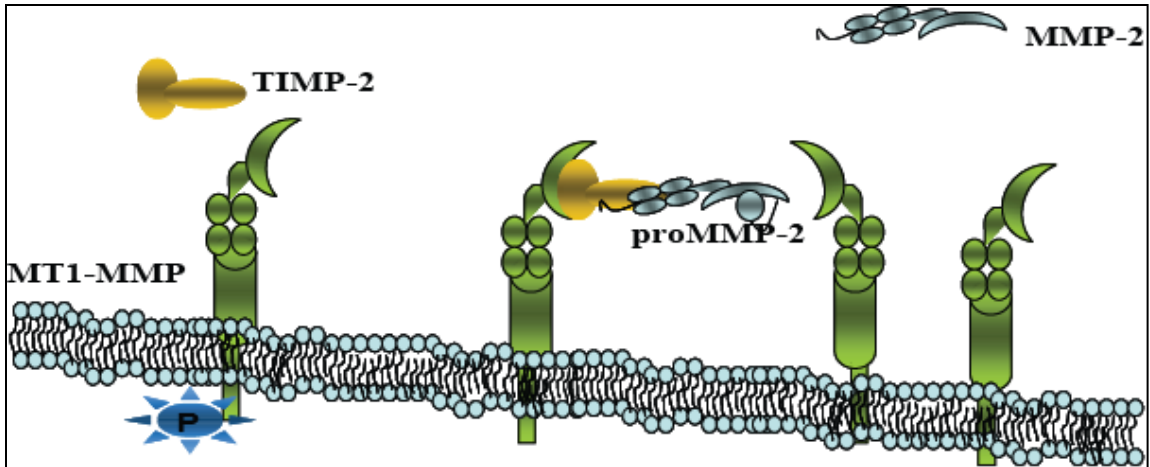
Figure 5: MT1-MMP at cell surface.
Adapted from Seiki 2003

The growing interest of the MT1-MMP field prompted the generation of a MT1-MMP null mouse by two separate groups a decade ago. The MT1-MMP null mouse was the first example of an MMP knockout with a severe baseline phenotype (Fingleton B 2010). These mice are severely runted and live only several weeks after birth (Holmbeck 1999; Zhou 2000). This mouse model has been an invaluable resource for MMP studies. Through many studies, MT1-MMP appears to be a complex multifunctional molecule influencing different cell functions. Its importance during development is supported by the severe phenotype associated with MT1-MMP deficiency in mice (Zhou 2000; Hotary 2006). I will now discuss the phenotype of these mice and studies done to ascribe a role for MT1-MMP in developing tissue.

The role of MT1-MMP in Development

Bone development

MT1-MMP-deficient mice exhibit damages in skeletal development manifested by craniofacial dysmorphism, dwarfism, osteopenia and fibrosis (Holmbeck 1999; Zhou 2000). Furthermore, these mice (on a mixed background) die around 7-12 weeks of age. The functional significance of MT1-MMP during skeletal development is related to the requirement for ECM cleavage as regulated ECM degradation promotes cell migration and tissue stabilization. Detailed analyses suggest that the bone phenotype of the MT1-MMP null mice may, in part, result from the lack of cellular collagen degradation by osteocytes (Holmbeck 2005). As bone formation involves the highly regulated process of formation by osteoblasts and resorption by osteoclasts, the manifestation of this phenotype suggests that osteocytes were not able to create the right ECM environment for osteocytogenesis. These findings strongly suggest that MT1-MMP is an essential cellular collagenase important for organizing the ECM microenvironment, which cannot be substituted for by any other MMP during development. No other MMP gene KO mice have shown such drastic phenotypes within the long bone growth, soft tissue organization, molar root formation and eruption, and cartilage remodeling (Holmbeck 2004).

White Adipose Tissue

Studies have demonstrated that MT1-MMP coordinates adipocyte differentiation *in vivo*. In the absence of the protease, white adipose tissue development is aborted, leaving tissues populated by mini-adipocytes which render null mice lipodystrophic. While MT1-MMP preadipocytes display a defect *in vivo*, null progenitors retain the

31

ability to differentiate into functional adipocytes during 2-dimensional (2D) culture. By contrast, within the context of the 3-dimensional (3D) ECM, normal adipocyte maturation requires a MT1-MMP-mediated proteolysis that modulates pericellular collagen rigidity in a fashion that controls adipogenesis. It is believed that MT1-MMP acts as a 3D-specific adipogenic factor that directs the dynamic adipocyte-ECM interactions critical to white adipose tissue development (Chun 2006).

Muscle

MT1-MMP was identified as a major contributor for morphological differentiation in muscle tissue. Muscle development and differentiation proceeds through three distinct stages of proliferation, elongation and fusion. *In vitro* inhibition of MT1-MMP using short hairpin RNA effected muscle fusion by lessened myotube elongation. *In vivo* studies employing MT1-MMP null mice confirmed the role of MT1-MMP in myogenesis as mice had smaller myofibers in association with abnormal procession of laminin-211/221 in the basement membrane compared with those in the wild-type mice. These findings have lead to the belief that MT1-MMP is a multilateral regulator for muscle differentiation and maintenance through processing of stage-specific distinct ECM substrates (Ohtake 2006).

Lung development

Lung development in MT1-MMP null mice has been reported by two independent groups (Atkinson 2005; Oblander 2005). Findings reveal that development is arrested at the prealveolar stage, suggesting that MT1-MMP is required for the postnatal development of the alveolar septae. Interestingly, MMP-2 null mice lacked comparable

defects in with the lung, suggesting that MT1-MMP acts via mechanisms independent of pro-MMP-2 activation. MT1-MMP null mice also show reduced migration of lung endothelial cells and formation of three-dimensional structures on Matrigel (Oblander 2005).

Submanibular Gland

The submandibular salivary gland shows a classic branching morphogenesis and has been used as a model of organ culture *in vitro* for over 50 years (Borghese 1950). Oblander and colleagues have shown that MT1-MMP is required for the branching morphogenesis of the submandibular gland as two-week-old submandibular glands from MT1-MMP null mice were smaller than their control littermates and consistent with *in vitro* organ culture studies using E13.5 submandibular gland rudiments which branching in MT1-MMP null mice was subnormal compared to rudiments from control animals (Oblander 2005). A recent study has also found that MT2-MMP may play a role in this process as MT2-MMP dependent proteolysis of collagen IV regulates more protease expression and epithelial proliferation to promote branching morphogenesis (Rebustini IT 2009).

The role of MT1-MMP in the lung and submanibular gland is of particular importance towards understanding the role of the protein in kidney development. All three organs develop in a similar method and require the branching morphogenesis of an epithelial structure and MMPs have long been postulated to play a role in this process. In 1986, Nakanishi and colleagues conducted a seminal study that used exogenous collagenases to alter the morphogenesis of the salivary gland (Nakanishi 1986) to implicate a role for protease degradation during development. 5 years later, Ganser and

others were able to culture murine lung bud extracts and stimulate MMP activity through growth factors treatment. The stimulated branching was reversed in the presence of TIMP-1 to confirm a definite role for MMPs during branching morphogenesis (Ganser 1991). It is with the aforementioned knowledge that we wanted to determine the significance of MT1-MMP in kidney development.

Kidney

As described earlier, the roles of the gelatinases, MMP-9 and MMP-2, have been investigated in renal development. Despite the *in vitro* data suggesting a role of these gelatinases in branching morphogenesis, MMP-2- and MMP-9-deficient mice do not appear to have any obvious defects in renal development (Andrews 2000). Ota and colleagues have shown that the mesenchyme expresses all components except MT1-MMP at embryonic day 11, and MT1-MMP become concentrated in the induced mesenchyme after epithelial induction at embryonic day 12 (Ota 1998). Meanwhile they also observed MT1-MMP in the ureter bud at embryonic day 11, and found it localizes both in the ureteric bud and in the induced mesenchyme at embryonic day 12. MMP-2 also showed a weak expression in the ureter bud at this developmental stage, suggesting that either the ureteric bud or the mesenchyme subsequently produces MMP-2 as a ligand of MT1-MMP.

The ureteric bud tips appear to be the key areas for branching morphogenesis of the isolated ureteric bud, and increased expression of MT1-MMP and MMP-2 localizes to the isolated UB tips (Meyer TN 2004). The expression patterns of MT1-MMP are significant, as they suggest a role for the protein in ureteric bud development. It is

important to note, however, that there has been no evidence of this *in vivo* (Kanwar YS 1999).

MT1-MMP plays a broad spectrum of activity against ECM components such as type I, II and III collagens, ln-111, ln-332, nidogen, and perlecan. Therefore, it is possible that MT1-MMP plays a regulatory role in the maintenance of the renal basement membrane and in the absence of such regulation, kidney morphology is affected. Based on these data we hypothesize that ***MT1-MMP-mediated ECM remodeling regulates normal renal development.*** We will test this hypothesis in the following 2 aims:

Aim 1. Determine how lack of MT1-MMP leads to a renal phenotype.
The physiological role of MT1-MMP in kidney development is still unclear. We have found that MT1-MMP is highly expressed in the kidney and MT1-MMP null mice have postnatal defects. In this aim we will test the hypothesis that MT1-MMP cleavage of renal ECM components is critical for normal ureteric bud by **a**) analyzing embryonic and adult kidneys from wildtype and MT1-MMP-null mice to determine anatomical abnormalities, especially with reference to the composition and integrity of ECM components, and **b**) isolating collecting duct cells from wild type and MT1-MMP-null mice nephrons to determine the effects of lack of this enzyme on ECM-dependent cellular functions such as migration, adhesion and tubulogenesis.

Aim 2. Determine the relevant ECM substrates for MT1-MMP *in vivo*. MT1-MMP may i) directly cleave ECM components; ii) promote the activation of soluble MMPs; or iii) act synergistically with soluble MMPs. In this aim we will test the hypothesis that

MT1-MMP plays a major role in extracellular matrix turnover by determining *in vivo* whether there are differences in the composition of the renal basement membranes of the MT1-MMP null mice compared to their wild type counterparts.

CHAPTER II

MT1-MMP-MEDIATED ECM REMODELING REGULATES NORMAL RENAL DEVELOPMENT

Introduction

The kidney is composed of multiple nephrons that connect to collecting ducts which ultimately join together to form the ureter. The nephrons, which consist of the glomerulus and highly differentiated tubules, are derived from the metanephric mesenchyme, while the collecting system is derived from the UB. Due to the complexity of its specialized functions, different nephron segments have developed some of the most complex and specialized BM in the body whose formation and turnover are tightly controlled both spatially and temporally. The major constituents of these BMs are collagen IV, the laminins and heparan sulphates. The MMPs are important for the regulation of turnover and development of these BMs. 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 type IV collagens and laminins, which are major kidney BM component. Despite these *in vitro* findings mice harboring targeted null mutations for MMP-2 (Itoh 1997), MMP-9(Andrews 2000) and MMMP-2/MMP-9 mutant mice (Miosge 2002) had no renal abnormalities. In a disease model of angiotensin-II induced hypertension, MMP-9 was demonstrated to preserve vessel structure and alleviate blood pressure increases (Kalluri 2003), however progression of

anti-glomerular basement disease is not affected in either MMP-2 or MMP-9 null mice (Nart 2010). These minor effects on renal development and following renal injury suggest that there is redundancy of the gelatinases with respect to BM turnover of the kidney.

More recently MT1-MMP has been studied in this context. This enzyme not only has intrinsic proteolytic capabilities but also induces its effects by activating MMP-2 and MMP-13 (Itoh 2006). Numerous extracellular matrix (ECM) components, including type I, II and III collagens, fibronectin, vitronectin, laminins 111 and 332, fibrin and proteoglycans are substrates for MT1-MMP (Seiki 2003). In addition it can cleave other cell surface proteins such as CD44 (Kajita 2001), transglutaminase (Belkin 2001), low-density lipoprotein receptor related protein (Rozanov 2004), αv integrin (Deryugina 2002), and syndecan-1 (Endo 2003). These highly divergent substrates for MT1-MMP make it 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 collagenase activity (Holmbeck 1999). In addition they have submandibular gland branching morphogenesis abnormalities (Oblander 2005) as well as defects in lung development (Atkinson 2005; Oblander 2005), angiogenesis (Zhou 2000) and myeloid cell fusion (Gonzalo P 2010). These deficiencies have been 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 cytosolic tails.

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 (Kanwar YS 1999). In contrast to the gelatinase null mice, we 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. Both the glomeruli and the tubules were slightly dysmorphic and these renal abnormalities correlated with an increase in laminin 332, suggesting that lack of laminin 332 cleavage by MT1-MMP accounted for these abnormalities (Koshikawa 2004).

Although these data defined a role for MT1-MMP in renal development and suggested its role was to cleave 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 detail and demonstrate that when MT1-MMP null mice are bred onto a 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 an increase of multiple 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 these BM components. Thus our results suggest that pericellular cleavage of multiple BM components by MT1-MMP, which affects cell proliferation and migration plays a critical role in normal kidney development.

<center>**Experimental Procedures**</center>

Morphological analysis of MT1-MMP null mice.

MT1-MMP mice generated by Dr. M Seiki (University of Tokyo) were bred onto a pure C57/B6 background. Kidneys were isolated at different time points, fixed in 4% formaldehyde for 1 hour and embedded in paraffin. Paraffin tissue sections were stained with either hematoxylin and eosin or periodic acid-Schiff (PAS).

Glomerular counting

Glomeruli counts in the mutant and wild type mice were performed as described previously (Boyle 2007). Briefly, individual kidneys were isolated from 2 week old mice and minced into 2-mm cubes. Fragments were incubated in 5 ml of 6M HCl at 37°C for 90 min. Tissue was further homogenized by repeat and vigorous pipetting. 25 ml of H_2O was added and after overnight incubation at 4°C, glomeruli in 1 ml of this solution were counted in a 35-mm counting dish; each sample was counted 5 times. Total glomerular number per kidney was extrapolated mathematically from the mean of these five counts.

Organ culture

<center>40</center>

Embryonic kidneys were isolated from E12.5 mice and cultured on top of transwell filters as previously described. For the TIMP studies, TIMPs-1 or -2 were used at a concentration of 15μg/ml. Seventy two hours later, the kidneys were fixed in 4% paraformaldehyde and stained with fluorescein-conjugated E-cadherin antibodies (BD Transduction Laboratories), as described. Quantification of branching structures in 10 kidneys were performed as previously described.

Zymography assays

Gelatin zymograms of kidneys were performed as previously described. Briefly, equal amounts of plasma proteins (30 ug/lane) were loaded on a 10% SDS-PAGE containing 1 mg/ml gelatin and run under non-reducing conditions. The gels were incubated in 50 mM Tris–HCl, pH7.5, 0.1 M NaCl and 2.5% Triton-X100 for 2 h at room temperature, and then incubated in 50 mM Tris–HCl, 1 mM $CaCl_2$ and 0.02% NaN_3 for 18 h at 37^oC. The gels were stained with Commasie Blue to visualize MMP activity.

