Kidney International, Vol. 68 (2005), pp. 155–166

Renal expression of SIBLING proteins and their partner matrix metalloproteinases (MMPs)

KALU U.E. OGBUREKE and LARRY W. FISHER

Matrix Biochemistry Section, Craniofacial and Skeletal Disease Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, DHHS, Bethesda, Maryland

Renal expression of SIBLING proteins and their partner matrix metalloproteinases (MMP).

Background. Three members of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins have recently been shown to bind and activate specific promatrix metalloproteinases (MMPs) and to overcome the inhibition of tissue inhibitors of MMPs (TIMPs). Although usually associated with mineralized tissues, we have shown that the SIBLINGs and their MMP partners, when known, are coexpressed in salivary gland ductal cells. The present study examined the expression patterns of both the SIBLINGs and their MMP partners in adult kidney.

Methods. The expression patterns of all five SIBLINGs known to date, and their MMP partners were determined in monkey kidney using immunohistochemistry and in situ hybridization techniques.

Results. Bone sialoprotein (BSP) and its partner, MMP-2, were coexpressed in both the proximal and distal tubules. Osteopontin, as previously shown, was expressed in the distal tubules while its partner MMP-3 was expressed in both the proximal tubule and distal tubles. Dentin matrix protein-1 (DMP1) and MMP-9 were coexpressed throughout the nephron, including both parietal cells of Bowman's capsule and the thin limb of the loop of Henle. Dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE) were expressed in the proximal tubule and distal tubule, and proximal tubule, respectively.

Conclusion. In contrast to salivary gland in which all SIBLINGs and their MMP partners were coexpressed throughout the length of the ducts, these proteins were differentially expressed within the normal adult nephron. We hypothesize that the cells use the SIBLING/MMP pairs in the normal turnover of cell surface proteins and/or pericellular matrix proteins such as those in basement membranes.

The Small Integrin-Binding LIgand N-linked Glycoproteins (SIBLINGs) are a family of five secreted proteins characterized by common exon-intron features, the presence of the integrin-binding tripeptide, Arg-Gly-Asp (RGD), and several conserved phosphorylation and N-glycosylation sites [1]. The SIBLINGs include osteopontin (OPN; also known as uropontin in the kidney field [2, 3]); bone sialoprotein (BSP); dentin matrix protein-1 (DMP1); dentin sialophosphoprotein (DSPP); and matrix extracellular phosphoglycoprotein (MEPE). The SIBLING genes are clustered together on human chromosome 4 and mouse chromosome 5 [4]. The four acidic members of the family (BSP, DMP1, DSPP, and OPN) were discovered years ago associated with the matrices of bones and teeth by many laboratories [5] while the basic protein, MEPE, was discovered more recently in association with tumors that cause phosphate wasting [6].

With the exception of osteopontin, expression of the SIBLINGs in normal adults was generally thought to be limited to mineralized tissues (i.e., the bones and teeth). The presence of OPN in normal nonmineralizing tissues, including the kidney [7, 8] lactating breast [9], and immune cells [10], has been well documented. Its upregulation in renal tubules, particularly the distal tubules, has been demonstrated in renal patients and several models of renal injury suggesting that it is an important mediator of tubulointerstitial injury possibly by facilitating tissue remodeling and repair [11–15]. With respect to renal calcium stone matrix physiology, the role of osteopontin is less clear, with some reports indicating that it inhibits [8, 16–20], and others suggesting that it promotes, renal stone formation [20, 21].

Matrix metalloproteinases (MMPs) are a family of over 20 zinc-dependent enzymes that degrade a wide variety of proteins, including those in the extracellular matrix (ECM) [22]. They are secreted as proenzymes (pro-MMPs) that classically are transformed into active forms upon cleavage of a propeptide domain [23]. Recently, however, we have shown in vitro that at least three SIBLINGs can bind and activate specific pro-MMPs apparently without removing the propeptides [24]. Furthermore, the same SIBLING/MMP pairs are substantially

Key words: kidney, SIBLING, MMP, osteopontin, BSP, DSPP, DMP1, MEPE.

Received for publication December 17, 2004
and in revised form February 1, 2005
Accepted for publication February 21, 2005

more resistant to inhibition by tissue inhibitors of MMPs (TIMPs) [24]. The activities of MMPs have been implicated in a variety of normal and pathologic cellular processes, including organ involution during development, wound healing, and tumor cell invasion [25]. Normal epithelial cell expression of MMPs include placental trophoblasts [26–30], ameloblasts [31, 32], pancreatic ducts [33], breast ductal epithelium [34–36], and salivary gland ducts [37]. They have also been reported to be involved in renal development [38]. Reports of the precise localizations of MMP-2, MMP-3, and MMP-9 in primate normal adult kidney, however, are surprisingly sparse, perhaps due to the lack of specificity of many of the antibodies previously used for primate nephrons. There are reports of in vitro studies showing the presence of MMP-2 and MMP-9 in cell lines of the different segments of the nephron, including the glomerulus [39, 40]. In several papers in which various naturally occurring or induced kidney diseases have been studied, there are statements of baseline activities of these three MMPs but little evidence of specific sites of expression along the nephron [33, 41–43]. The studies of Piedagnel et al [39] directly probed the in vivo presence of two MMPs in normal rat kidney. The authors indicated that MMP-2 and -9 proteins were present in the collecting ducts of rat nephron [39].

Investigation of the localization of the SIBLINGs and their partner MMPs in normal kidney was initiated after our discovery that the SIBLINGs and their MMP partners were coexpressed in the epithelial cells of the ducts of normal salivary glands [37]. The two SIBLINGs without known MMP partners, DSPP and MEPE, were also expressed in the relatively simple ductal system of the salivary glands [37] even though they are usually thought to be limited to bones and teeth. The coexpression of all SIBLINGs and their MMP partners throughout the entire length of the salivary gland ducts, however, make it difficult to differentiate among the different roles that the individual SIBLING-MMP pairs may play in the normal physiology of mature, metabolically active ducts. As presented here, the nephron, with its more complex physiology, exhibits differential expression of both the SIBLINGs and their MMP partners, thereby suggesting that the proteins may have distinct but related functions. We propose that these functions may include the enzymatic digestion of cell surface and/or pericellular matrix proteins damaged by oxidative byproducts invariably produced by such active cells.

METHODS

Tissue collection

Fresh surgical waste kidneys of monkey (*M. fascicularis*) were obtained from the Tissue Distribution Program of the National Primate Research Center (University of Washington, Seattle, WA, USA). Mouse kidneys were obtained as surgical wastes from normal mice previously culled from a breeding pool. Sagittal slices of the tissue specimens were fixed in 10% neutral-buffered formalin for 24 to 48 hours and routinely processed for paraffin embedding. Normal human kidney and placenta (for MMP antibody positive controls) paraffin blocks without patient identifiers were obtained from the Mid-Atlantic Division of the Cooperative Human Tissue Network (Charlottesville, VA, USA) under an NIH-approved (exempt) protocol. Three micron sections were cut and processed under strict RNAse-free conditions. Sections of rat kidney (69687-3) were purchased from Novagen (Madison, WI, USA).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Human kidney total RNA (64097-1) was purchased from BD Biosciences (Palo Alto, CA, USA). One microgram of total RNA was reverse transcribed and amplified by PCR for each gene product using the Superscript III One-Step RT-PCR System (Invitrogen, Carlsbad, CA, USA) and the intron-spanning oligonucleotide pairs described below. The cDNAs were reverse transcribed for 15 minutes at 60°C, denatured at 94°C for 2 minutes, and then amplified for 45 cycles of 30 seconds each (denaturation 94°C, annealing 60°C, and extension 68°C) with a final extension at 68°C for 7 minutes. Products were electrophoresed on a 6% polyacrylamide gel in Tris-borate-ethylenediaminetetraacetic acid (EDTA) (TBE) buffer, stained with ethidium bromide, and photographed. Standard are ΦX174 DNA digested with *Hae*III (New England Biolabs, Beverly, MA, USA). The oligonucleotides used were as follows: BSP forward (exon 6), GT TAGCTGCAATCCAGCTTCC; BSP reverse (exon 7), GAGAGGTTGTTGTCTTCGAGG; DMP1 forward (exon 5), GGCAGTAAAGTTAGCTCAGAGG; DMP1 reverse (exon 6), CTCTCTTCACTGGCTTGTATG; DSPP forward (exon 4), GAATCAGAGACACATGCT-GTTGG; DSPP reverse (exon 5), GGGATCATCTCC TTGCATGG; osteopontin (OPN) forward (exon 5), GTTTCGCAGACCTGACATCC; OPN reverse (exon 6), CATGGCTGTGGAATTCACGG; MEPE forward (exon 3), CTGTGTGGAAGAGCAGAGG; MEPE reverse (exon 4), GCTGATAGCATCATCTCCATCC; MMP-2 forward (exon 12), CCAAGCTCATCGCAG ATGCC; MMP-2 reverse (exon 13), TACAGTCAGC ATCTATTCTTGGG; MMP-3, forward (exon 9), GAG AAGAGAAATTCCATGGAGC; MMP-9 forward (exon 12), GACGTGAAGGCGCAGATGG; and MMP-9 reverse (exon 13), CCTTTCCTCCAGAACA-GAATACC.

Immunohistochemistry

Mouse monoclonal and rabbit polyclonal antisera to the SIBLINGs have been previously described to work in paraffin sections of monkey tissues [37] and are

Table 1. Antisera and riboprobes

Antisera				
Antigen	ID	Description	Dilution	Type[a]
Human BSP[a]	LFMb-25	Carboxy-terminal region	10 μg/mL	Mouse IgG_1
Human DSPP[a]	LFMb-21	DPP domain CSRGDASYNSDESKDNG	2.5 μg/mL	Mouse IgG_{2b}
Human OPN[a]	LFMb-14	Just C-terminal to RGD	2.5-10 μg/mL	Mouse IgG_{2b}
Human MEPE	LFMb-33	Within last exon	3.3 μg/mL	Mouse IgG_1
Human MEPE[a]	LF-155	Within last exon	1:200 dilution	Rabbit polyclonal
Human DMP1	LFMb-31	Peptides (Jain et al 2002)	20 μg/mL	Mouse IgG_1
Human BSP[a]	LF-84	(Mintz et al 1993)	1:200 dilution	Rabbit antibody[b]
Mouse OPN	LF-175	Bacterial recombinant	1:200 dilution	Rabbit polyclonal
Human MMP-2	AB807	(Chemicon, Temecula, CA)	1:100 dilution	Rabbit polyclonal[b]
Human MMP-3	MAB3306	(Chemicon, Temecula, CA)	10ug/mL	Mouse IgG_1
Human MMP-9	RB-1539	(Lab Vision, Fremont, CA)	1:200 dilution	Rabbit polyclonal

Riboprobes				
Target	ID	Description	Insert	Plasmid/Reference
Human:				
BSP	B6-5g	Full-length coding cDNA	1.2 kbp	pBluescript (Fisher et al 1990)
DMP1	hDMP1 - E5 - 3	Coding in last exon by PCR	1.4 kbp	pBluescript (Ogbureke, Fisher 2004)
DSPP	hDSPP-1	DSP portion of DSPP exon 4 by PCR	0.7 kbp	pBluescript (Ogbureke, Fisher 2004)
DSPP	hDPP510	(Nonrepeat) DPP of exon 4 by PCR	215 pb	pBluescript (Ogbureke, Fisher 2004)
MEPE	hMEPEx45	Full-length cDNA lacking exons 4 and 5	1.6 kbp	pBluescript (Ogbureke, Fisher 2004)
OPN	OP10	Full-length cDNA lacking exon 4	1.5 kbp	pBluescript (Young et al 1990)
MMP-2	hMMP2	Portion last exon by PCR	~320 bp	pBluescript (Ogbureke, Fisher 2004)
MMP-3	hMMP3	portion last exon by PCR	~340 bp	pBluescript (Ogbureke, Fisher 2004)
MMP-9	hMMP9	portion last exon by PCR	~320 bp	pBluescript (Ogbureke, Fisher 2004)

Abbreviations are: BSP, bone sialprotein; DSPP, dentin sialophosphoprotein; OPN, osteopontin; MEPE, matrix extracellular phosphoglycoprotein; DMP, dentin matrix protein; MMP, matrix metalloproteinase; PCR, polymerase chain reaction.

[a]Cross to mouse on paraffin sections.

[b]Affinity purified.

summarized in Table 1. Although only monoclonal antibody results are shown for SIBLINGs, each result was verified using the appropriate rabbit polyclonal antiserum. Also, a series of commercial mouse monoclonal and rabbit polyclonal antibodies suggested to work in paraffin sections of human tissues were screened for their ability to detect MMP-2, MMP-3, or MMP-9 in sections of human placenta. Two commercial monoclonals (one each for MMP-2 and MMP-3) worked under our conditions as did two polyclonals (for MMP-2 and MMP-9) (Table 1). The two antibodies to MMP-2 (polyclonal, AB807, and monoclonal, MAB 13431) and the monoclonal antibody to MMP-3 (MAB 3306) were purchased from Chemicon (Temecula, CA, USA). Antibody to human Tamm-Horsfall protein (THP) was purchased from Cedarlane (Hornby, Ontario, Canada). Affinity-purified rabbit polyclonal antisera to mouse aquaporins 1, 3, and 4 with known cross-reactivity to human and monkey kidneys [44] (generous gifts from Dr. Mark Knepper, NIDDK, NIH, Bethesda, MD, USA) were used to verify the identity of the proximal tubule (aquaporin 1), thin limb of Henle (aquaporin 1), and collecting duct (aquaporins 3 and 4) when morphology alone was insufficient.

Immunostaining was performed at room temperature using the Zymed ST5050 automated system (Zymed Lab Inc., San Francisco, CA, USA). In brief, paraffin sections of monkey kidney were manually dewaxed in xylene and rehydrated through graded ethanol (100%, 95%, and 70%) and water. Endogenous peroxidase activity was then inhibited by treating the sections with 3% hydrogen peroxide in methanol for 20 minutes at room temperature. Sections were thereafter washed three times in phosphate-buffered saline (PBS) for at least 5 minutes each and covered with PBS + Tween-20 (PBS-T) before loading the slides on to the preprogrammed ST5050 automated immunohistochemistry machine. The ST5050 was programmed to incubate each slide for one hour with corresponding SIBLING/MMP antibody diluted in 10% normal goat serum in PBS (see Table 1 for specific dilutions). The sections thereafter went through a 4 × 1-minute wash cycle with PBS-T before incubation with SuperPicTure Polymer horseradish peroxidase (HRP)-conjugated broad-spectrum secondary antibody (87-8963) (Zymed Lab. Inc.) for 10 minutes. The sections were passed through another wash cycle and then developed with AEC Single Solution chromogen (00-1122) (Zymed Lab. Inc.) for 2 minutes. All steps were done at room temperature. Sections were then lightly counterstained manually with Mayer's hematoxylin for 10 to 20 seconds before applying an overlay of Clearmount (Zymed Lab. Inc.) glaze over the sections. After air-drying, slides were coverslipped with Histomount (Zymed Lab. Inc.). Negative controls included the substitution of primary antibody with nonimmune rabbit serum or mouse IgG control (08–6599) (Zymed Lab. Inc.). Photographic images of representative results were captured

using the Axioplan2 Universal microscope equipped with an Axiovision digital camera and the company's Axiovision program (Carl Zeiss GmbH, Jena, Germany).