Immunoblotting

30 µg total protein was electrophoresed by SDS-PAGE and subsequently transferred to nitrocellulose membranes. Membranes were incubated with different primary antibodies followed by the appropriate HRP-conjugated secondary antibodies. Immunoreactive bands were identified using enhanced chemiluminescence according to the manufacturer's instructions. The following antibodies were used: Collagen IV (Biodesign International, 1:500), Laminin-β1 chain (Mab 5A2 a gift from Dale

Abrahamson, 1:100) Laminin-α3 chain (a gift from Vito Quaranta, 1:1000), Laminin-α5

chain (Chemicon International, AB8948, 1:800), Nidogen (a gift from Peter Yurchenco

as described by Li et al., 2005, 1:500), and Perlecan (Endorepellin mAb a gift from Peter

Yurchenco as described by Yurchenco et al., 1987, 1:250). FAK (Santa Cruz

Biotechnology, sc558, 1:1000).

Immunohistochemistry.

Antibodies used were Collagen IV antibody (BioDesign International, 1:400),

Laminin α3 chain (a gift from Vito Quaranta, 1:100), Laminin α5 chain (Chemicon

International, AB8948, 1:500), Nidogen (a gift from Peter Yurchenco as described by Li

et al., 2005, 1:500), and Perlecan (Endorepellin mAb a gift from Peter Yurchenco as

described by Yurchenco et al., 1987, 1:500), ERK (1:1000, 9102), pERK (1:1000,

9101S), p38 MAPK (1:1000, 9212), pp38 MAPK (1:1000, 9211S) antibodies were

purchased from Cell Signaling.

For basement membrane staining, 5um kidney sections were cut from paraffin

blocks and treated sequentially with 100, 95, and 70% alcohol for 5 min each. Sections

were quenched with 1.25% H_2O_2 in methanol for 15 min, then incubated for 90 min

with %3 BSA. Sections were then incubated with the aforementioned primary antibodies.

A 2-step method for signal amplification was used (BioGenex Laboratories) following

primary antibody incubation. Slides were then washed in PBS then diaminobenzidine

was added as a chromogen.

For Laminin-β1 chain (Mab 5A2 a gift from Dale Abrahamson, 1:500), antibody was applied on 0.2% paraformaldehyde fixed tissue (10 min. RT) which was subsequently permeabilized with 0.5% Triton X-100 in PBS for 5 min., washed 3x, then labeled as described above.

Immunofluorescence

For immunofluorescence, 5 µm kidney sections were fixed in 4% paraformaldehyde for 1 hour at room temperature, blocked with 1% BSA in PBS and then stained with the following primary antibodies: anti-E-cadherin (1:50,BD BioSciences Pharinogen); anti-ZO1 (1:200, Zymed). Detection of bound primary antibodies was accomplished with Alexa Fluor 488 anti-mouse IgG and Alexa Fluor 647 goat anti-rabbit IgG or (Molecular Probes), respectively. Slides were then analyzed under an epifluorescence microscope.

Generation of MT1-MMP-expressing cell line

Innermedullary collecting duct (IMCD) cells were isolated and cultured from MT1-MMP null mice as described by Husted, 1988. The cells were then transfected with either MT1-MMP or E240A MT1-MMP, which has no proteolytic activity. Levels of MT1-MMP expression in the reconstituted cells were verified by flow cytometry utilizing an anti-rabbit MT1-MMP antibody (Chemical International, AB8345).

Small interference RNA assay

MT1-MMP expression in ureteric bud cells was knocked down utilizing small interfering RNA duplexes corresponding to the target sequences 5'CAUCUGUGACGGGAACUUtt3' and 5'AGUACUACCGGUUCAAUGAtt3' which are commercially available as siRNAs s69919 and s69920 respectively from Ambion. Oligonucleotides were transfected into UB cells using the Lipofectamine 2000 system (Invitrogen Corporation).

Cell migration

Cell migration was assayed as previously described (Chen 2004). Briefly, transwells with 8 µm pores were coated with different ECM components and $1\text{x}10^6$ cells were added to the upper well in serum-free medium. The cells that migrated through the filter after 4 hours were counted. Five random fields were analyzed per treatment. Three independent experiments were performed in triplicate for each substrate tested.

Cell proliferation

For 2-Dimensional analysis, 5×10^3 cells were plated in 96-well plates on various ECM components and maintained in DMEM (10% FBS). After 12 hours, the cells were incubated in DMEM (2% FBS) for 24 hours and then pulsed with 1 µCi/well [^{3}H] thymidine (PerkinElmer Life Sciences). Twenty-four hours later, the cells were solubilized and radioactivity was measured using a scintillation counter. For manual counting assays, 6×10^4 cells were plated per plate on collagen and maintained in a serum-free environment.

For 3-Dimensional analysis, UB or IMCD cells were embedded within a 3-D collagen matrix containing Collagen I and Matrigel and maintained in DMEM (10% FBS). After 12 hours, the cells were incubated in DMEM (2% FBS) for 48 hours and then pulsed with 1 µCi/well [^{3}H] thymidine (PerkinElmer Life Sciences). 48 hours later, the gels were removed from the plate and dialyzed in PBS for 24 hours to remove unincorporated [^{3}H] thymidine. The gels were subsequently solubilized in 100µl of 20% SDS. Radioactivity was measured using a scintillation counter.

Tubulogenesis Assays

Tubulogenesis of UB-derived cells was performed in 3D ECM gels as previously described (Chen et al., 2004). The gels were composed of 0.1 mg/ml Collagen I in DMEM containing 20 mM HEPES (pH 7.2). For the Matrigel/Collagen I gels, a 1:1 mixture of the collagen solution described above was mixed with growth factor-reduced Matrigel, giving a final concentration of 0.5 mg/ml of Collagen I and 0.5 mg/ml of

Matrigel (Sakurai et al., 1997). One hundred microliters of medium supplemented with 10% FBS were added to the gels after they had solidified. Assays were performed at least in triplicate, and error bars represent SE. P values were calculated with Student's t-test.

MTT Assays

The viability of siRNA treated cells was estimated by MTT Cell Viability Kit (Sigma Chemical Co., St. Louis, MO). Briefly, 5mg/ml MTT (3-[4-,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) stock solution was added to each well in an amount equal to 10% of the culture volume in triplicates and incubated for 4 hours at 37ºC. After the incubation period, the resulting formazan crystals were dissolved in MTT solvent (0.1N HCL in anhydrous isopropanol) in an amount equal to the original cell culture volume. Absorbance was measured at a wavelength of 570 nm and background absorbance at 690nm was subtracted.

<div align="center">

Results

</div>

MT1-MMP null kidneys are small with a severe proliferation and mild branching morphogenesis defect.

We previously examined the kidneys of MT1-MMP mice bred on a mixed background that died at 10 weeks and found them to be small, dysmorphic and dysplastic. To define the kidney developmental abnormalities further, we bred MT1-MMP mice onto a pure C57/B6 background. These mice die at approximately 15 days at which time they are significantly smaller than their wild type controls and have musculoskeletal defects as

previously described. At the time of death the kidneys were small, but the size was

proportional to the decreased size of the mice **(Figure 6A).**

Figure 6: MT1-MMP affects postnatal kidney development.
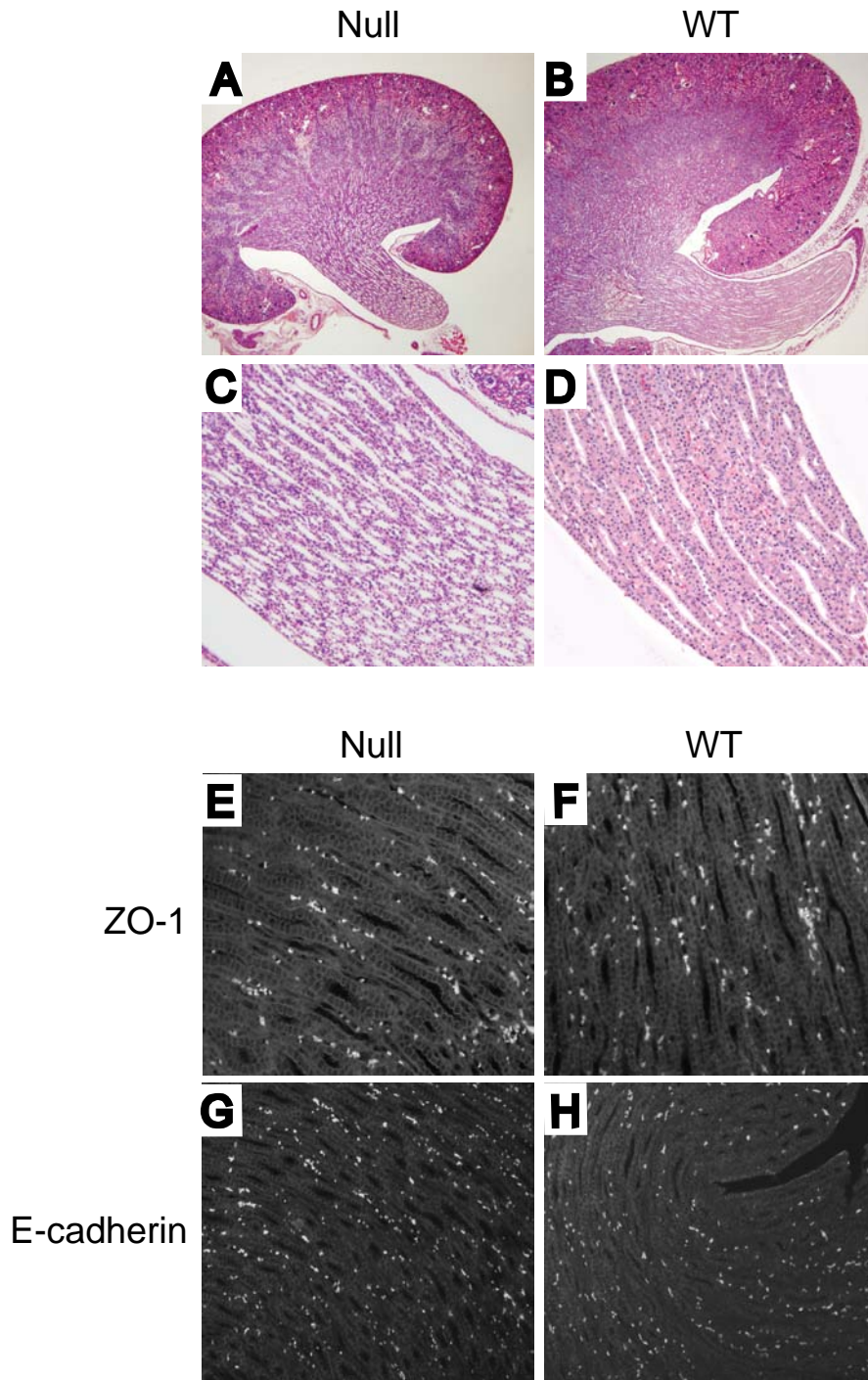**Collecting ducts within the papilla are slightly irregular showing tubular disorganization as**
well as dilation in MT1-MMP null mice (A, C) as compared the wildtype (B, D). ZO-1 and

E-cadherin staining of P14 kidneys showed no differences in localization and cell polarity between null (E and G, respectively) and wildtype (E and H, respectively) littermates.

On light microscopy, the parenchyma of MT1-MMP null kidneys was less dense than their wild type controls, suggesting there was a UB branching morphogenesis defect as well as decreased nephron formation. The branching defect was clearly evident in the papilla of the kidney **(Figure 6C),** where the tubules were loosely packed, dilated and dysmorphic. To our surprise, the tubular abnormalities were not due to defects in cellular polarity when we looked at E-cadherin and ZO-1 **(Figure 6E-H)** expression patterns. The cortex of the MT1-MMP null mice was small, the cortico-medullary junction poorly delineated and fewer glomeruli were evident **(Figure 7A)**. The decreased number of glomeruli was confirmed by glomerular counts **(Figure 7C)**. As MT1-MMP is expressed in the UB at E11.5 and MM at E12.5 we assessed the morphology of the kidneys from E12.5 till birth. The MT1-MMP null kidneys were smaller than wildtype kidneys at all time points and this was associated with a moderate UB branching defect and MM induction (examples at E13.5 and E17.5 **Figure 8A,C**). The UB branching defect in the MT1-MMP null mice was confirmed in *in vitro* organ cultures of E12.5 kidneys **(Figure 8E)**, which exhibited a significant decrease in UB branches in the mutant mice. MT1-MMP null kidneys proliferated approximately half as much as wild type kidneys when measured by Ki67 staining **(Figure 9A)** and there was no difference in apoptosis (data not shown).
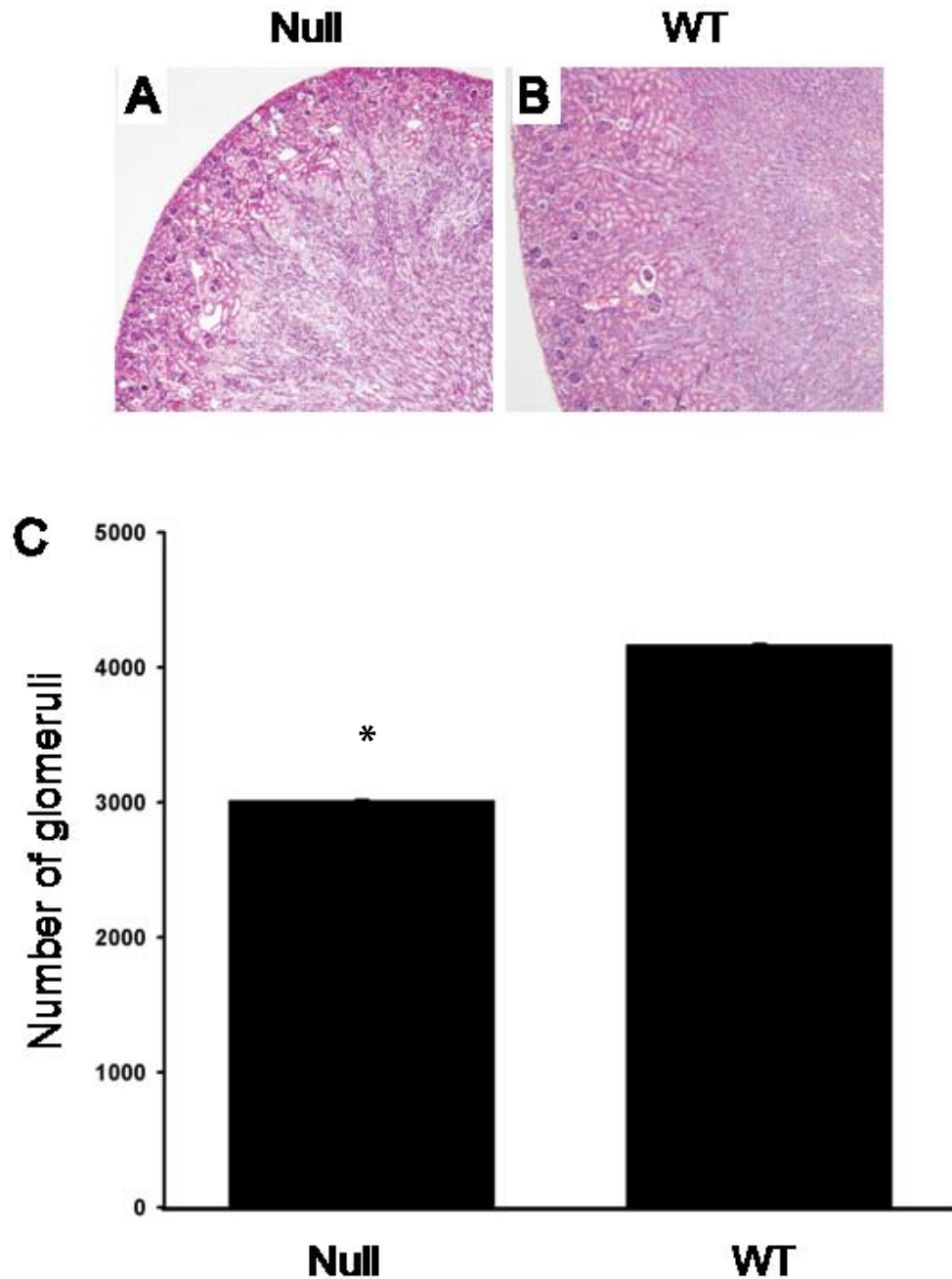
Figure 7: MT1-MMP affects glomerular development.
PAS staining revealed that MT1-MMP null mice (A) have a poorly delineated cortico-medullary junction with fewer glomeruli than wildtype littermates (B). The number of glomeruli in the cortices from whole kidneys of MT1-MMP null and wildtype mice were counted and expressed as the average+/- the standard deviation. A significant difference in the number of glomeruli between genotypes was present (p<0.05) (C). The mean and ±s.d. between MT1-MMP null and wildtype animals are shown.