In situ hybridization

A summary of the antisense and sense riboprobes for the SIBLINGs and MMPs is shown in Table 1. Human SIBLING and MMP probes that cross-hybridized with corresponding monkey mRNAs were used for the monkey kidney sections. We have recently described in detail the methods used for the synthesis of the probes for nonradioactive detection of mRNA transcripts [37]. In brief, digoxigenin (DIG) labeling of the probes was carried out using the DIG RNA Labeling Mix (Roche Diagnostic GmbH, Mannheim, Germany), the appropriately linearized cDNA plasmid, and the corresponding RNA polymerases. For in situ detection of transcripts with the 5-bromo-4-chloro-3-indoyl phosphate/nitro blue (BCIP/NBT) final color reagent, the InnoGenex^{TM} Universal ISH Kit SH-3018-01 (InnoGenex, San Ramon, CA, USA) was used following the manufacturer's instructions. Temperature control during the in situ hybridization was carried out with the aid of the OmniSlide Thermal Cycler (Thermo Electron Corp., Milford, MA, USA). All aqueous solutions were constituted in RNAse-free water. The amount of sense probe used as negative control was always greater than that used for the antisense (as determined by comparing the relative strength of the DIG labeling using serial dilutions and dot blots on charged nylon membranes with subsequent detection using alkaline phosphatase–conjugated anti-DIG antibodies). Fast Red was used as the nuclear counterstain. As with the immunohistochemistry results, representative images of posthybridization sections were captured using the Axioplan2 Universal Microscope system.

Segment notations and scoring system for SIBLING/MMP expression

There are many accepted notations of the different segments of the primate nephron and we have chosen the nomenclature illustrated in Figure 1A. A threshold intensity of the AEC red/brown-colored staining was based on negative control sections treated with either preimmune rabbit serum for polyclonal antibodies or inactive mouse IgG antibody as indices for negative staining of the various kidney structures (example, Fig. 2L). Positive staining for either the SIBLINGs or MMPs was based on consistent staining of any structure type visibly exceeding the threshold observed for the negative controls. Tubular profiles were scored as positive for SIBLING/MMP, only when 50% or more of the cells of a tubular cross-section in the cortex or medulla exhibited staining intensity exceeding the baseline established by the negative control staining. Tubules not visualized in their entirety were not assessed. Concurrent with assessment of the intensity of staining, homogeneity of staining was assessed before assigning numerical score. Depending on the degree to which the intensity of staining exceeded the baseline (control), positive staining was semiquantitatively recorded as +, ++, or +++ in ascending order of intensity. Segments exhibiting focal staining were scored as +/−.

RESULTS

RT-PCR demonstrates presence of SIBLINGs and MMPS

The presence of the five SIBLINGs as well as MMP-2, MMP-3, and MMP-9 in kidney was first determined by RT-PCR of total RNA obtained from normal adult human kidney. The oligonucleotide pairs used in this experiment each spanned an intron to distinguish cDNA copied from mRNA by the reverse transcriptase from a larger band that would have been the result of contaminating genomic DNA. In each case, a band corresponding to the mRNA for each of the SIBLINGs and the three MMPs was observed (Fig. 1B).

Immunohistochemical and in situ localization of SIBLING/MMP

A summary of the semiquantitative distribution of the SIBLINGs and their partner MMPs in the various segments of the nephron, as determined by immunohistochemistry is presented in Table 2. While the entire kidney was subject to microscopic observation for both protein and mRNA content, for brevity only the three major morphologic regions (cortical labyrinth, medullary rays, and the medullary pyramid) that present all of the segments of the various nephrons are illustrated in the histologic results. Red/brown coloration of tissue elements represents positive immunohistochemistry after staining with AEC chromogen, while bluish coloration reflects nuclear counterstain with hematoxylin. For the in situ hybridization results, the purplish/blue staining represents positive results for the specific mRNA after staining with BCIP/NBT chromogen, while red coloration reflects the Nuclear Fast Red counterstain.

Osteopontin/MMP3

Osteopontin was restricted to the distal convoluted tubules and distal straight tubules in the cortex (Fig. 2A) and medullary regions (Figs. 2B and 3C) of the nephron as summarized as the red color in the schematic (Fig. 2D and Table 2). The identity of osteopontin-positive tubules was verified by showing localization of the distal tubules protein, THP, within the same tubules in serial or near-serial sections (Fig. 2E). Except for the most distal portion of the distal convoluted tubule abutting the collecting duct

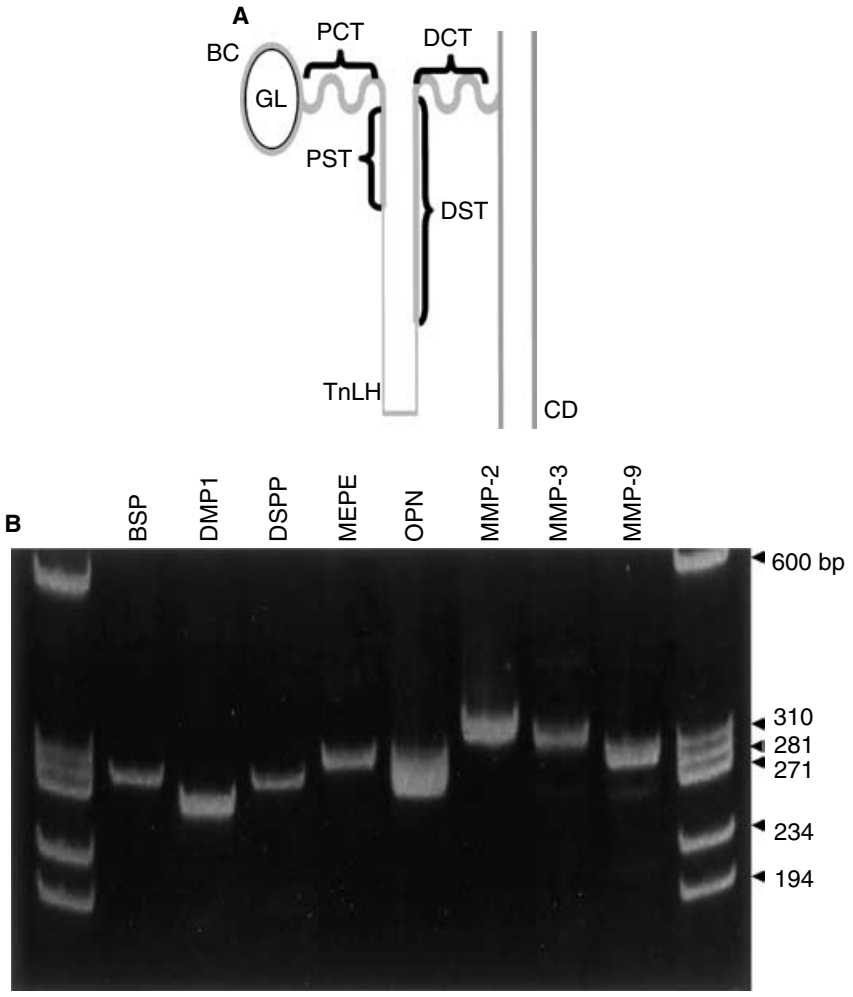

Fig. 1. (*A*) Schematic of the functional segments of the nephron. Abbreviations are: BC, Bowman's capsule; GL, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; TnLH, thin limb of the loop of Henle; DST, distal straight tubule; DCT, distal convoluted tubule; CD, collecting duct. (*B*) The presence of the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) and matrix metalloproteinase (MMP)-2, MMP-3, and MMP-9 in normal kidney tissue determined by reverse transcription-polymerase chain reaction (RT-PCR) of total RNA. Abbreviations are: BSP, bone sialprotein; DSPP, dentin sialophosphoprotein; OPN, osteopontin; MEPE, matrix extracellular phosphoglycoprotein; DMP1, dentin matrix protein.

(in which THP shows only weak staining), OPN and THP were both highly expressed along the full length of the distal tubules (Fig. 2A and E, arrows). As was the case in the salivary ducts, the OPN protein was observed particularly concentrated along the basal portions of the cells irrespective of the antibody that was used and whether the kidney was that of adult monkey (Fig. 2A, arrow), rat (Fig. 2I to K) human (data not shown), or mouse (data not shown). The mRNA for OPN was limited to the same distal tubule segments as for the protein (Fig. 2F and G). Typical nonreactive IgG antibody negative control (Fig. 2L) and osteopontin mRNA riboprobe sense strand (Fig. 2H) are shown.

MMP-3, previously shown to be bound and activated by OPN in vitro, also localized along the distal tubules (distal straight tubule and distal convoluted tubule) at both the protein (Fig. 2M to O) and mRNA (Fig. 2Q to S) levels. Interestingly, MMP-3 was also present in the proximal tubules (proximal convoluted tubule and proximal straight tubule) (Fig. 2M and N, arrows), segments that do not express OPN. Figure 2P (red) schematic summarizes this distribution of MMP-3 along the nephron. Figure 2T is a typical representation of the sense strand negative control for MMP-3 mRNA. The wider scope of MMP-3 distribution than that of its SIBLING partner, OPN, illustrates the point that the partner MMP is always found where a SIBLING is expressed, but the MMP can also be found in locations without its SIBLING partner.

BSP/MMP2

BSP and its partner, MMP-2, were more widely distributed than OPN/MMP-3 in the adult monkey nephron (Fig. 3). The staining pattern of BSP and MMP-2 by both antisera and in situ hybridization indicated a weak but clear presence in the proximal convoluted tubule, proximal straight tubule, distal straight tubule, and distal convoluted tubule in both the cortex and medulla (Fig. 3A to P). The collecting duct demonstrated focal staining for BSP protein (Fig. 3C, asterisk), but local synthesis could not be confirmed by in situ localization of its respective mRNA (Fig. 3G, asterisk). The immunohistochemistry distributions of BSP and MMP-2 are summarized as red areas in Figure 3D and L schematics,

Table 2. Small integrin-binding ligand N-linked glycoprotein (SIBLING) and matrix metalloproteinase (MMP) expression in monkey nephron

	Glomerulus	Proximal convoluted tubule	Proximal straight tubule	Thin limb of Henle	Distal straight tubule	Distal convoluted tubule	Collecting duct
BSP	–	+ +	+ +	–	+ +	+ +	+/–
MMP-2	–	+ +	+ +	–	+ +	+ +	+/–
OPN	–	–	–	–	+ +	+ + +	–
MMP-3	–	+	+	–	+ +	+ +	–
DMP1	+ (PC)	+ +	+	+ +	+	+	–
MMP-9	+ (PC)	+ +	+	+ +	+	+	–
DSPP	–	++	+	–	+	+	+/–
MEPE	–	+ +	+	–	–	–	–
THP[a]	–	–	–	–	+	+++	–

Abbreviations are: BSP, bone sialprotein; DSPP, dentin sialophosphoprotein; OPN, osteopontin; MEPE, matrix extracellular phosphoglycoprotein; DMP, dentin matrix protein; MMP, matrix metalloproteinase; PC, parietal cells; THP, Tamm-Horsefall protein; +, some expression; –, no expression; + +, increased expression; + + +, mid-spread, strong expression; +/–, focal and/or low level expression by immunohistochemistry not verifiable at mRNA level.

[a]Positive control for osteopontin localization.

respectively. Figure 3H and P represent sense strand control results for BSP and MMP-2 mRNA, respectively. The presence of BSP and MMP-2 proteins but not their mRNA in the collecting duct suggests that these secreted proteins may be released at least in part into the lumen from more upstream nephron segments later to be bound to the lumen surfaces of the collecting duct. The thin limb of Henle and glomeruli were all negative for BSP and MMP-2. Unlike OPN, BSP and MMP-2 protein distribution encompassed the entire basoluminal width of the cell, presumably due to the localization of these secreted protein within the rough endoplasmic reticulum, Golgi, and various secretory vesicles.

DMP1/MMP-9

Immunohistochemistry staining (Fig. 4A to C) and in situ hybridization (Fig. 4E to G) of DMP1 show that this protein was expressed in virtually all segments of the nephron as summarized in Figure 4D schematic. Immunohistochemistry staining in the distal tubules was less intense and more luminal than in the proximal tubules. Unlike any other SIBLING, DMP1 was also clearly expressed in the thin limb of Henle (Fig. 4B, F, and G) and in the parietal epithelial cells of Bowman's capsule (Fig. 4A and E, arrows). MMP-9 distribution mirrored that of its SIBLING partner, DMP1, by its expression in the proximal tubules, distal tubules, thin limb of Henle, collecting duct, and corresponding parietal epithelial cells of Bowman's capsule in serial/near-serial sections (Fig. 4I to O) (Fig. 4L schematic, red). As with the other SIBLINGs and MMPs, cells within the glomerular tuft, all connective tissues, and all blood vessels were negative for DMP1 and MMP-9. Figure 4H and P represent sense strand (negative) controls for DMP1 and MMP-9 mRNA, respectively.

DSPP

DSPP staining was intense in the proximal tubules and less intense but clearly present within the distal tubules at both the protein (Fig. 3Q to S) and mRNA levels (Fig. 3U to W). Focal staining was also observed in the collecting duct (Fig. 3D) at the immunohistochemistry level but like that for BSP/MMP-2, local expression could not be verified at the mRNA level by in situ hybridization (Fig. 3W). Figure 3T (red) is a schematic summary of DSPP distribution, while Figure 3X represents sense strand (negative) control for DSPP mRNA.

MEPE

Figure 4T schematic (red) summarizes the distribution of the fifth SIBLING, MEPE, and indicates the immunolocalization of MEPE strictly to the proximal tubules (Fig. 4Q and R) with significantly more intensity in the proximal convoluted tubules than the proximal straight tubules. This distribution was also verified by the demonstration of its mRNA to the proximal tubules by in situ hybridization, although the difference in intensity observed by immunohistochemistry was not reflected at the mRNA level (Fig. 4U). The glomeruli, thin limb of Henle, distal tubules, and collecting duct were all negative for MEPE at both protein and mRNA levels (Fig. 4Q to V). Interestingly, MEPE distribution tended to be on the luminal surface and/or in the luminal half of the proximal tubule cells (Fig. 4X).