While MT1-MM is most noted as a protease, it has also been shown to activate intracellular ERK to affect cell function. To further investigate the proliferation defect in our MT1-MMP mice, we assessed the expression of activated ERK and p38 in MT1-MMP null and wildtype animals using total kidney lysates. Our findings suggest that there is no difference in the relative levels of protein activation **(Figure 9D)**. Therefore, proliferative defect in MT1-MMP null mice is not associated with defects in the proliferation-promoting intracellular signaling cascades.
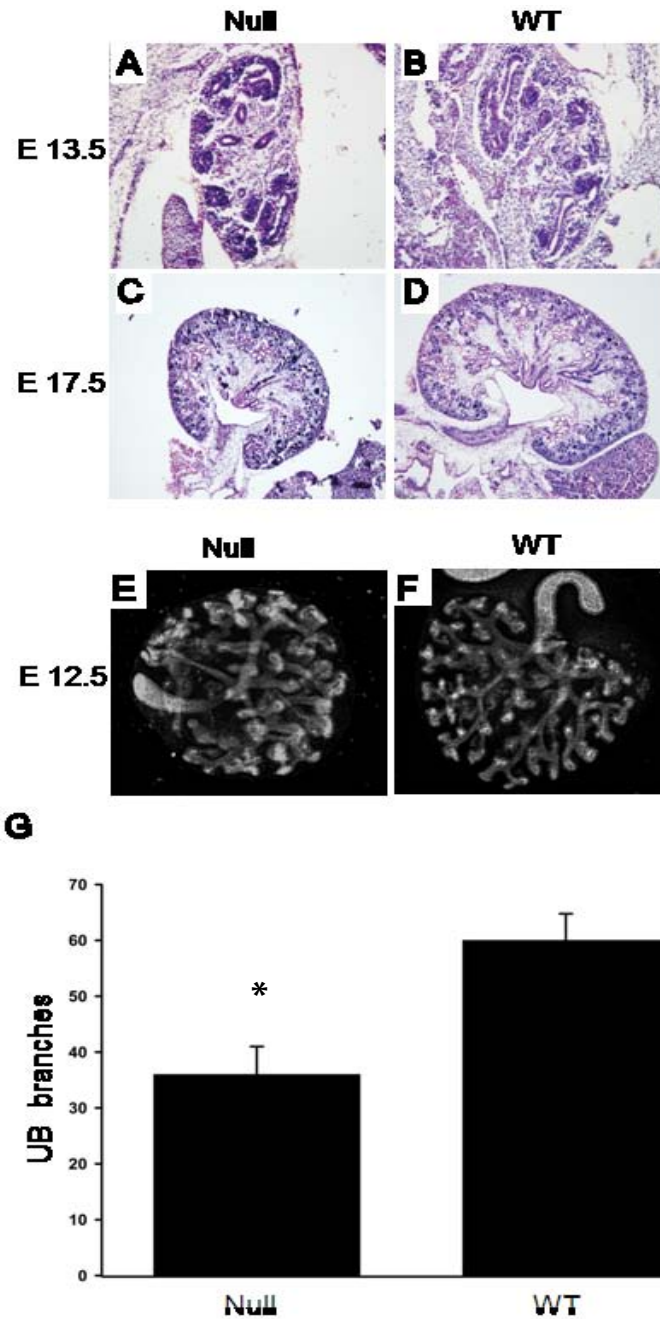
Figure 8: MT1-MMP affects embryonic kidney development.
Diminished kidney parenchyma and delayed development of tubular structures of the UB is present in E13.5 and E7.5 MT1-MMP null mice kidneys (A and C, respectively). Cultures of E12.5 kidneys of MT1-MMP null (E) and wildtype mice (F) were performed on transwells, as described in the *Experimental Procedures*. The kidneys were stained with antibodies directed against E-cadherin. The number of branches in 10 kidneys of MT1-MMP 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)(G).

The branching morphogenesis kidney phenotype of the MT1-MMP mice is

independent of MMP-2 and MMP-9 activity.

MT1-MMP exerts its proteolytic effects on ECM by both its intrinsic enzymatic functions as well as by activating proMMP-2. To define which of these mechanisms caused the developmental abnormalities in the MT1-MMP null mice, we performed zymography to define the activity of the gelatinases on kidneys from MT1-MMP null and wildtype mice. There was no difference in the amount or activity of MMP-2 and MMP-9 between these genotypes, suggesting that the observed renal phenotype is independent of MMP-2 and MMP-9 activity **(Figure 10A and B)**. To confirm that the gelatinases did not play a role in the MT1-MMP induced effects on renal development, we cultured E 12.5 kidneys from wildtype animals in either TIMP-1, which specifically inhibits MMP-2 and MMP-9 activity, or TIMP-2 which inhibits MT1-MMP, MMP-2 and MMP-9 activity **(Figure 10C-E)**. There were no differences in UB branching morphogenesis of kidneys grown in the presence or absence of TIMP-1; however it was decreased by approximately 33% ($p < 0.01$) by TIMP-2.
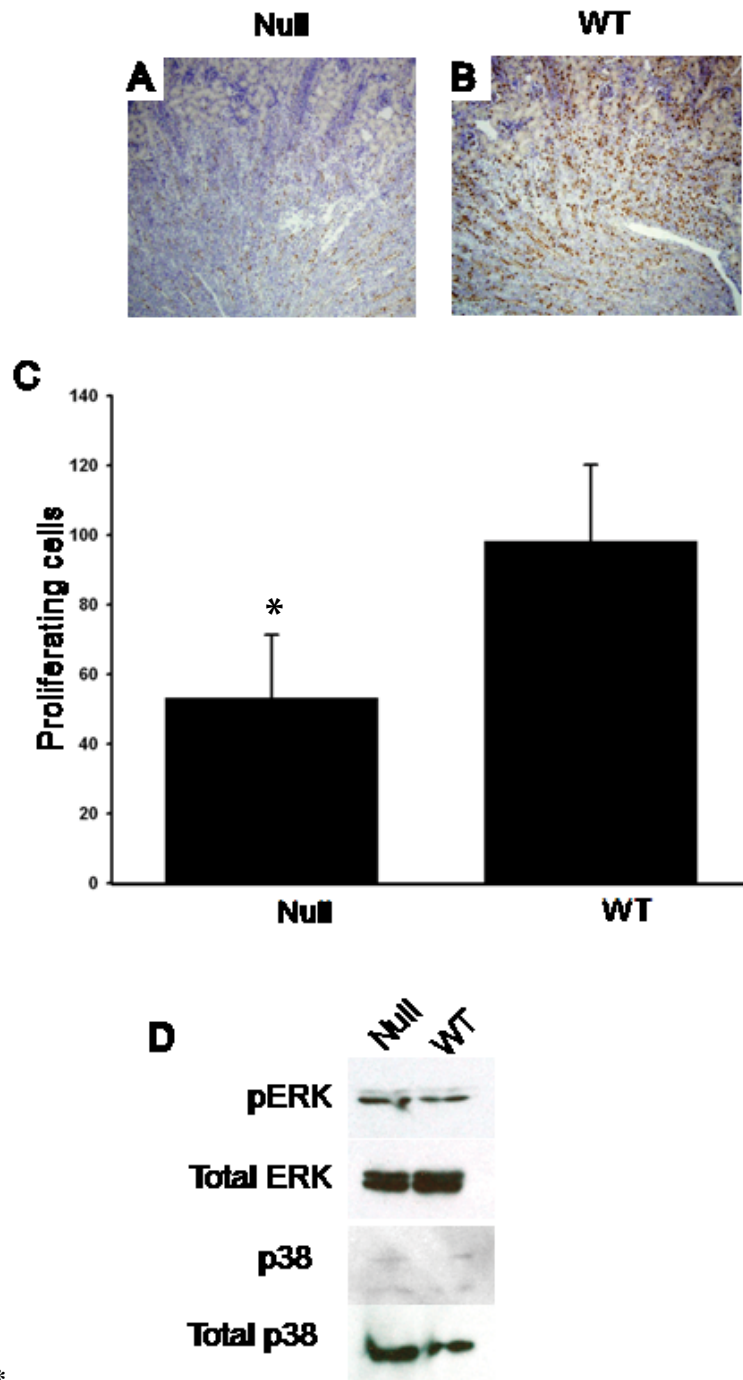
Figure 9: MT1-MMP affects cellular growth.
Ki67 staining was performed on kidneys of MT1-MMP null (A) and wildtype (B) newborn mice. The number of Ki67-positive cells were quantified and expressed as mean ± s.d. of five high power fields of three different mice (C). Total kidney lysates (30 μg) were immunoblotted for for levels of activated and total ERK and p38 MAPK. A representative experiment is shown (D).

Together, these results confirm that MT1-MMP does not increase gelatinase

activity in the kidney and that the gelatinases are not required for UB branching

morphogenesis of the of the kidney.
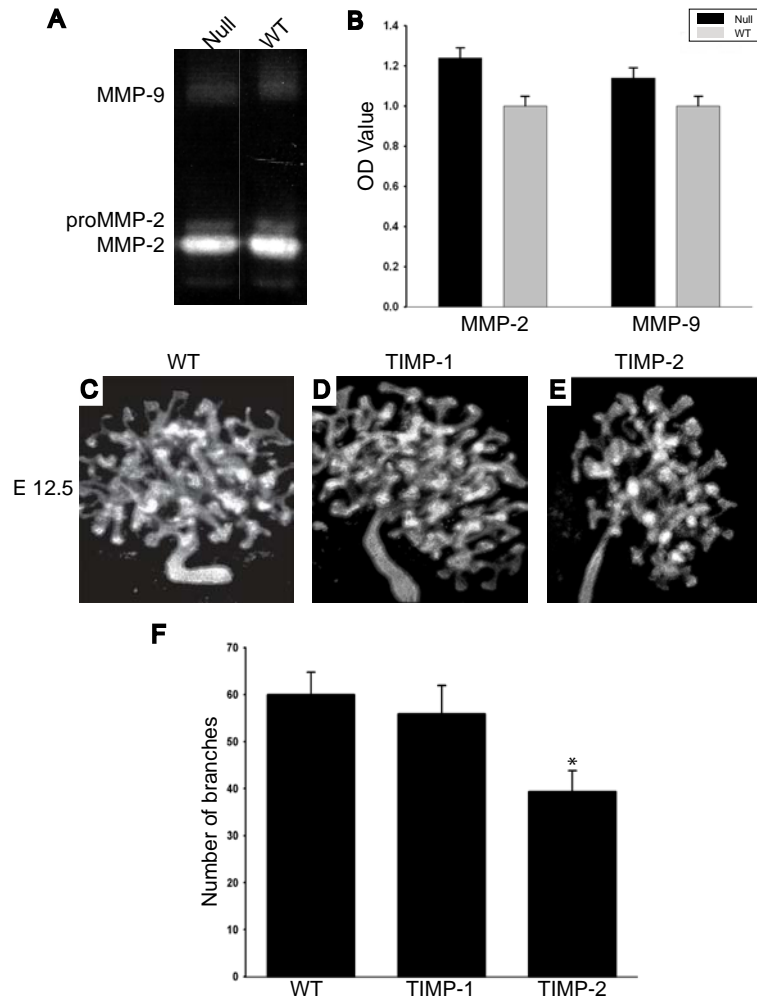
Figure 10: MT1-MMP inhibition attenuates UB branching.
Gelatin zymography of total kidney tissue lysates from week-old MT1-MMP null and
wildtype mice show activated MMP-9 and MMP-2 at comparative levels (A). Bar graph
shows quantified densitometry data (B). Cultures of E12.5 kidneys of MT1-MMP null and
wildtype mice were untreated (C) or treated with TIMP-1 (D) or TIMP-2 (E) as described
in the *Experimental Procedures*. The number of branches were counted in 10 kidneys from
both genotypes and expressed as mean ± s.d. differences between TIMP-1 and TIMP-2
treated kidneys (F).

MT1-MMP proteolytic activity is required for renal tubular cells to proliferate and undergo tubulogenesis in vitro.

In addition to the moderate branching morphogenesis defect, a severe proliferative deficiency was evident in the MT1-MMP-null mouse kidneys. To investigate the mechanisms of both these defects, we made use of a well described UB cell line derived from E12.5 mouse kidneys that is able to undergo tubulogenesis in three dimensional collagen/matrigel gels. These cells endogenously express MT1-MMP **(Figure 11A)**, which we were able to knock down utilizing siRNA. When the siRNA treated UB cells were placed in 3-dimensional gels they exhibited a moderately severe (50%) branching morphogenesis defect **(Figure 11B and C)**. Furthermore, the tubules were smaller suggesting these cells had a significant proliferative defect. We therefore next determined the ability of these cells to proliferate in 3-D gels by thymidine incorporation assays. As predicted the siRNA treated UB cells treated had a severe proliferative disorder **(Figure 12A)**. To define whether there was a specific MT1-MMP-dependent substrate that regulated the cell proliferation, we performed thymidine incorporation assays on siRNA treated UB cells on various ECM substrates that are expressed in the basement membranes of the kidney in 2-dimensional cultures **(Figure 12B)**. Interestingly, under these conditions siRNA treated UB cells proliferated as well as untreated cells irrespective of the ECM substrate (undigested and trypsin digested collagen IV, laminins 111, 332 or 511/521). To ensure that siRNA treatment, itself, did not influence proliferation, siRNA-treated cells underwent MTT analysis. As predicted the knockdown system did not interfere with cell viability **(Figure 12C)**.
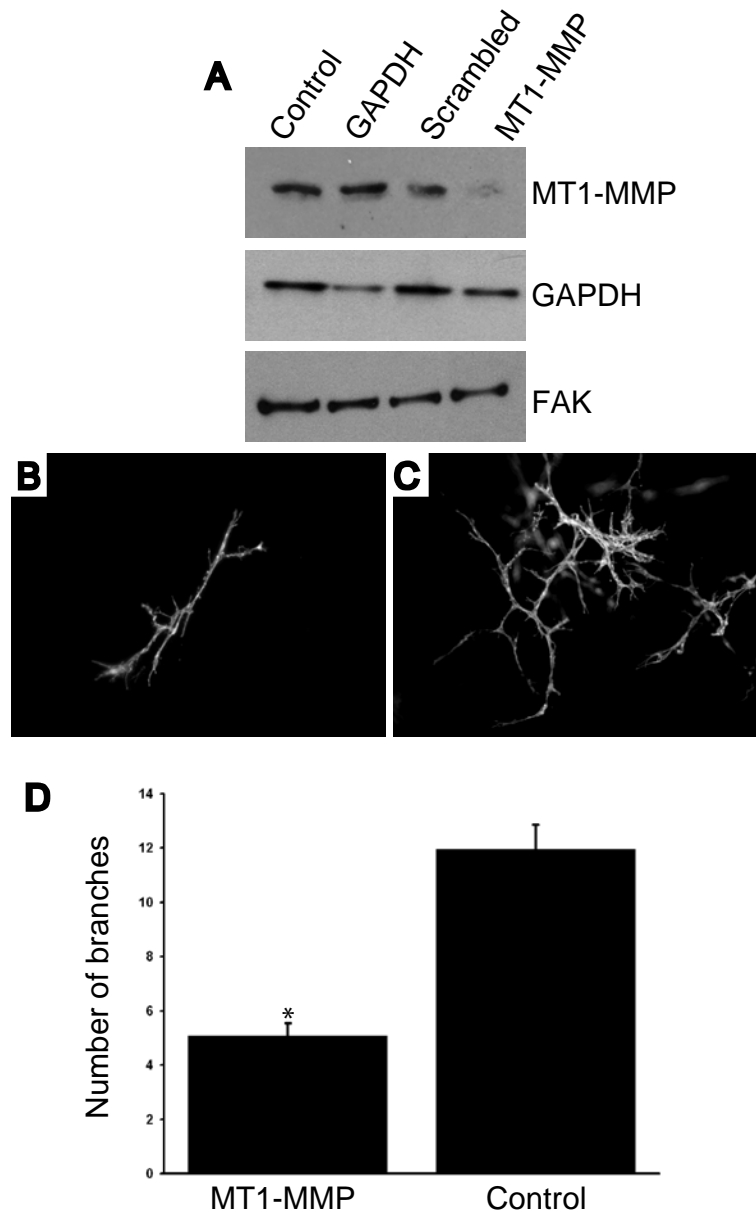
Figure 11: Silencing MT1-MMP results in abnormalities in UB growth and tubulogenesis.

UB cells were transfected with empty vector, double-strand irrelevant small interfering siRNA oligonucleotides (GAPDH and scrambled siRNAs) or siRNA oligonucleotides against MT1-MMP. Western blot analysis was performed 72 hours later utilizing a primary antibody against the catalytic domain of MT1-MMP as described in *Experimental Procedures* (A). UB cells were subjected to 72 hours of MT1-MMP siRNA targeted constructs and subsequently cultured in 3D-Collagen 1 and Matrigel gels (B), and compared to UB cells treated with the scrambled construct as that the same concentration (D). Images were recorded 12 days after culture. The number of branches in 10 kidneys in each group was counted and expressed as the average +/- the standard deviation. * denotes a significant decrease in branch number in kidneys grown in the presence of TIMP-2 relative to control (p<0.05) (D).

While the results presented demonstrate that MT1-MMP expression is required for renal tubule cells to proliferate in a 3-dimensional matrix, they do not define the requirement of the proteolytic activity of MT1-MMP. We therefore isolated inner medullary collecting duct cells from MT1-MMP null mice at postnatal day 15, which were reconstituted with either human MT1-MMP or a proteolytically inactive E240A MT1-MMP mutant. Cells were sorted for equal expression by flow cytometry **(Figure 13A)**. We verified there was increased MMP activation in the MT1-MMP null cells reconstituted with wild type MT1-MMP by performing zymography **(Figure 13B).** When these cells were placed in 3-dimensional collagen/matrigel gels **(Figure 13C)** or collagen gels (data not shown) the MT1-MMP null IMCD cells reconstituted with wild type MT1-MMP proliferated significantly more than either the MT1-MMP null IMCD cells or E240A reconstituted cells.
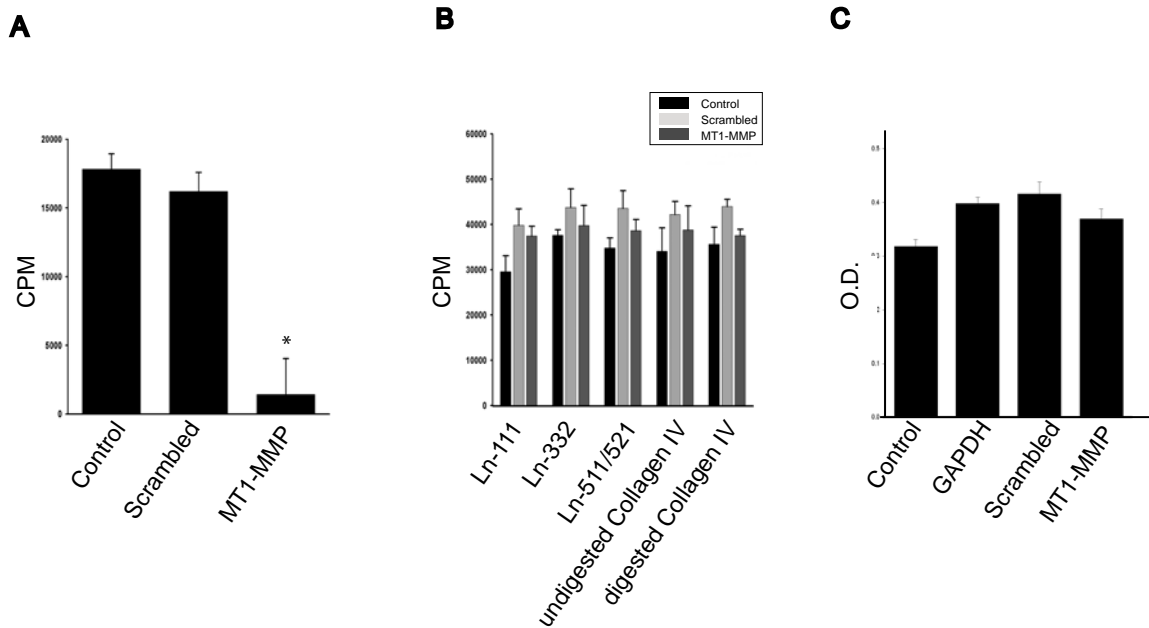
Figure 12: Silencing MT1-MMP results in abnormalities in UB growth, 3D.
UB cells were transfected with either an empty vector (control) or double-strand irrelevant small interfering (si) RNA oligonucleotides against GAPDH, a scrambled peptide or MT1-MMP. Western blot analysis was performed 72 hours later utilizing primary antibodies against MT1-MMP, GAPDH or focal adhesion kinase (FAK) which was used as a loading control. A representative of four separate experiments is shown. (B-C) UB cells either treated with siRNA directed against MT1-MMP or a scrambled siRNA were placed in 3D-collagen I/matrigel gels and allowed to undergo branching morphogenesis as described in the methods. The number of branches in the different UB cell populations was counted and expressed as the average number of branches/tubular structure +/ the standard deviation (A). * denotes that significantly fewer branches were observed in the UB cells treated with siRNA directed against MT1-MMP (p<0.01). (B) UB cells treated with siRNA directed against MT1-MMP and scrambled siRNA were placed in 3D-collagen I/matrigel gels and proliferation was determined utilizing tritiated thymidine as described in the methods. The average and standard deviation of 4 experiments is shown. * denotes a significant decrease in proliferation in UB cells treated with siRNA (p<0.05) (F). When control UB cells and UB cells treated with siRNA directed against MT1-MMP or scrambled siRNA were placed in onto laminin-111, laminin-332, laminin-511, trypsin digested collagen IV (α1α1α2) and undigested collagen IV (α1α1α2) shown 3D-collagen I/matrigel gels no differences in proliferation was seen as determined by tritiated thymidine (B). UB cells treated with siRNA constructs were tested for viability using the MTT assay as described in *Experimental Procedures.* The average and standard deviation of 3 experiments is shown (C).

Together these results demonstrate that MT1-MMP is required for normal branching morphogenesis of UB cells *in vitro* and for proliferation of renal tubular cells in 3-dimensional ECM matrices. Furthermore, in this model system that phenocopies our *in vivo* data, we demonstrate the requirement of the proteolytic function of MT1-MMP for renal tubular cell proliferation in 3-dimensional matrices.
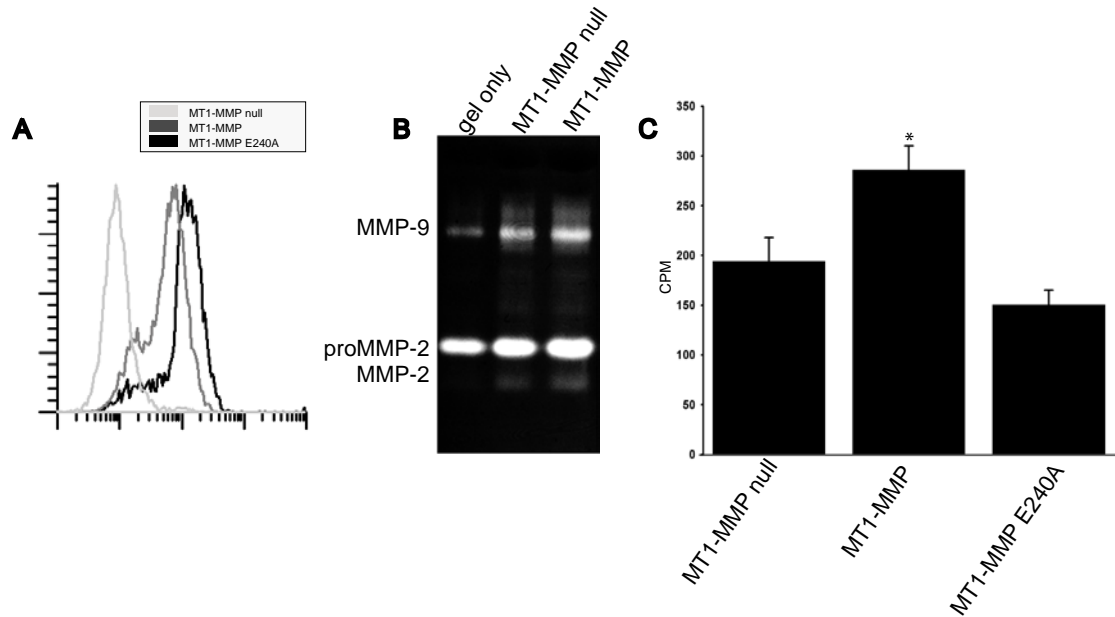
Figure 13: MT1-MMP-dependent proteolysis enhances for IMCD
Flow cytometry was performed MT1-MMP null IMCD containing pcDNA3.1 mammalian empty vector, pcDNA3.1 contain Human MT1-MMP-flag tagged, or pcDNA3.1 Human MT1-MMP E240A cells utilizing antibodies directed against the catalytic domain of MT1-MMP (A). Gelatin zymography of CD cells isolated from MT1-MMP null mice and reconstituted with MT1-MMP (MT1-MMP) were grown in 3D collagen gels. Gelatin zymography of cell lysates show activated MMP-9 and MMP-2 at comparative levels (B). MT1-MMP-null, MT1-MMP-null reconstituted with MT1-MMP (MT1-MMP) or an E240A mutant (MT1-MMP E240A) inner medullary collecting duct cells were placed in 3D-collagen I/matrigel gels and proliferation was determined utilizing tritiated thymidine as described in the methods. The average and standard deviation of 4 experiments is shown. * denotes a significant increase in proliferation in IMCD cells expressing MT1-MMP (p<0.05) (C).

Kidney basement membranes of MT1-MMP null mice have excessive amounts of ECM components.

We previously demonstrated that the dysmorphic dysgenic renal phenotype of the MT1-MMP null mice correlated with increased laminin 332 in renal tubules. However in the experiments described above, we show that MT1-MMP is also required for renal tubular cells to proliferate and undergo tubulogenesis in 3-dimensional collagenI/matrigel gels which do not contain laminin 322. Together these results suggest that renal tubule cell MT1-MMP exerts its proteolytic effects on multiple ECM components.

To examine this possibility further, we performed immunohistochemistry on kidneys on MT1-MMP-null and wild type mice to define whether there were differences in the amount of the predominant ECM components of the kidney basement membranes *in vivo* namely; collagen IV ($\alpha 1.\alpha 2.\alpha 1.$), laminins 111, 332, 511/521, entactin/nidogen and sulfated proteoglycans. Relative to the wildtype controls, all components of the BM were increased in either the tubules and/or the glomeruli of the MT1-MMP null animals **(Figure 14A).** These increases were confirmed on immunoblots of whole kidney lysates of MT1-MMP-null mice **(Figure 14B).** Thus, MT1-MMP regulates the amount of deposition of all the major components of the renal basement membranes. Surprising, despite the increase in BM deposition in null animals, MT1-MMP null and wildtype kidney glomerular basement membranes were of similar thickness in electron microscopy analysis **(Figure 15A)** with no evidence of glomerular function abnormalities as evidenced by urine analysis **(Figure 15B)**.

MT1-MMP proteolytic cleavage of laminin and collagen IV BM components is required for renal tubular epithelial cell migration.