DISCUSSION

To our knowledge this is the first report of the localization of all five SIBLINGs in the tubular epithelial cells of the normal nephron by immunohistochemistry and in situ hybridization. MMP expression in the kidney has been widely noted, although reports of their expression in duct epithelial cells of normal kidney have been less than

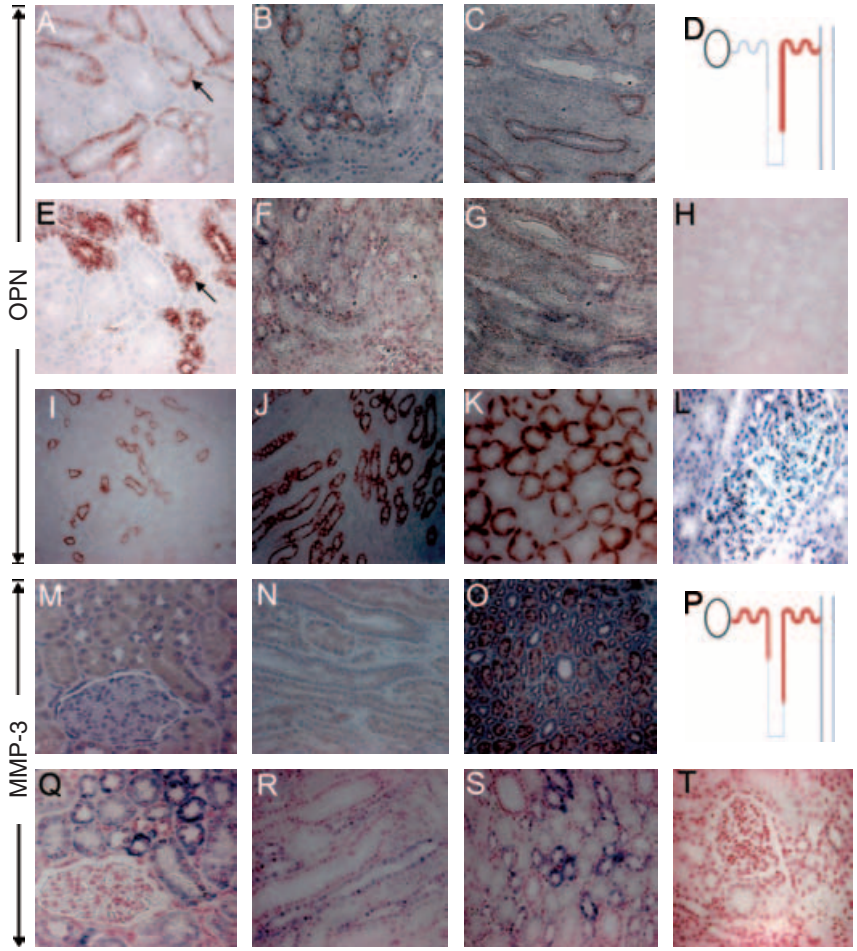

Fig. 2. Osteopontin (OPN) and matrix metalloproteinase (MMP)-3 localization in monkey and rat kidneys. Immunolocalization (reddish/brown) of OPN (*A* to *C*) to the distal tubules of monkey kidney with prominent basal localization (A, arrow). Distal convoluted tubules in the cortical labyrinth (A) and distal straight tubules in the medullary rays (B and C) are positive for osteopontin monoclonal antibody LF-Mb14, and negative for all other segments, including the glomeruli; schematic (*D*) summarizes OPN segmental localization (red). Positive control (*E*, arrow) showing distal tubule localization of Tamm-Horsefall protein (THP) but without basal polarity [contrast with (A)]. Antibody results for OPN were verified by in situ hybridization (*F* and *G*) (purple/blue) on corresponding serial sections. Immunolocalization of rat OPN to the distal tubules (*I* to *K*) in the cortical labyrinth (I), medullary rays (J), and renal pyramid (K), using LF-Mb14 antibody; representative preimmune IgG negative control (*L*), and in situ hybridization sense strand (*H*) (negative control) are also shown. Immunolocalization of MMP-3 to the distal tubules and proximal tubules (*M* to *O*) in the cortical labyrinth (M), medullary ray (N), and medullary pyramid (O). In situ hybridization (*Q* to *S*) verifying the presence of MMP-3 mRNA. Schematic summary (*P*) (red) of MMP-3 localization and in situ hybridization with sense strand (*T*) (negative control) is shown.

direct and clear. Piedagnel et al [39] did report the localization of MMP-2 and MMP-9 in the collecting ducts in normal rabbit kidney using sheep polyclonal antisera made against the corresponding human proteins. Other reports were results involving in vitro studies carried out on cells thought to represent distinct segments of the nephron [33, 40–43, 45]. Additional studies have been reported on the expression of the MMPs during nephrogenesis, or their profiles in specific kidney pathologies [40, 43, 46–49]. To our knowledge this is also the first report distinctly delineating the localization of MMP-3 as well as showing the localization of MMP-2 and MMP-9 in normal primate adult kidneys by immunohistochemistry and in situ hybridization.

Following our recent report that the SIBLINGs were colocalized with their partner MMPs along the entire length of the ducts of salivary glands [37], we hypothesized that the SIBLINGs may also be present in distantly related structures such as the kidney. The nephron, being a more complex structure than the salivary duct system, also offered the possibility that the proteins may be differentially expressed in different functional segments. Indeed, it has long been known that OPN, a member of the SIBLING family, is expressed only in kidney distal tubules in many species [8]. At least three members of the integrin-binding SIBLING family of secreted proteins bind and activate specific pro-MMPs and can even prevent TIMPs from inhibiting their corresponding active MMPs in vitro [24].

In primate salivary glands, both the SIBLINGS and the MMPs were always located throughout the ductal system and were never expressed in the acini [37]. In rodents, however, all of the SIBLINGs (except DSPP) and their MMP partners were also expressed in the acini [37]. Furthermore, in the special, androgen-dependent granulated convoluted tubules of the male mice, none of the SIBLINGs or MMPs were expressed [37]. Thus, SIBLINGs and their cognate MMP are consistently expressed together in a spatially restricted fashion suggesting that the SIBLING-MMP pairing may be important in vivo. However, because all five of the SIBLINGs and the three MMPs partners are coexpressed in the same cells, hypotheses of what the individual parings of SIBLNGs and MMP may be doing (for example, what are the local substrates of the activated MMP) are difficult to postulate. We reasoned, therefore, that kidney is an ideal tissue to