We next wanted to assess whether the lack of MT1-MMP-dependent BM proteolysis affects cellular processes known to be required for renal development. We therefore measured cell migration of UB cells where MT1-MMP was knocked down by siRNA on the BM components Ln-111, Ln-332, and Ln-511/521, and Collagen IV **(Figure 16A).** Depleting MT1-MMP significantly decreased haptotactic migration on all these substrates but not on trypsin cleaved collagen IV. Conversely when the full length but not the R/A mutant proteolytically dead MT1-MMP was transduced into MT1-MMP null IMCD cells, there was significantly increased cell migration on all these substrates. **(Figure 16B)**. Thus the proteolytic activity of MT1-MMP increases renal tubular epithelial cell migration on the major ECM components found in renal tubule BMs.
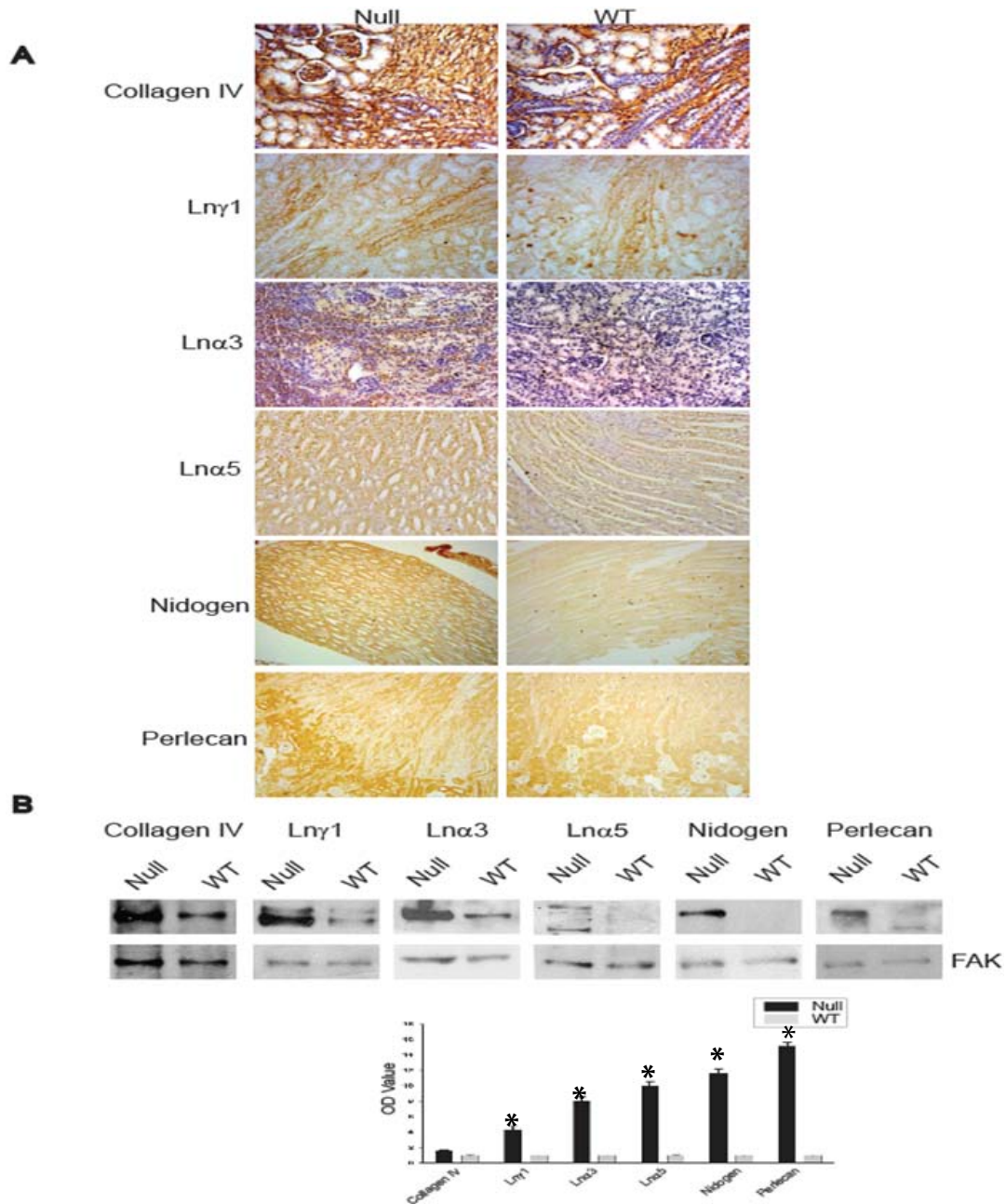
Figure 14: MT1-MMP modulates renal basement membrane composition.
Mouse kidneys at different stages of postnatal development were stained for collagen IV, laminins 111, 332, 511/521, entactin/nidogen and sulfated proteoglycans expression using Immunohistochemistry techniques mentioned in *Experimental Procedures* section (A). Total kidney lysates (30 μg) were immunoblotted for proteins described above. Membranes were incubated with anti-FAK antibody to confirm equal loading (B). Immunoblots of the BM proteins and FAK from MT1-MMP and wild type mice were scanned, normalized and expressed as the relative intensity of MT1-MMP mice compared to wild type mice. The averages and standard deviations of 4 different mice are shown. * denotes a significant increase in the amount of basement membrane proteins in the MT1-MMP-null mice (C).

Conclusion

MT1-MMP remodeling of the pericellular ECM environment plays a critical role in bone (Holmbeck 1999), lung (Atkinson 2005; Oblander 2005) and submandibular gland development (Oblander 2005). We now demonstrate that MT1-MMP also plays a role in kidney development. Kidneys from MT1-MMP null mice exhibit a severe proliferation and a mild to moderate UB branching defect with decreased nephrogenesis. These morphological defects are associated with increased amounts of the principal BM components. No defects in MMP-2 and MMP-9 activation were evident in the kidneys suggesting that the developmental abnormalities were due to a defect in MT1-MMP proteolysis of BM components. MT1-MMP-dependent proteolysis is shown to be required for kidney branching morphogenesis in organ culture, renal tubular cell proliferation in 3D matrix gels and cell migration on specific BM components. Thus, MT1-MMP regulates normal renal development due to its ability to cleave renal BM components.
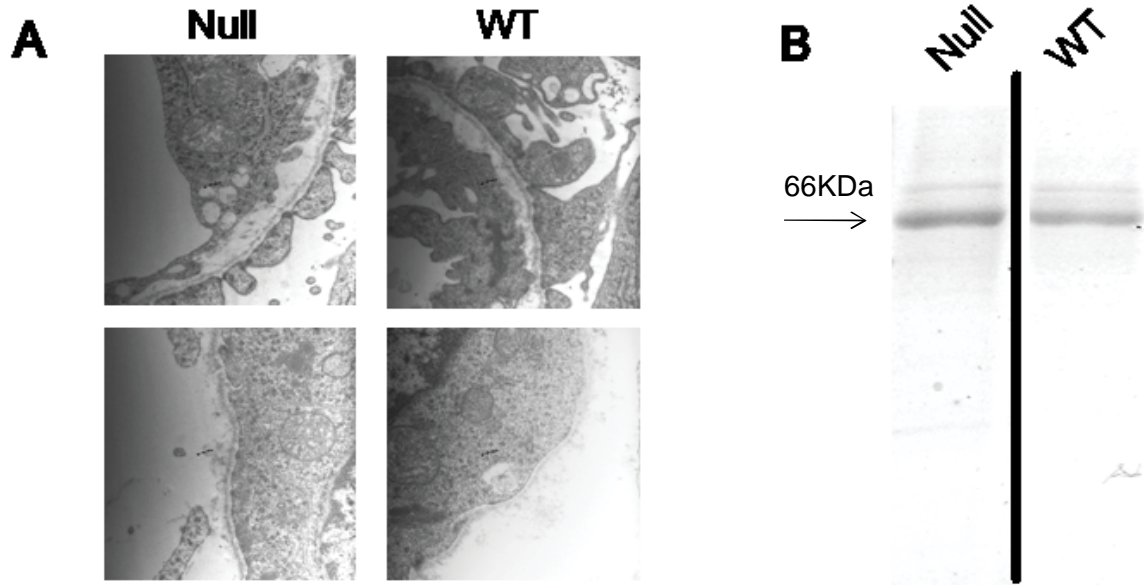
Figure 15: MT1-MMP null mouse kidneys have functional BM.
Embryonic and newborn kidneys were isolated, fixed in glutaraldehyde, and examined by electron microscopy to determine structural abnormalities. GBMs appear to be similar in null and wildtype animals (A). Urine was extracted from MT1-MMP null and wildtype mice and analyzed for protein by SDS-PAGE and subsequent Coomassie staining (5 μl/lane) Albumin (66KDa) concentrations are similar in MT1-MMP null and wildtype mice(B).

MT1-MMP is the only MMP shown to play a significant role in renal development both *in vivo* and *in vitro*. We demonstrate a branching defect in the MT1-MMP null mice that occurs early in development. This is consistent with the recent observation that MT1-MMP is a downstream target of ETS transcription factors Etv4 and Etv5, which are positively regulated by Ret signaling in the ureteric bud tips (Lu BC 2009). The Ret-GDNF axis is one of the critical determinants for the initiation and subsequent branching of the UB in renal development (Schuchardt A 1994; Pichel JG 1996; Zehnder 2005; Roy 2009).

The UB branching morphogenesis defect in MT1-MMP null mice, which is recapitulated in an *in vivo* organ culture model, is similar to that seen in the submandibular gland but not in the lung where branching is normal (Oblander 2005). Our data confirm previous studies demonstrating a role for MT1-MMP in UB branching morphogenesis *in vitro* (Kanwar YS 1999). However, contrary to these *in vitro* reports, our data demonstrates that MT1-MMP modulates renal branching morphogenesis by its inherent proteolytic activity and does not require MT1-MMP/TIMP-2-dependent MMP-2 activation. These results are similar to the observation that MMP-2 activation occurs in fibroblasts isolated from MT1-MMP null animals (Miner 1999) and verifies that like other physiological situations, MMP-2 activation is not required for normal renal development.
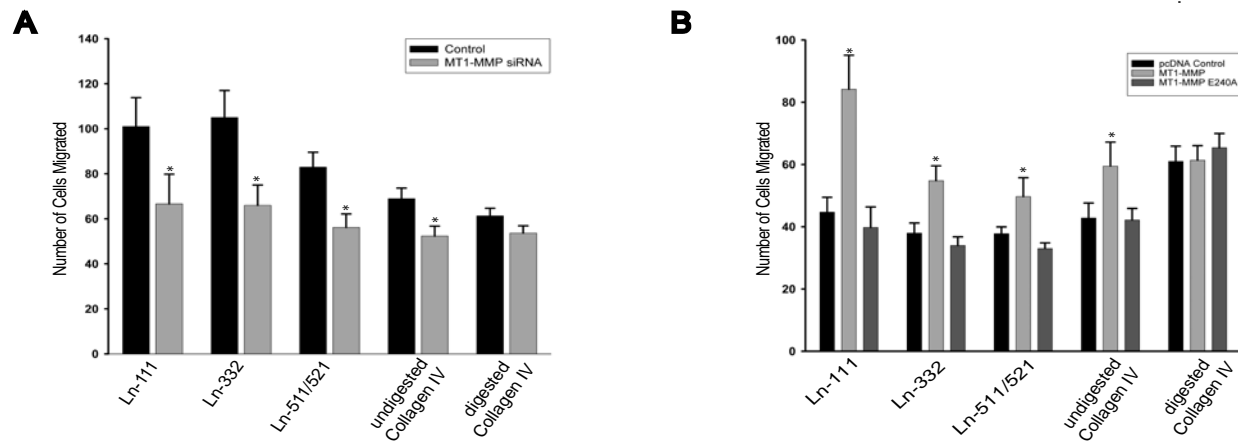
Figure 16: MT1-MMP proteolytic activity stimulates renal cell migration.
UB cells were subjected to either a scrambled siRNA or siRNA directed at MT1-MMP silencing and allowed to migrate on digested and undigested collagen IV ($\alpha1\alpha1\alpha2$), laminin-111, laminin-332 or laminin 521 (all at 10 μg/ml) for 4 hours. The cells were counted at the end of this time and the number is expressed as cells/high power field. Values are the mean and standard deviation of three experiments performed in triplicate. * denotes statistically significant differences (P<0.05) between the two cell populations (A) IMCD cells that were either null for MT1-MMP or were reconstituted with MT1-MMPor MT1-MMP E240/A were allowed to migrate on digested and undigested collagen IV ($\alpha1\alpha1\alpha2$), laminin-111, laminin-332 or laminin 521 (all at 10 μg/ml) for 4 hours. The cells were counted at the end of this time and the number is expressed as cells/high power field. Values are the mean and standard deviation of three experiments performed in triplicate. * denotes statistically significant differences (P<0.05) between MT1-MMP null IMCD cells reconstituted with human MT1-MMP or MT1-MMP E240A(B).

One of the most striking abnormalities in the MT1-MMP null kidneys is the proliferation defect, which was also seen in our *in vitro* organ (data not shown) and 3D cell culture systems but not in 2D cell culture irrespective of the ECM on which the cells were plated. These results are consistent with *in vitro* studies where pericellular collagenolysis has been shown to be required for cellular proliferation (Hotary 2003). The mechanisms whereby this mediates proliferation is unclear, however, it was recently demonstrated that MT1- and MT2-MMP dependent cleavage of NC1 domains of collagen IV is required for the proliferation and branching of the submandibular gland (Rebustini IT 2009). In this system, it was proposed that NC1 domains signal via $\beta1$ integrins to induce epithelial proliferation by the induction of epithelial HB-EGF and FGF1. Another study showed that HB-EGF is a substrate for MT1-MMP, thus it is possible that MT1-MMP increases cell proliferation by inducing both ECM proteolysis and processing HB-EGF to a more active form (Koshikawa, in press).

The kidney is the only organ to show BM abnormalities in the MT1-MMP null mice. We previously demonstrated an increase in the $\gamma2$ laminin chain (Koshikawa 2004) and in the current study we show that all the major components of the BM are increased *in vivo*. Our study is the first demonstration of increased nidogen and perlecan deposition in the MT1-MMP mice and is consistent with the ability of MT1-MMP to digest these ECM components *in vitro* (d'Ortho 1997). Our observation of increased BM components in the kidney in the MT1-MMP mice contrasts with the submandibular gland where the BM component increases were only seen when MT2-MMP siRNA was used (Rebustini IT 2009). In this context the $\alpha2$ chains of collagen IV and laminin $\alpha5$ were increased due

67

to both a decrease in degradation and an increase in production. The increased collagen IV in the MT1-MMP null kidney was less than that of other BM components, suggesting that MT2-MMP might be the major MT-MMP for collagen IV degradation. The role of MT2-MMP in renal development has not been defined, however based on the data from the submandibular gland it is likely that its expression is increased in the MT1-MMP-null mice.

In conclusion, we have shown that MT1-MMP plays an important role in renal development, where it mediates it regulates both cell proliferation and branching morphogenesis. These affects are at least in part mediated by the direct ability of MT1-MMP to proteolytically cleave multiple ECM components. Based on the critical role of MT2-MMP in submandibular gland development *in vitro*, it is likely that both MT1-, MT2- and perhaps MT4-MMP regulate renal development. The specific roles of these different MT-MMPs will only be defined when floxed mice for these proteases are generated so that compound and cell specific mutants can be analyzed in detail.

Discussion

2D versus 3D

It is interesting that MT1-MMP stimulates UB cell growth in a 3D and not 2D-dependent manner. Much like our embryonic UB cell model system, cancer cells have been shown to proliferate at accelerated rates within the confines of a three-dimensional (3D) extracellular matrix (ECM) that is rich in type I collagen. Specifically, Hotary and colleagues were able to show that MT1-MMP confers tumor cells with a distinct 3D growth advantage *in vitro* and *in vivo*. This growth advantage conferred by MT1-MMP

68

requires pericellular proteolysis of the ECM, as proliferation is fully suppressed when

tumor cells are suspended in 3D gels of protease-resistant collagen.

Pericellular degradation is believed to require changes within the cell shape or

cytoskeleton reorganization (Hotary 2003). It is probable that this kind of rearrangement

is not afforded in a 2-dimensional system. Studies have suggested that cell geometry or

cytoskeleton reorganization can largely be determined by two core cellular properties,

cell-cell adhesion and cell contractility (Montell 2008). I have been able to show that

MT1-MMP does not affect the cell-cell interactions in our *in vivo* mouse model through

E-cadherin and ZO-1 localization. However, cell contractility has not been explored. It

is important to mention, here, that cultured MT1-MMP null IMCD cells differ in shape

than IMCD cells that have been transfected with MT1-MMP. With MT1-MMP

expression, cells appear to form more expansive cellular extensions and projections on 2-

D substratum compared to null control cells. As the ability for a cell to generate cell

protrusions while contributes to the movement of the cells (Montell 2008), this

unpublished observation identifies a MT1-MMP-dependent cell geometry which is

probably exaggerated within a 3-D matrices.

UB tip is a developmental model for invasion

Our developmental research exploring the importance of MT1-MMP-mediated

ECM turnover is of interest in the scope of cancer biology. The regulated process

involving ECM degradation has been shown to be disregulated during cancer metastasis

(Kalluri 2003; Nart 2010). The major family of ECM degrading enzymes involved in

both kidney organogenesis and tumorigenesis is the matrix metalloproteinases (MMPs)

family (Lelongt 1997; Hornebeck 2002). Furthermore, in different cancer invasion models, functional loss of MT1-MMP is poorly compensated by other enzymes (Hotary 2000; Sabeh 2004). Our findings provide much insight to a MT1-MMP-dependent mechanism in develop and cancer.

Invadopodia, or invasive foot processes, are actin-rich structures that function in degradation of the extracellular matrix (Buccione 2004); these cellular processes are believed to play a role in tumor cell metastasis. Within the findings of our study, it is important to identify the UB as a multi-cellular protrusion. Whereas single cell movement can occur independently of protease function and migrate in an amoeboid fashion, multi-cellular movement requires manipulation of the ECM.

According to studies on the UB, the formation of cellular processes is a critical part of the morphological change that occurs during tubulogenesis (Sakurai 1997). Much like invadopodia, the UB outgrowth of the Woffian duct must invade through the basement membrane to enable organ formation. UB and invadopodia processes have two commonalities: 1) movement is concerted and directional and 2) MT1-MMP has been localized to the leading edge of both invading structures. Under this comparison, it is acceptable that kidney development studies can further the fields knowledge on tumor invasion and metastasis.

The proteolytic turnover of the ECM mediated by MMPs is another important factor in the regulation of tumorigenesis. Overexpression of collagenase in the skin of transgenic mice enhances chemically induced carcinogenesis (D'Armiento 1995), and stromelysin-1 can trigger mammary epithelial cell transformation and tumorigenesis (Sympson 1995; Lochter 1997). Conversely, the loss of stromelysin-3 inhibits chemical

70

carcinogenesis and stroma-dependent tumor implantation (Lukashev 1998; Masson 1998).

We have now shown that the loss of MT1-MMP attenuates cell proliferation as well as

protease-stimulated migration on ECM substrates. Additionally, we have shown that

MT1-MMP promotes cellular proliferation and UB branching within 3-D matrices. Our

work suggests that the presence of abundant MT1-MMP at the tips of UB is a potential

mechanism for tubule formation and subsequent growth through extracellular matrix

action.

Future Directions

MT1-MMP Synergy

Weiss and colleagues have proposed that a triad of membrane tethered proteases

namely, MT1-, MT2-, and MT3-MMP trigger agents that independently confer cancer

cells with the ability to proteolytically efface the BM scaffolding and propagate

transmigration (Hotary K 2007). Furthermore, studies involving MT2-MMP activity in

submanibular development have also led us to consider that other membrane bound

proteases during kidney development (Rebustini IT 2009). While the expression or

importance of MT2- and MT3-MMP have not been explored in renal development, it is

possible that MT1-MMP acts in synergy with MT2- and MT3-MMP. It would be

interesting to utilize our *in vivo* mouse model as well as our *in vitro* cell systems to

determine the relative expression levels of membrane tethered MMPs, namely MT2- and

MT3-MMPs during renal development using RT-PCR analysis. Additionally *in vitro*

proteolysis analysis would determine whether MT1-MMP directly cleaves ECM

components and/or acts synergistically with MT2-MMP and/or MT3-MMP. These

studies would also allude to any amount of compensation in the absence of MT1-MMP.

Despite the homology of the overall domain structures of MT1-, MT2- and MT3-MMP, the potency of MT1-MMP has been distinct from the other MT-MMPs (Nagase 1999). Thus, while these studies may reveal MT-MMP synergy, it likely that MT1-MMP will play the major role in basement membrane remodeling.

While our current MT1-MMP null mouse model was appropriate at this present study, our null animals die at 2 weeks old and would present extensive limitations to a study on MT-MMP synergies. A more appropriate *in vivo* model for these studies would be using the conditional knock out Cre-loxP system. As the MT1-MMP null phenotype is characterized by moderate a UB branching defect with decreased nephrogenesis, the best system would be mice expressing HoxB7-Cre. Under the control of the HoxB7, MT1-, MT2-, and MT3-MMP expression could be ablated from the UB and its derivatives.

Once this system is established, further investigation to determine the relevant ECM substrates in renal development would compliment our current study. We would assess MT-MMP-dependent basement membrane remodeling by isolating basement membrane from wildtype, MT1-, MT2-, MT3-MMP knockout animals at various embryonic and adult time points. These studies would determine whether there are differences in the cleavage products within the renal basement membranes of the MT1-MMP null mice compared to MT2- and MT3- null mice. It would serve to support the finding of the *in vitro* proteolysis analysis mentioned earlier. Additionally, UB cells isolated from MT1-, MT2-, and MT3-MMP floxed mice, would be an excellent tool to

determine the MT-MMPs that are critical regulators of early cellular processes including

migration, proliferation and even tubulogenesis.

MT1-MMP and growth factors

GDNF, a growth factor found to regulate *Mmp14* expression (Lu BC 2009), is

important for UB cell proliferation, cell survival and branching of the epithelium. We

have shown that MT1-MMP also influences UB tubulogenesis, in part, through

modulating proliferation through a 3-D dependent pathway. It is quite possible that

MT1-MMP-mediated ECM degradation collaborates with GDNF signaling to stimulate

UB morphogenesis. To test this hypothesis, we would first have to determine the relative

expression levels of GDNF and Ret in MT1-MMP null and wildtype littermates at

embryonic time points. This would determine whether MT1-MMP expression can

modulate the GDNF pathway. We could then treat isolated MT1-MMP null and wildtype

kidneys at E12.5 with exogenous GDNF and/or Ret inhibitors to determine whether the

MT1-MMP null phenotype could be rescued. Collectively, these findings should confirm

gene expression data that expresses a link between MT1-MMP and GDNF.

HGF signaling is required for UB elongation and has been linked to MT1-MMP

stimulated tubulogenesis (Kadono Y 1998). This is important as MT1-MMP expression

at the UB tip, *in vivo* (Kanwar YS 1999) and *in vitro* (Meyer TN 2004) suggests a

potential mechanism for UB outgrowth and subsequent elongation. In the discussion of

growth factor modulation, it would be interesting to assess the level of crosstalk between

MT1-MMP and HGF in renal development. Reports have shown that the addition of

exogenous HGF to MDCK cells overexpressing MT1-MMP showed initial scattering of

cells from the cavity into the gel, but organized branching tubules were not formed

(Kadono Y 1998), similar to reports looking at HGF and MT2-MMP (Hotary 2000).

These studies suggest that HGF may stimulate MT1-MMP and MT2-MMP-dependent

matrix degradation at a rate permissive for invasion, not branching morphogenesis.

However, our studies show the MT1-MMP, independent of HGF, modulates

branching during tubulogenesis. Concomitant with our findings, the overexpression of

MT1-MMP in mammary epithelial cells lead to the development of roughly twice the

number of branch sites (Ha 2001). It is possible that MT1-MMP stimulates tubule

branching while HGF stimulation induces MT-MMP dependent invasion.

We could utilize our siRNA system in our UB cells to inhibit MT1-MMP and/or

MET in the presence or absence of HGF within 3D cultures. These experiments could

distinguish the roles of HGF and MT1-MMP in the process of tubulogenesis as the two

molecules are linked but the connection is not well defined. The proposed experiments

would provide more insight to the actual influence of HGF on MT1-MMP-dependent UB

morphogenesis.

Adhesion and Migration Equilibrium

Our laboratory is globally interested in cell-ECM interactions during renal

development and disease. Primarily, our lab focuses on the role of integrins as ECM

receptors including integrins with laminin-binding activity. Our MT1-MMP analysis has

shown a correlation between lack of Ln-γ2 chain cleavage, increased accumulation of

uncleaved Ln-332 and renal abnormalities in the MT1-MMP null mice. Based on this

data, we believe that the abnormalities present in the MT1-MMP mice are due, in part, to

aberrations of Ln-332 cleavage. However, it is also known that full length Ln-332 plays

are role in epithelial cellular processes such as cell spreading, migration, and proliferation.

We have shown that Ln-332 is expressed during renal organogenesis, but there is

no evidence that determines whether the Ln-332 is present as uncleaved or cleaved form.

Furthermore, little is known about the function of Ln-332 in renal development. While

recent studies suggest a role for Ln-332 in glomerulogenesis (Abrass CK 2006), our lab

has observed severely dysplastic kidneys of Ln-332 null mice with abnormalities in all

components of the kidney.

Therefore, it is possible that the importance of MT1-MMP mediated processing of

Ln-332 is due in part to the critical role of Ln-332 during renal development. By

characterizing the renal phenotype of embryonic and newborn kidneys from wildtype and

Ln-332-null mice (LAMα3- and LAMγ2- null mice) to determine anatomical

abnormalities and then using embryonic UB and collecting duct cells to assess the

importance of Ln-332 as a migratory substrate, the importance of the MT1-MMP

substrate, laminin-332 could be determined. Our preliminary findings are intriguing as

we are the first group to show that 42% of mice lacking the α3 subunit fail to develop a

right kidney. These findings have led us to suggest a role for Ln-332 in UB induction

and/or branching.

BIBILIOGRAPHY

Abrass CK, B. A., Ryan MC, Carter WG, Hansen KM. (2006). "Abnormal development of glomerular endothelial and mesangial cells in mice with targeted disruption of the lama3 gene. ." <u>Kidney Int. </u>**70**(6): 1062-1071.

Andrews, K., Betsuyaku, T, Rogers, S, Shipley, JM, Senior, RM, Miner, JH. (2000). "Gelatinase B (MMP-9) is not essential in the normal kidney and does not influence progression of renal disease in a mouse model of Alport syndrome." <u>Am J Pathol</u> **157**(1): 303-311.

Apte, S., Fukai, N, Beier, DR, Olsen, BR. (1997). "The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. ." <u>J Biol Chem. </u>**272**(41): 25511-25517.

Apte, S., Hayashi, K, Seldin, MF, Mattei, MG, Hayashi, M, Olsen, BR (1994). "Gene encoding a novel murine tissue inhibitor of metalloproteinases (TIMP), TIMP-3, is expressed in developing mouse epithelia, cartilage, and muscle, and is located on mouse chromosome 10." <u>Dev Dyn</u> **200**: 177-197.

Atkinson, J., Holmbeck, K, Yamada, S, Birkedal-Hansen, H, Parks, WC, Senior, RM. (2005). "Membrane-type 1 matrix metalloproteinase is required for normal alveolar development." <u>Developmental Dynamics </u>**232**: 1079-1090.

Bader, B., Smyth N, Nedbal S, Miosge N, Baranowsky A, Mokkapati S, Murshed M, Nischt R. (2005). "Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice." <u>Mol Cell Biol. </u>**25**(15): :6846-6856.

Bader B, S. N., Nedbal S, Miosge N, Baranowsky A, Mokkapati S, Murshed M, Nischt R (2005). "Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice." <u>Mol Cell Biol </u>**25**: 6846–6856.

Bair, E., Chen, ML, McDaniel, K, Sekiguchi, K, Cress, AE, Nagle, RB, Bowden, GT. (2005). "Membrane type 1 matrix metalloprotease cleaves laminin-10 and promotes prostate cancer cell migration." <u>Neoplasia</u> **7**(4): 380-389.

Barasch, J., Yang, J, Qiao, J, Tempst, P, Erdjument-Bromage, H, Leung, W, Oliver, JA. (1999). "Tissue inhibitor of metalloproteinase-2 stimulates mesenchymal growth and regulates epithelial branching during morphogenesis of the rat metanephros." <u>J Clin Invest</u> **103**(9): 1299-1307.

Belkin, A., Akimov, SS, Zaritskaya, LS, Ratnikov, BI, Deryugina, EI, Strongin, AY. (2001). "Matrix-dependent proteolysis of surface transglutaminase by membrane-type

metalloproteinase regulates cancer cell adhesion and locomotion." <u>J Biol Chem</u> **276**(21): 18415-18422.

Borghese, E. (1950). "Explantation experiments on the influence of the connective tissue capsule on the development of the epithelial part of the submandibular gland of Mus musculus." <u>J Anat</u> **84**(1950): 303-321.

Boyle, S., Shioda, T, Perantoni, AO, de Caestecker, M. (2007). "Cited1 and Cited2 are differentially expressed in the developing kidney but are not required for nephrogenesis." <u>Dev Dyn</u> **236**(8): 2321-2330.

Breitkrentz D, M. N., Roswitha N. (2009) (2009). " Basement membranes in skin: unique matrix structures with diverse functions?" <u>Histochem Cell Biol </u>**132**: 1-10.

Brinckerhoff, C. (1991). "Joint destruction in arthritis: metalloproteinases in the spotlight." <u>Arthritis Rheum.</u> **37**(9): 1073-1075.

Buccione, R., Orth, J. D., and McNiven, M. A. (2004). "Foot and mouth: podosomes, invadopodia and circular dorsal ruffles." <u>Nat. Rev. Mol. Cell Biol.</u> **5**: 647-657.

Butkowski, R., Langveld, JPM, Wieslander, J, Hamilton, J, Hudson, BG: 1987 262:7874–7877, (1987). "Localization of the Goodpasture epitope to a novel chain of basement membrane collagen. ." <u>J Biol Chem </u>**262**: 7874-7877.

Cao, J., Sato, H., Takino, T. and Seiki, M. (1995). "The C-terminal region of membrane type matrix metalloproteinase is a functional transmembrane domain required for pro-gelatinase A activation. ." <u>J. Biol. Chem. </u>**270**: 801-805.

Chen, D., Roberts, R, Pohl, M, Nigam, S, Kreidberg, J, Wang, Z, Heino, J, Ivaska, J, Coffa, S, Harris, RC, Pozzi, A, Zent, R. (2004). "Differential expression of collagen- and laminin-binding integrins mediates ureteric bud and inner medullary collecting duct cell tubulogenesis. ." <u>Am J Physiol Renal Physiol.</u> **287**(4): 602-611.

Chun, T., Hotary, KB, Sabeh, F, Saltiel, AR, Allen, ED, Weiss, SJ. (2006). "A pericellular collagenase directs the 3-dimensional development of white adipose tissue." <u>Cell</u> **125**(3): 577-591.