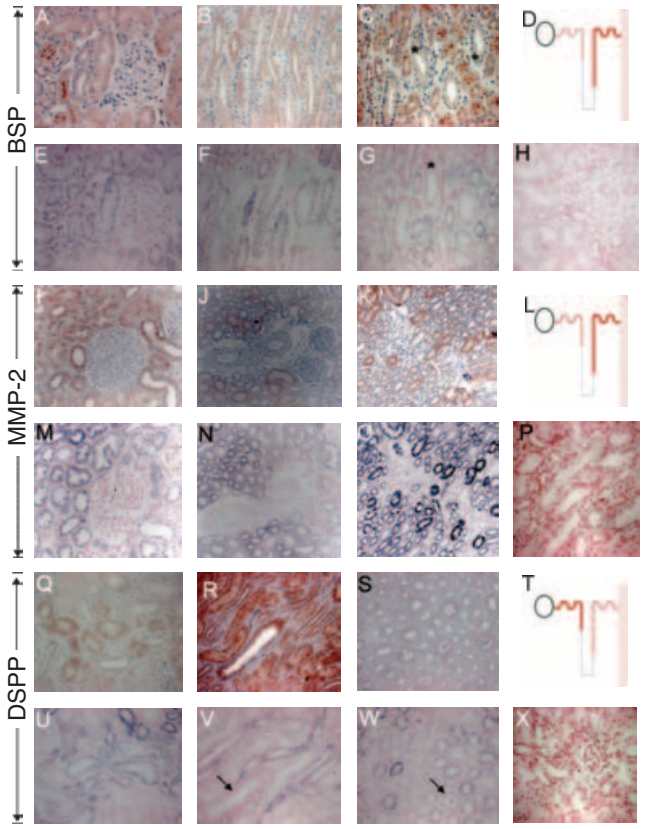

Fig. 3. Localization of bone sialoprotein (BSP), matrix metalloproteinase (MMP)-2, and dentin sialophosphoprotein (DSPP). Immunolocalization of BSP (reddish/brown) to proximal tubule and distal tubule (*A* to *C*). Distal convoluted tubule and proximal convoluted tubule in the cortical labyrinth (A), distal straight tubule and proximal straight tubule in the medullary rays (B and C) are positive for BSP monoclonal antibody LF-Mb25; collecting duct (C, *) is focally positive for BSP also seen with polyclonal (LF-84) antibody (data not shown). In situ hybridization (purple/blue) on corresponding serial sections (*E* to *G*) also showed the presence of BSP mRNA. Collecting duct (G, *) is negative for BSP mRNA. BSP sense strand control was negative (*H*) for BSP mRNA. MMP-2 immunolocalization (*I* to *K*) in the cortex (I and J) and medulla (K) showing positive staining of the proximal tubule and distal tubule was essentially similar to, and verified on corresponding serial sections by in situ hybridization (*M* to *O*). MMP-2 sense strand (*P*) was negative for MMP mRNA. The schematics (*D* and *L*) summarize BSP and MMP-2 localization, respectively (intense uniform red indicates "strong" expression; hatched red indicates "some" expression; and dotted red indicates "focal/low" level expression as explained in Table 2 legends). Like BSP, DSPP immunolocalized to the proximal tubule and distal tubule (*Q* and *R*), and to the collecting duct (*S*) without in situ hybridization verification of DSPP mRNA in the collecting duct (*V* and *W*, arrows). DSPP mRNA in the proximal tubule and distal tubule was verifiable by in situ hybridization (*U* and V). Immunolocalization of DSPP is summarized in the schematic (*T*) (intense uniform red indicates "strong" expression; hatched red indicates "some" expression, and dotted red indicates "focal/low" expression as explained in Table 2 legends). Sense strand in situ hybridization for DSPP mRNA is also shown (*X*).

investigate the possibility that the SIBLINGs and their MMP partners may be differentially expressed along a ductal system more complex than that found in the salivary gland.

Although our observations for adult human and monkey kidneys were virtually the same for the SIBLINGs

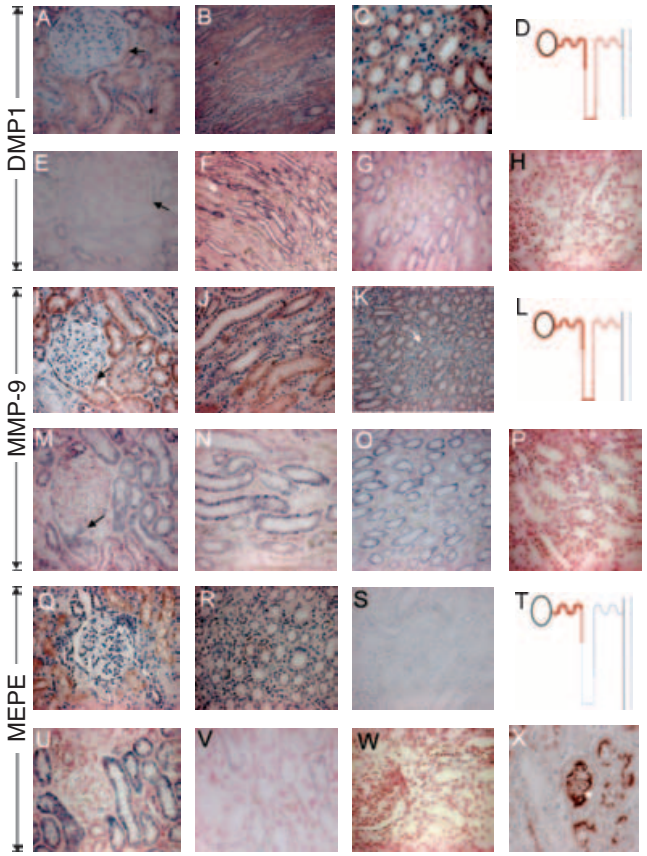

Fig. 4. Localization of dentin matrix protein-1 (DMP1), matrix metalloproteinase-9 (MMP-9), and matrix extracellular phosphoglycoprotein (MEPE). DMP1 and MMP-9 showed similar immunolocalization (*A* to *C* and *I* to *K*) as summarized in their respective schematics (*D* and *L*) as well as corresponding mRNA distribution (*E* to *G* and *M* to *O*). DMP1 immunolocalized to the proximal tubule and distal tubule (A to C), parietal cells of Bowman's capsule (A), and the thin limb of Henle (B). The distal tubuless show reduced expression of DMP1 as summarized in the schematic (*D*) (intense uniform red indicates "strong expression; " hatched red indicates "some" expression as explained in Table 2 legends). The distribution of DMP1 mRNA to the proximal tubule and distal tubule (E and F), the parietal cells of Bowman's capsule (E), and to the thin limb of Henle (F and G) mirrors that of MMP-9 (M to O). The sense strands for DMP1 (*H*) and MMP-9 (*P*) in situ hybridization for their respective mRNA was negative. Immunolocalization of MEPE was limited to the proximal tubule (*Q*) with more intensity in the proximal convoluted tubule as summarized in the schematic (*T*) (intense uniform red indicates "strong" expression; hatched red indicates "some" expression as explained in Table 2 legends). Luminal localization (*X*, *) is also shown. Other segments of the medullary nephron (*R*) were essentially negative. MEPE mRNA distribution corresponded to its protein localization in the proximal tubule (*U*) with absence in the other segments (*V*). Representative preimmune IgG antibody negative control (*S*) and sense strand in situ hybridization for MEPE mRNA negative control are also shown. Black arrows show Bowman's capsule parietal cell staining, while white arrows show thin loop of Henle staining.

and their partner MMPs, results of the monkey sections are shown here because the small size of the *M. fascicularis* kidney permitted all three morphologic regions (cortex medulla and renal calyx) to be probed on a single section. Except for DMP1 and MMP-9, which also showed staining of parietal cells of the Bowman's capsule, SIBLING and MMP localization were confined to the tubular epithelial cells of the nephron. The other glomerular cells, including tuft cells, connective tissue, blood vessels, and the rest of the kidney parenchyma, were always devoid of protein and mRNA of the SIBLINGs and their MMP partners.

Our observation of the localization of OPN is consistent with earlier reports [45, 50, 51] to the extent that OPN is localized to the distal tubules. However, using several different antibodies and four species (monkey and rat data presented, human and mouse data not shown) OPN was consistently localized to the basal rather than the luminal (apical) region of the distal tubule suggested by other authors [20]. Salivary gland striated ducts similarly had a clear basal staining with antibodies to OPN [37]. MMP-3, although found in the same distal tubule cells, did not have a particularly basal distribution pattern. Brown et al [50] reported that OPN might be present in the human collecting duct, but we did not observe any evidence of OPN expression in the epithelial cells of the adult kidney collecting duct either at the protein or mRNA level in any of the species studied. Because OPN is known to be in the urine of many species [52], occasionally one may expect to find luminal material that may sustain a positive immunostaining reaction.

Following pathologic stimuli, reports of the expression of OPN in the proximal tubules in addition to the normal expression in the distal tubules [11–13, 15, 20] has been reported. Although the report of Xie et al [20] had indicated that OPN was present in the descending portion of the thin limb of Henle, and not in the distal tubule of normal rat kidney, there was neither immnunohistochemistry nor in situ hybridization results included to substantiate this observation. We were unable to verify the presence of OPN in the rat thin limb of Henle and clearly found it in the location seen for all other reported species, the distal tubules.

As indicated in the results, mRNAs to BSP and its protease partner MMP-2 were not detectable in the collecting duct by in situ hybridization in serial sections even though their respective proteins were demonstrable by immunohistochemistry. As suggested by Tanney et al [38] for MMP-2 observed in the collecting duct at protein but not mRNA levels during nephrogenesis, it can be hypothesized that at least some of the BSP made in the proximal tubule or distal tubule was secreted into the urine and became bound to the luminal surfaces of some collecting duct cells. Piedagnel et al [39] detected the presence of MMP-2 only in the collecting duct segment of the nephron and therefore suggested that MMP-2 is a specific marker for the collecting duct segment. With sufficiently sensitive reagents, however, MMP-2 was also localized to the proximal tubule and distal tubule suggesting that MMP-2 is an unlikely specific marker for normal collecting ducts. The portions of the kidney that are positive for the BSP/MMP-2 pair, proximal tubule + distal tubule (and perhaps collecting duct), are the ones generally thought to be involved in active ion transport. Thus they are similar to the salivary gland duct system in both shared physiologic function and expression of BSP/MMP-2. The portions of the nephron that are negative for BSP/MMP-2, the glomerulus and thin limb of Henle region, are not generally considered to be involved in high, active transport and may share little or no physiologic functions with elements of the salivary gland ductal system.

DMP1, and its MMP-9 partner, localized to all segment types and notably, also in the parietal cells of the Bowman's capsule. With respect to MMP-9, this result contrasted with those of Piedagnel et al [39] who reported the expression of MMP-9 in only the collecting duct segment of normal rat kidney as well as those of Knowlden et al [53] who reported localization in normal glomerular mesangial cells based on human cell culture studies.

The two SIBLINGs, DSPP and MEPE (with yet to be identified MMP partners), also localized to specific segments of the nephron. The apparently stronger intensity of DSPP staining in the proximal tubule luminal surface than in the distal tubule suggests that DSPP is secreted into the lumen of the nephron, and possibly binds to specific luminal surfaces of this nephron segment. Similarly, the apparent difference in intensity of MEPE in the proximal convoluted tubule and proximal straight tubule observed by immunohistochemistry may be due to the presence of a surface binding protein for MEPE on the luminal surface of the proximal convoluted tubule.

While MMPs were traditionally thought to be limited to areas of development, wound repair, cellular migration, or matrix turnover, they now are increasingly thought to be involved in the homeostasis of normal tissue elements. It has been suggested that the segmental preferences for expression of the MMPs in the nephron reflect distinct transcriptional control mechanisms arising from major differences in the structures of the 5′ regulatory regions of these genes [54]. Given the SIBLING/MMP partnership we postulate, by extension, that the presence of similar major differences in the structure of the 5′ regulatory regions of the SIBLING genes may account for transcriptional control differences causing each SIBLING to be localized to specific segments of the nephron. It is predictable that such control mechanisms may, in disease conditions, alter the expression

of a particular SIBLING or MMP at additional segmental locations to that in normal conditions. For example, in certain pathologic conditions, OPN is expressed in the proximal convoluted tubule in addition to its normal expression in the distal tubule.

Our current results support the hypothesis that all of the SIBLINGs appear to have a function(s) in addition to control of mineralization. A final hypothesis of the function(s) of the SIBLINGs must eventually include the following: (*1*) retained ability to bind to integrins throughout evolution; (*2*) coexpression with MMPs (for at least three of the five known SIBLINGs); and (*3*) the distribution of the proteins within the mineralized and nonmineralized tissues.

The physiology of the nephron is one of the most highly studied and best understood complex structures in nature. While work remains to be done, the basic functions of the different segments of the nephron are well understood. The epithelial cells produce and maintain cell surface proteins, basement membranes, and other pericellular matrices that are critical to the different functions performed by the different segments. While proteins such as type IV collagen and laminin are always part of the basement membrane, other gene products probably differ among the segments so that the local microenvironments best fit the different physiologic functions being performed. For example, due to the filtering properties of the glomerular basement membrane, it is clearly different in content than that found in the proximal tubule or the loop of Henle. Furthermore, the assembly, maintenance, and local turnover of these different basement membranes, pericellular matrices, and cells surface proteins are probably the responsibility of each epithelial cell. Proteases would certainly be required for these processes and this paper shows that each segment of the nephron expresses one or more the three MMPs investigated. Activation of these MMPs historically has required the removal of their inhibitory propeptides, but it has recently been shown that three members of the SIBLING family of proteins can bind and activate their partner MMPs without removal of the propeptides in vitro. The fact that we were able to show that every time a nephron cell produces a specific SIBLING protein, it also invariably is making its partner MMP clearly suggested that the active SIBLING-MMP complex will be formed locally. The differential expression of the three SIBLING-MMP complexes suggests that there are likely different target proteins that each cell is modifying. MMP-9 is known to turnover type IV collagen so the coexpression of DMP-1 and MMP-9 along the entire length of the nephron suggests that this complex may be involved in the turnover of this ubiquitous basement membrane component. The more restricted pattern of BSP/MMP-2 and OPN/MMP3 partners may reflect the turnover of a different basement membrane or pericellular proteins that are also restricted to those segments of the nephron. The question of whether these complexes are involved in the routine turnover of normal, undamaged pericellular proteins or are used to specifically remove proteins damaged by the oxidative byproducts invariably generated by these highly metabolically active cells must be answered in future studies.

In recent years it has also become widely accepted that MMPs are often involved in the processing of a variety of cell surface proteins. We find this particularly exciting because BSP has recently been shown to bridge MMP-2 to $\alpha_v\beta_3$ integrin, thereby making it an excellent candidate for modifying cell surface proteins [55]. OPN bridges MMP-3 to cell surfaces and DMP1 does the same for MMP-9 [manuscript in preparation]. The different segments of the nephron each have many different cell surface proteins such as water channels; ion pumps and channels; protein, peptide and amino acid transporters; assorted carbohydrate transporters and lectins; and numerous receptors involved in signal transduction to name a few. Some of these cell surface proteins require proteolytic events to be activated while others are inactivated prior to recycling by the cell. Proteases may also degrade proteins bound to various receptors allowing the disengagement of the receptor from the bound protein. The SIBLING/MMP/cell surface receptor complexes, because they may already be localized to the specific cell surfaces, offer unique opportunities for cells to specifically modify various cell surface proteins under highly controlled spatial conditions. The expression of MMP-3 in both the proximal and distal tubules while its SIBLING partner, OPN, is expressed only in the distal tubule offers insight into the different roles that MMP-3 may play in the normal kidney. The soluble form of MMP-3 may be involved in the turnover of local matrix components of the proximal tubule while in the distal tubule, where OPN is expressed, it may also be held on the cell surface where it can modify specific cell surface proteins.

The kidney offers a unique opportunity in future studies to identify the specific protein substrates for each SIBLING-MMP partnership and will also serve to verify the identity of the MMP partners for MEPE and DSPP as they are proposed. Furthermore, the future documentation of the up- or down-regulation of these sometimes destructive gene products in various kidney diseases may offer new opportunities for intervention and prevention. For example, in diseases in which both the MMP and its SIBLING partner are up-regulated, any synthetic MMP protease inhibitor that may be proposed to be used to treat the disorder should first be shown to work in the presence of that MMP's SIBLING partner.

ACKNOWLEDGMENT

We acknowledge Ms. Li Li for assistance with histologic tissue preparation and immunohistochemistry. This work was supported by the Division of Intramural Research, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA.