Costantini, F. S., R. (2006). "GDNF/Ret signaling and the development of the kidney. ." <u>Bioessays </u>**28**: 117-127.

D'Armiento, J., DiColandrea, T., Dalal, S.S., Okada, Y., Huang, M.-T., Conney, A.H., Chada, K. (1995). "Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis." <u>Mol and Cell Biol</u> **15**(10): 5732-3739.

d'Ortho, M., Will, H, Atkinson, S, Butler, G, Messent, A, Gavrilovic, J, Smith, B, Timpl, R, Zardi, L, Murphy, G. (1997). "Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases." 250 **3**: 751-757.

Davies J, F. C. (2002). "Genes and Proteins in Renal Development." Exp Nephrology **10**: 102.

Deryugina, E., Ratnikov, BI, Postnova, TI, Rozanov, DV, Strongin, AY. (2002). "Processing of integrin alpha(v) subunit by membrane type 1 matrix metalloproteinase stimulates migration of breast carcinoma cells on vitronectin and enhances tyrosine phosphorylation of focal adhesion kinase." J Bio Chem. **277**(12): 9749-9756.

Ekblom, P., Alitalo, K, Vaheri, A, Timpl, R, Saxen, L (1980). "Induction of a basement membrane glycoprotein in embryonic kidney: possible role of laminin in morphogenesis." Proc Natl Acad Sci U S A. **77**: 485-489.

Ekblom P, E. M., Fecker L, Klein G, Zhang HY, Kadoya Y, Chu ML, Mayer U, Timpl R: (1994). "Role of mesenchymal nidogen for epithelial morphogenesis in vitro." Development **120**: 2003-2014.

Ekblom, P., Lehtonen, E, Saxen, L, Timpl, R (1981). "Shift in collagen type as an early response to induction of the metanephric mesenchyme." J Cell Biol **89**: 276-283.

Endo, K., Takino, T, Miyamori, H, Kinsen, H, Yoshizaki, T, Furukawa, M, Sato, H. (2003). "Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration." J Bio Chem. **278**(42): 40764-40770.

Eremina, V., Baelde, HJ., and Quaggin, SE. (2007). "Role of the VEGF—a signaling pathway in the glomerulus: evidence for crosstalk between components of the glomerular filtration barrier." Nephron Physiol **106**: 32-37.

Fingleton B, L. C. (2010). "A new dress code for MMPs: cleavage optional." Dev Cell. **18**(1): 3-4.

Fox, J. W., Mayer, U., Nischt, R., Aumailley, M., Reinhardt, D., Wiedemann, H., Mann, K., Timpl, R., Krieg, T., Engel, J. and Timpl, R. (1991). " Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen type IV." EMBO J **10**: 3137-3146.

Ganser, G., Stricklin, GP, Matrisian, LM. 1991 Dec;35(4):453–461 (1991). "EGF and TGF alpha influence in vitro lung development by the induction of matrix-degrading metalloproteinases." Int J Dev Biol. **35**(4): 453-461.

Gersdorff, N., Kohfeldt, E, Sasaki, T, Timpl, R, Miosge, N. (2005). "Laminin gamma3 chain binds to nidogen and is located in murine basement membranes. ." <u>J Biol Chem.</u> **280**(23): 22146-22153.

Golubkov, V., Chekanov, AV, Savinov, AY, Rozanov, DV, Golubkova, NV, Strongin, AY. (2006). "Membrane type-1 matrix metalloproteinase confers aneuploidy and tumorigenicity on mammary epithelial cells. ." <u>Cancer Res.</u> **66**(21): 10460-10465.

Gonzalo P, G. M., Hernández-Riquer MV, Pollán A, Grande-García A, Bartolomé RA, Vasanji A, Ambrogio C, Chiarle R, Teixidó J, Risteli J, Apte SS, del Pozo MA, Arroyo AG. (2010). "MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling." <u>Dev. Cell</u> **18**(1): 77-89.

Grobstein, C. (1955). "Tissue disaggregation in relation to determination and stability of cell type." <u>Ann N Y Acad Sci.</u> **2**(60): 1095-1107.

Ha , H., Moon, HB, Nam, MS, Lee, JW, Ryoo, ZY, Lee, TH, Lee, KK, So, BJ, Sato, H, Seiki, M, Yu, DY. (2001). "Overexpression of membrane-type matrix metalloproteinase-1 gene induces mammary gland abnormalities and adenocarcinoma in transgenic mice." <u>Cancer Res.</u> **61**(3): 984-990.

Hartman HA, L. H., Patterson LT. (2007). "Cessation of renal morphogenesis in mice." <u>Dev Biol.</u> **310**(2): 379-387.

Harvey P, C. I., Jaurand MC, Warn RM, Edwards DR.   (2000). "Hepatocyte growth factor/scatter factor enhances the invasion of mesothelioma cell lines and the expression of matrix metalloproteinases." <u>Br J Cancer.</u> **83**(9): 1147-1153.

Hata, D., Miyazaki, M, Seto, S, Kadota, E, Muso, E, Takasu, K, Nakano, A, Tamai, K, Uitto, J, Nagata, M, Moriyama, K, Miyazaki, K. (2005). "Nephrotic syndrome and aberrant expression of laminin isoforms in glomerular basement membranes for an infant with Herlitz junctional epidermolysis bullosa." <u>Pediatrics</u> **116**(4): 601-607.

Holliday, M., Barratt, TM., and Avner, ED. (1994). Pediatric nephrology 3rd edition. Baltimore, Williams & Wilkins**:** 3-24.

Holmbeck, K. (2005). "Collagenase in cranial morphogenesis." <u>Cells Tissues Organs.</u> **181**(3-4): 154-165.

Holmbeck, K., Bianco, P, Caterina, J, Yamada, S, Kromer, M, Kuznetsov, SA, Mankani, M, Robey, PG, Poole, AR, Pidoux, I, Ward, JM, Birkedal-Hansen, H. (1999). "MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover." <u>Cell</u> **99**(1): 81-92.

Holmbeck, K., Bianco, P, Yamada, S, Birkedal-Hansen, H.   (2004). " MT1-MMP: A tethered collagenase." <u>J Cell Physiol.</u> **200**: 11-19.

Hornebeck, W., Emonard, H, Monboisse, JC and Bellon, G (2002). "Matrix-directed regulation of pericellular proteolysis and tumor progression." <u>Semin Cancer Biol</u> **12**: 231-241.

Hotary, K., Allen, E, Punturieri, A, Yana, I, Weiss, SJ. (2000). "Regulation of cell invasion and morphogenesis in a three-dimensional type I collagen matrix by membranetype matrix metalloproteinases 1, 2, and 3. ." <u>J Cell Biol</u> **149**: 1309-1323.

Hotary, K., Allen, ED, Brooks, PC, Datta, NS, Long, MW, Weiss, SJ. (2003). "Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix." <u>Cell</u> **114**(1): 33-45.

Hotary, K., Li, XY, Allen, E, Stevens, SL, Weiss, SJ. (2006). "A cancer cell metalloprotease triad regulates the basement membrane transmigration program." <u>Genes Dev</u> **20**(19): 2673-2686.

Hotary K, L. X., Allen E, Stevens SL, Weiss SJ. (2007). "A cancer cell metalloprotease triad regulates the basement membrane transmigration program." <u>Genes Dev</u> **20**(19): 2673-2686.

Hudson, B., Reeders, ST, Tryggvason, K. (1993). "Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis." <u>J Bio Chem.</u> **268**(35): 26033-26036.

Hudson BG, T. K., Sundaramoorthy M, Neilson EG. (2003). "Alport's syndrome, Goodpasture's syndrome, and type IV collagen. ." <u>N Engl J Med.</u> **348**(25): 2543-2556.

Hurst DR, S. M., Ghaffari MA, Jin Y, Tschesche H, Fields GB, Sang QX. (2004). "Catalytic- and ecto-domains of membrane type 1-matrix metalloproteinase have similar inhibition profiles but distinct endopeptidase activities." <u>Biochem</u> **377**(3): 775-779.

Itoh, T. I., T; Gomi, H; Nakao, S; Suzuki, T; Itohara, S. (1997). "Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice." <u>J Biol Chem.</u> **272**(36): 22389-22392.

Itoh, Y., Ito, N, Nagase, H, Seiki, M. (2008). "The second dimer interface of MT1-MMP, the transmembrane domain, is essential for ProMMP-2 activation on the cell surface. ." <u>J Biol Chem</u> **283**(19): 13053-13062.

Itoh, Y., Nagase, H. (2002). "Matrix metalloproteinases in cancer." <u>Essays Biochem</u> **38**: 21-36.

Itoh, Y., Seiki, M. (2006). "MT1-MMP: a potent modifier of pericellular microenvironment." <u>J Cell Physiol.</u> **206**(1): 1-8.

Jarad G, C. J., Shaw AS, Miner JH. (2006). "Proteinuria precedes podocyte abnormalities inLamb2-/- mice, implicating the glomerular basement membrane as an albumin barrier." J Clin Invest. **16**(8): 2272-2279.

JH., M. (1998). "Developmental biology of glomerular basement membrane components." Curr Opin Nephrol Hypertens **7**(1): 13-19.

Kadono Y, S. K., Namiki M, Watanabe Y, Seiki M, Sato H. (1998). "Membrane type 1-matrix metalloproteinase is involved in the formation of hepatocyte growth factor/scatter factor-induced branching tubules in madin-darby canine kidney epithelial cells. ." Biochem Biophys Res Commun **251**(3): 681-687.

Kajita, M., Itoh, Y, Chiba, T, Mori, H, Okada, A, Kinoh, H, Seiki, M. (2001). "Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration." J Cell Biol **153**(5): 893-904.

Kalluri, R. (2003). "Basement membranes: structure, assembly and role in tumour angiogenesis." Nat Rev Cancer. **3**(6): 422-433.

Kalluri, R., Sun, MJ, Hudson, BG, Neilson, EG (1996). "The Goodpasture autoantigen: Structural delineation of two immunologically privileged epitopes on 3(IV) chain of type IV collagen. ." J Biol Chem **271**: 9062-9068.

Kanwar YS, C. F., Kumar A, Wada J, Ota K, Wallner EI. (1997). "Role of extracellular matrix, growth factors and proto-oncogenes in metanephric development." Kidney Int **52**(3): 589-606.

Kanwar YS, O. K., Yang Q, Wada J, Kashihara N, Tian Y, Wallner EI. (1999). "Role of membrane-type matrix metalloproteinase 1 (MT-1-MMP), MMP-2, and its inhibitor in nephrogenesis." Am J Physiol **277**(6 Pt 2): 934-947.

Kanwar YS, W. J., Lin S, Danesh FR, Chugh SS, Yang Q, Banerjee T, Lomasney JW. (2004). "Update of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis." Am J Physiol Renal Physiol. **286**(2): 202-215.

Kanwar YS, W. J., Lin S, Danesh FR, Chugh SS, Yang Q, Banerjee T, Lomasney JW. (2004). "Update of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis. ." Am J Physiol Renal Physiol. **286**(2): 202-215.

Klein G, L. M., Timpl R, Ekblom P. (1988). "Role of laminin A chain in the development of epithelial cell polarity. ." Cell **55**(2): 331-341.

Koshikawa, N., Schenk, S, Moeckel, G, Sharabi, A, Miyazaki, K, Gardner, H, Zent, R, Quaranta, V. (2004). "Proteolytic processing of laminin-5 by MT1-MMP in tissues and its effects on epithelial cell morphology." FASEB J. **18**(2): 364-366.

Kramer, J. (2005). "Basement membranes." WormBook.: 1-15.

Lehti, K., Lohi, J , Juntunen, MM , Pei, D, Keski-Oja, J. (2002). "Oligomerization through hemopexin and cytoplasmic domains regulates the activity and turnover of membrane-type 1 matrix metalloproteinase." J Bio Chem. **277   10   8440-8**.

Lelongt, B., Legallicier, B, Piedagnel, R, Ronco, PM. (2001). " Do matrix metalloproteinases MMP-2 and MMP-9 (gelatinases) play a role in renal development, physiology and glomerular diseases?" Curr Opin Nephrol Hypertens. **10**(7): 7-12.

Lelongt B, M. H., Dalecki TM, and Kanwar YS. (1988). "Role of proteoglycan in metanephric development." Dev Biol **128**: 256-276.

Lelongt, B., Trugnan, G, Murphy, G, Ronco, PM. (1997). "Matrix metalloproteinases MMP2 and MMP9 are produced in early stages of kidney morphogenesis but only MMP9 is required for renal organogenesis in vitro." J Cell Biol. **136**(6): 1363-1373.

Lemmink, H., Nillesen, WN, Mochizuki, T, Schroder, CH, Brunner, HG, van Oost, BA, Monnens, LAH, Smeets, HJM (1996). "Benign familial hematuria due to mutation of the type IV collagen 4 gene." J Clin Invest **98**: 1114-1118.

Li, S., Edgar, D., Fassler, R., Wadsworth, W. & Yurchenco, P. D. , 2003. 4: p. 613-24. (2003). "The role of laminin in embryonic cell polarization and tissue organization. ." Dev Cell **4**: 613-624.

Liu, Y. (2002). "Hepatocyte growth factor and the kidney." Curr Opin Nephrol Hypertens **11**(1): 23-30.

Lochter, A., Galosy, S., Muschler, J. , Freedman, N., Werb, Z., Bissell, M.J (1997). "Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells." Journal of Cell Biology **139**(7): 1861-1872.

Lu BC, C. C., Chi X, Kuure S, Kuo R, Bates CM, Arber S, Hassell J, MacNeil L, Hoshi M, Jain S, Asai N, Takahashi M, Schmidt-Ott KM, Barasch J, D'Agati V, Costantini F (2009). "Etv4 and Etv5 are required downstream of GDNF and Ret for kidney branching morphogenesis. ." Nat Genet. 2009 **41**(12): 1295-1302.

Lukashev, M., Werb, Z. (1998). "ECM signalling: orchestrating cell behaviour and misbehaviour. ." Trends Cell Biol. **8**(11): 437-441.

Masson, R., Lefebvre, O., Noel, A., El Fahime, M., Chenard, M.-P., Wendling, C., Kebers, F., Rio, M.-C. (1998). "In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy." Journal of Cell Biology **140**(6): 1535-1541.

Matrisian, L. (1990). "Metalloproteinases and their inhibitors in matrix remodeling." Trends Genet. **6**(4): 121-125.

Matrisian, L. (1992). "The matrix-degrading metalloproteinases." Bioessays. **14**(7): 455-463.

Mazzone, M., Baldassarre, M, Beznoussenk,o G, Giacchetti, G, Cao, J, Zucker, S, Luini, A, Buccione, R. (2004). "Intracellular processing and activation of membrane type 1 matrix metalloprotease depends on its partitioning into lipid domains." J Cell Sci **117**(26): 6275-6287.

Meyer TN, S. C., Bush KT, Stuart RO, Rose DW, Shah MM, Vaughn DA, Steer DL, Nigam SK. (2004). "Spatiotemporal regulation of morphogenetic molecules during in vitro branching of the isolated ureteric bud: toward a model of branching through budding in the developing kidney." Dev Biol. **275**(1): 44-67.

Miner, J. (1998). "Developmental biology of glomerular basement membrane components." Curr Opin Nephrol Hypertens **7**(1): 13-19.

Miner, J. (1999). "Renal basement membrane components." Kidney Int **56**(6): 2016-2024.

Miner JH, L. C. (2000). "Defective glomerulogenesis in the absence of laminin alpha5 demonstrates a developmental role for the kidney glomerular basement membrane." Dev Biol. **217**(2): 278-289.

Miosge N, K. F., Heinemann S, Kohfeldt E, Herken R, Timpl R. (2000). "Ultrastructural colocalization of nidogen-1 and nidogen-2 with laminin-1 in murine kidney basement membranes." Histochem Cell Biol **113**: 115-124.

Miosge, N., Sasaki, T, Timpl, R. (2002). "Evidence of nidogen-2 compensation for nidogen-1 deficiency in transgenic mice." Matrix Biology **21**(7): 611-621.

Montell, D. (2008). "Morphogenetic cell movements: diversity from modular mechanical properties." Science **322**(5907): 1502-1505.

Moss, N., Wu, YI, Liu, Y, Munshi, HG, Stack, MS. (2009). "Modulation of the membrane type 1 matrix metalloproteinase cytoplasmic tail enhances tumor cell invasion and proliferation in three-dimensional collagen matrices." Biol Chem. **284**(30): 19791-19799.

Murray, P., Edgar, D. (2000). "Regulation of programmed cell death by basement membranes in embryonic development." J Cell Biology **150**(5): 1215-1221.

Nagase, H., Woessner, JF. (1999). "Matrix metalloproteinases." J Bio Chem. **274**(21491-21494).

Nagase, H. a. M., G. (2008). <u>Tailoring TIMPs for selective metalloproteinase inhibition.</u> New York Springer Science.

Nakanishi, Y., Sugiura, F, Kish,i J, Hayakawa, T. (1986). "Collagenase inhibitor stimulates cleft formation during early morphogenesis of mouse salivary gland." <u>Dev Biol.</u> **113**(1): 201-206.

Nart , D., Yaman, B, Yilmaz, F, Zeytunlu, M, Karasu, Z, Kiliç, M. (2010). "Expression of matrix metalloproteinase-9 in predicting prognosis of hepatocellular carcinoma after liver transplantation." <u>Liver Transpl.</u> **16**(5): 621-630.

Nyalendo, C., Michaud, M, Beaulieu, E, Roghi, C, Murphy, G, Gingras, D, Béliveau, R. (2007). "Src-dependent phosphorylation of membrane type I matrix metalloproteinase on cytoplasmic tyrosine 573: role in endothelial and tumor cell migration. ." <u>J Biol Chem</u> **282**(21): 15690-15699.

Oblander, S., Zhou, Z, Gálvez, BG, Starcher, B, Shannon, JM, Durbeej, M, Arroyo, AG, Tryggvason, K, Apt,e SS. (2005). "Distinctive functions of membrane type 1 matrix-metalloprotease (MT1-MMP or MMP-14) in lung and submandibular gland development are independent of its role in pro-MMP-2 activation." <u>Dev Biol.</u> **277**(1): 255-269.

Ohtake, Y., Tojo, H, Seiki, M. (2006). "Multifunctional roles of MT1-MMP in myofiber formation and morphostatic maintenance of skeletal muscle." <u>J Cell Sci.</u> **15**(118(Pt. 8)): 3822-3832.

Ohuchi, E., Imai, K, Fujii, Y, Sato, H, Seiki, M and Okada, Y (1997). "Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. ." <u>J Bio Chem.</u> **272**: 2446-2451.

Ota, K., Stetler-Stevenson, WG, Yang, Q, Kumar, A, Wada, J, Kashihara, N, Wallner, EI, Kanwa,r YS. (1998). "Cloning of murine membrane-type-1-matrix metalloproteinase (MT-1-MMP) and its metanephric developmental regulation with respect to MMP-2 and its inhibitor. ." <u>Kidney Int</u> **54**: 131-142.

Park, Y., Ryu, HS, Choi, DS, Chang, KH, Park, DW, Min CK. (2003). " Effects of hepatocyte growth factor on the expression of matrix metalloproteinases and their tissue inhibitors during the endometrial cancer invasion in a three-dimensional coculture." <u>Int J Gynecol Cancer</u> **13**(1): 53-60.

Pei, D., Weiss, SJ. (1996). "Transmembrane-deletion mutants of the membrane-type matrix metalloproteinase-1 process progelatinase A and express intrinsic matrix-degrading activity." <u>J Bio Chem.</u> **15**: 9135-9140.

Pichel JG, S. L., Sheng HZ, Granholm AC, Drago J, Grinberg A, Lee EJ, Huang SP, Saarma M, Hoffer BJ, Sariola H, Westphal H. (1996). "Defects in enteric innervation and kidney development in mice lacking GDNF." <u>Nature</u> **382**: 73-76.

Pohl, M., Sakurai, H, Bush, KT, Nigam, SK. (2000). "Matrix metalloproteinases and their inhibitors regulate in vitro ureteric bud branching morphogenesis." <u>Am J Physiol Renal Physiol.</u> **279**(5): 891-900.

Pöschl E, F. J., Block D, Mayer U, Timpl R. (1994). "Two non-contiguous regions contribute to nidogen binding to a single EGF-like motif of the laminin gamma 1 chain." <u>EMBO J</u> **13**(16): 3741-3747.

Pöschl E, S.-S. U., Brachvogel B, Saito K, Ninomiya Y, Mayer U. (2004). "Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development." <u>Development</u> **131** (7): 1619-1628.

Powell, K. (2005). "Contacting the Matrix. ." <u>J Cell Biol.</u> **170**(7).

Rebustini IT, M. C., Lassiter KS, Surmak A, Szabova L, Holmbeck K, Pedchenko V, Hudson BG, Hoffman MP. (2009). "MT2-MMP-dependent release of collagen IV NC1 domains regulates submandibular gland branching morphogenesis." <u>Dev Cell</u> **17**(4): 482-493.

Reidy K, R. N. (2009). "Cell and Molecular Bology of Kidney development." <u>Semin Nephrol</u> **29**(321-337).

Reponen, P., Sahlberg, C, Huhtala, P, Hurskainen, T, Theslef,f I, Tryggvason, K., (1992). "Molecular cloning of murine 72-kDa type IV collagenase and its expression during mouse development. ." <u>J Biol Chem</u> **267**: 7856-7862.

Roy, R., Yang, J., Moses, MA. (2009). "Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer." <u>J Clin Oncol.</u> **27**(31): 5287-5297.

Rozanov, D., Hahn-Dantona, E, Strickland, DK, Strongin, AY. (2004). "The low density lipoprotein receptor-related protein LRP is regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) proteolysis in malignant cells." <u>J Biol Chem</u> **279**(6): 4260-4268.

Sabeh, F., Ota, I, Holmbeck, K, Birkedal-Hansen, H, Soloway, P, Balbin, M, Lopez-Otin, C, Shapiro, S, Inada, M, Krane, S, Allen, E, Chung, D, Weiss, SJ. (2004). "Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP." <u>J Cell Biol</u> **157**(4): 769-781.

Sakurai, H., Barros, EJ, Tsukamoto, T, Barasch, J, Nigam, SK. (1997). "An in vitro tubulogenesis system using cell lines derived from the embryonic kidney shows dependence on multiple soluble growth factors." <u>Proc Natl Acad Sci U S A.</u> **94**(12): 6279-6284.

Sariola, H. (1985). "Interspecies chimeras: an experimental approach for studies on embryonic angiogenesis." Med Biol 43-65.

Sato, H., Takino, T, Kinoshita, T, Imai, K, Okada, Y, Stetler Stevenson, WG, Seiki, M. (1996). "Cell surface binding and activation of gelatinase A induced by expression of membrane-type-1-matrix metalloproteinase (MT1-MMP). ." FEBS Lett. **385**(3): 238-240.

Sato, H., Takino, T, Okada, Y, Cao, J, Shinagawa, A, Yamamoto, E, Seiki, M. (1994). "A matrix metalloproteinase expressed on the surface of invasive tumour cells." Nature **370**(6484): 61-65.

Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E. and Seiki, M. (1994). "A matrix metalloproteinase expressed on the surface of invasive tumour cells." Nature **370**: 61-65.

Saus, J., Wieslander, J, Langeveld, JPM, Quinones, S, Hudson, BG: . 1988 263:13374–13380 (1988). "Identification of the Goodpasture antigen as the 3(IV) chain of collagen IV." J Biol Chem **263**: 13374-13380.

Saxẻn, L. (1987). Organigeneiss of the kidney. Cambridge, UK, University Press.

Saxẻn, L. a. S., H. (1987). "Early organogenesis of the kidney." Pediatr Nephrol **1**: 385-392.

Schubert, C. (2006). "Remodeling in the kidney." Nat Med. **12**(4): 391.

Schuchardt A, D. A. V., Larsson-Blomberg L, Costantini F, Pachnis V. (1994). "Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret." Nature **367**: 380-383.

Seiki, M. (2003). "Membrane-type 1 matrix metalloproteinase: a key enzyme for tumor invasion." Cancer Lett. **194**(1): 1-11.

Shah, M., Sampogna, RV, Sakurai, H, Bush, KT, Nigam, SK. (2004). "Branching morphogenesis and kidney disease." Development **131**(7): 1449-1462.

Skinner MA, S. S., Reeves JG, Jackson ME, Freemerman AJ. (2008). "Renal Aplasia in Humans Is Associated with RET Mutations." AM J Hum Genet **82**(2): 344-351.

Smyth, N., Vatansever, HS, Murray, P, Meyer, M, Frie, C, Paulsson, M, Edgar, D. (1999). "Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation." J Cell Biology **144**(1): 151-160.

Somerville RP, O. S., Apte SS (2003). "Matrix metalloproteinases: old dogs with new tricks." Genome Biology **4**(6): 216.

St John PL, W. R., Yin Y, Miner JH, Robert B, Abrahamson DR. (2001). "Glomerular laminin isoform transitions: errors in metanephric culture are corrected by grafting. ." <u>Am J Physiol Renal Physiol. </u>**280**(4): 695-705.

Steer, D., Shah, MM, Bush, KT, Stuart, RO, Sampogna, RV, Meyer, TN, Schwesinge,r C, Bai, X, Esko, JD, Nigam, SK. (2004). "Regulation of ureteric bud branching morphogenesis by sulfated proteoglycans in the developing kidney." <u>Dev Biol. </u>**272**(2): 310-327.

Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A. and Goldberg, G. I. (1995). . 270, 5331-5338. (1995). "Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease." <u>J. Biol. Chem. </u>**270**: 5331-5338.

Sympson, C. J., Bissell, M.J., Werb, Z. (1995). "Mammary gland tumor formation in transgenic mice overexpressing stromelysin-1." <u>Semin Cancer Biol</u> **6**(3): 159-163.

Takino, T., Sato, H., Yamamoto, E. and Seiki, M. (1995). "Cloning of a human gene potentially encoding a novel matrix metalloproteinase having a C-terminal transmembrane domain." <u>Gene </u>**155**: 293-298.

Tanney, D., Feng, G, Pollock, AS, Lovett, DH. (1998). "Regulated expression of matrix metalloproteinases and TIMP in nephrogenesis. ." <u>Dev Dyn </u>**213**: 121-129.

Timpl, R., Brown, JC. (1996). "Supramolecular assembly of basement membranes." <u>Bioessays</u> **18**(2): 123-132.

Turner, N., Mason, PJ, Brown, R, Fox, M, Povey, S, Rees, A, Pusey, CD (1992). "Molecular cloning of the human Goodpasture antigen demonstrates it to be the 3 chain of type IV collagen." <u>J Clin Invest</u> **89**: 592-601.

<u>Uekita, T., Itoh, Y, Yana, I, Ohno, H. Seiki, M.</u> (2001). "Cytoplasmic tail-dependent internalization of membrane-type 1 matrix metalloproteinase is important for its invasion-promoting activity." <u>J Cell Biol.</u> **155**(7): 1345-1356.

VanSaun, M., Matrisian, LM. (2006). "Matrix metalloproteinases and cellular motility in development and disease." <u>Birth Defects Res C Embryo Today.</u> **78**(1): 69-79.

Velasco, G., Pendás, AM, Fueyo, A, Knäuper, V, Murphy ,G, López-Otín, C. (1999). " Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members." <u>J. Biol. Chem.</u> **274**: 4570-4570.

Vu TH, W. Z. (1998). Gelatinase B. Structure regulation and function. In: Matrix metalloproteinases. . San Diego, Academic Press**:** 115-140.

Wallner EI, Y. Q., Peterson DR, Wada J, Kanwar YS. (1998). "Relevance of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis." <u>Am J Physiol.</u> **275**: 467-477.

Wang, P., Nie, J, and Pei, D. (2004). "The Hemopexin Domain of Membrane-type Matrix Metalloproteinase-1 (MT1-MMP) is not required for its activation of proMMP2 on cell surface but is essential for MT1-MMP-mediated invasion in three-dimensional type I Collagen. ." <u>J Biol Chem.</u> **279**(49): 51148-51155.

Woolf, A., Kolatsi-Joannou, M, Hardman, P, Andermarcher, E, Moorby, C, Fine, LG, Jat, PS, Noble, MD, Gherardi, E. (1995). "Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros." <u>J Cell Biol</u> **128**(1-2): 171-184.

Yana, I., Weiss, SJ. (2000). "Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases." <u>Mol Biol Cell</u> **11**(7): 2387-2401.

Yu, M., Sato, H., Seiki, M. and Thompson, E. W. (1995). "Complex regulation of membrane-type metalloproteinase expression and matrix metalloproterinase-2 activation by concanavalin A in MDA-MB-231 human breast cancer cells." <u>Cancer Res.</u> **55**: 3272-3277.

Zehnder, A., Adams, JC, Santi, PA, Kristiansen, AG, Wacharasindhu, C, Mann, S, Kalluri, R, Gregory, MC, Kashtan, CE, Merchant, SN. (2005). "Distribution of type IV collagen in the cochlea in Alport syndrome." <u>Arch Otolaryngol Head Neck Surg.</u> **131**(11): 1007-1013.

Zent, R., Bush, KT, Pohl, ML, Quaranta, V, Koshikawa, N, Wang, Z, Kreidberg, JA, Sakurai, H, Stuart, RO, Nigám, SK. (2001). "Involvement of laminin binding integrins and laminin-5 in branching morphogenesis of the ureteric bud during kidney development." <u>Dev Biol.</u> **238**(2): 289-302.

Zhai, Y., Hotary, KB,Nan,B, Bosch, FX, Nubia Muñoz N,, Weiss, SJ, and Cho, KR (2005). "Expression of Membrane Type 1 Matrix Metalloproteinase Is Associated with Cervical Carcinoma Progression and Invasion " <u>Cancer Res.</u> **97**: 6543-6550.

Zhou, Z., Apte, SS, Soininen, R, Cao, R, Baaklini, GY, Rauser, RW, Wang, J, Cao, Y, Tryggvason, K. (2000). "Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I." <u>Proc Natl Acad Sci U S A.</u> **97**(8): 4052-4057.