Reprint requests to Larry W. Fisher, Matrix Biochemistry Section, Craniofacial and Skeletal Disease Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive, Building 30, Room 223, Bethesda, MD 20982-4320.
E-mail: lfisher@dir.nidcr.nih.gov

REFERENCES

1. Fisher LW, Torchia DA, Fohr B, *et al*: Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. *Biochem Biophys Res Commun* 280:460–465, 2001
2. Min W, Shiraga H, Chalko C, *et al*: Quantitative studies of human urinary excretion of uropontin. *Kidney Int* 53:189–193, 1998
3. Hoyer JR, Asplin JR, Otvos L: Phosphorylated osteopontin peptides suppress crystallization by inhibiting the growth of oxalate crystal. *Kidney Int* 60:77–82, 2001
4. Fisher LW, Fedarko NS: Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res* 44 (Suppl 1):33–40, 2003
5. Robey PG: Bone proteoglycans and glycoproteins, in *Principles of Bone Biology*, edited by Bilezikian JP, Raisz LA, Rodan GA, San Diego, Academic Press, 2002, pp 225–238
6. Rowe PS, de Zoysa PA, Dong R, *et al*: MEPE, a new gene expressed in bone marrow and tumors causing osteomalacia. *Genomics* 67:54–68, 2000
7. Hudkins KL, Giachelli CM, Cui Y, *et al*: Osteopontin expression in fetal and mature human kidney. *J Am Soc Nephrol* 10:444–457, 1999
8. Shiraga H, Min W, VanDusen WJ, *et al*: Inhibition of calcium oxalate crystal growth in vitro by uropontin: Another member of the aspartic acid-rich protein superfamily. *Proc Natl Acad Sci USA* 89:426–430, 1992
9. Senger DR, Perruzzi CA, Papadopoulos A, Tenen DG: Purification of a human milk protein closely similar to tumor-secreted phosphoproteins and osteopontin. *Biochim Biophys Acta* 996:43–48, 1989
10. Patarca R, Wei FY, Singh P, *et al*: Dysregulated expression of the T cell cytokine Eta-1 in CD4-8- lymphocytes during the development of murine autoimmune disease. *J Exp Med* 172:1177–1183, 1990
11. Giachelli CM, Pichler R, Lombardi D, *et al*: Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. *Kidney Int* 45:515–524, 1994
12. Pichler R, Giachelli CM, Lombardi D, *et al*: Tubulointerstitial disease in glomerulonephritis. Potential role of osteopontin (uropontin). *Am J Pathol* 144:915–926, 1994
13. Young BA, Burdmann EA, Johnson RJ, *et al*: Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity. *Kidney Int* 48:439–448, 1995
14. Persy VP, Verstrepen WA, Ysebaert DK, *et al*: Differences in osteopontin up-regulation between proximal and distal tubules after renal ischemia/reperfusion. *Kidney Int* 56:601–611, 1999
15. Diamond JR, Kees-Folts D, Ricardo SD, *et al*: Early and persistent up-regulated expression of renal cortical osteopontin in experimental hydronephrosis. *Am J Pathol* 146:1455–1466, 1995
16. Worcester EM, Blumenthal SS, Beshensky AM, Lewand DL: The calcium oxalate crystal growth inhibitor protein produced by mouse kidney cortical cells in culture is osteopontin. *J Bone Miner Res* 7:1029–1036, 1992
17. Asplin JR, Arsenault D, Parks JH, *et al*: Contribution of human uropontin to inhibition of calcium oxalate crystallization. *Kidney Int* 53:194–199, 1998
18. Lieske JC, Leonard R, Toback FG: Adhesion of calcium oxalate monohydrate crystal to renal epithelial cells is inhibited by specific anions. *Am J Physiol* 268:F604–F612, 1995
19. Worcester EM, Beshensky AM: Osteopontin inhibits nucleation of calcium oxalate crystals. *Ann NY Acad Sci* 760:375–377, 1995
20. Xie Y, Sakatsume M, Nishi S, *et al*: Expression, roles, receptors, and regulation of osteopontin in the kidney. *Kidney Int* 60:1645–1657, 2001
21. Konya E, Umekawa T, Iguchi M, Kurita T: The role of osteopontin on calcium oxalate crystal formation. *Eur Urol* 43:564–571, 2003
22. Nagase H, Woessner JF, Jr.: Matrix metalloproteinases. *J Biol Chem* 274:21491–21494, 1999
23. Carmeliet P, Moons L, Lijnen R: Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet* 17:439–444, 1997
24. Fedarko NS, Jain A, Karadag A, Fisher LW: Three small integrin binding ligand N-linked glycoproteins (SIBLINGs) bind and activate specific matrix metalloproteinases. *FASEB J* 18:734–736, 2004
25. McCawley LJ, Matrisian LM: Matrix metalloproteinases: They're not just for matrix anymore! *Curr Opin Cell Biol* 13:534–540, 2001
26. Nuttal RK, Sampieri CL, Pennington CJ, *et al*: Expression of the entire MMP and TIMP gene families during mouse tissue development. *FEBS Lett* 563:129–134, 2004
27. Ahmed N, Riley C, Oliva K, *et al*: Expression and localization of avB6 integrin in human parturition. *Mol Human Reproduction* 10:173–179, 2004
28. Li W, Alfaidy N, Challis JR: Expression of extracellular matrix metalloproteinase inducer in human placenta and fetal membranes at term labor. *J Clin Endocrinol Metab* 89:2897–2904, 2004
29. Merchant SJ, Crocker IP, Baker PN, *et al*: Matrix metalloproteinase release from placental explants of pregnancies complicated by intrauterine growth restriction. *J Soc Gynecol Investig* 11:97–103, 2004
30. Isaka K, Usuda S, Ito H, *et al*: Expression and activity of matrix metalloproteinase 2 and 9 in human trophoblasts. *Placenta* 24:53–64, 2003
31. Goldberg M, Septier D, Bourd K, *et al*: Immunohistochemical localization of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the forming rat incisor. *Connect Tissue Res* 44:143–153, 2003
32. Yoshiba N, Yoshiba K, Stoetzel C, *et al*: Temporospatial gene expression and protein localization of matrix metalloproteinases and their inhibitors during mouse molar tooth development. *Dev Dyn* 228:105–112, 2003
33. Maatta M, Soini Y, Liakka A, Autio-Harmainen H: Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: Implication for tumor progression and clinical prognosis. *Clin Cancer Res* 6:2726–2736, 2000
34. Djonov V, Hogger K, Sedlacek R, *et al*: MMP-19: Cellular localization of a novel metalloproteinase within normal breast tissue and mammary gland tumours. *J Pathol* 195:147–155, 2001
35. Rudolph-Owen LA, Matrisian LM: Matrix metalloproteinases in remodeling of the normal and neoplastic mammary gland. *J Mammary Gland Biol Neoplasia* 3:177–189, 1998
36. Rudolph-Owen LA, Cannon P, Matrisian LM: Overexpression of the matrix metalloproteinase matrilysin results in premature mammary gland differentiation and male infertility. *Mol Biol Cell* 9:421–435, 1998
37. Ogbureke KUE, Fisher LW: Expression of SIBLINGs and their partner MMPs in salivary glands. *J Dent Res* 83:664–670, 2004
38. Tanney DC, Feng L, Pollock AS, Lovett DH: Regulated expression of matrix metalloproteinases and TIMP in nephrogenesis. *Dev Dyn* 213:121–129, 1998
39. Piedagnel R, Murphy G, Ronco PM, Lelongt B: Matrix metalloproteinase 2 (MMP2) and MMP9 are produced by kidney collecting duct principal cells but are differentially regulated by SV40 large-T, arginine vasopressin, and epidermal growth factor. *J Biol Chem* 274:1614–1620, 1999
40. Norman JT, Gatti L, Wilson PD, Lewis M: Matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases expression by tubular epithelia and interstitial fibroblasts in the normal kidney and in fibrosis. *Exp Nephrol* 3:88–89, 1995
41. Junaid A, Amara FM: Osteopontin: Correlation with interstitial fibrosis in human diabetic kidney and PI3-kinase-mediated enhancement of expression by glucose in human proximal tubular epithelial cells. *Histopathology* 44:136–146, 2004

42. Norman JT, Lewis MP: Matrix metalloproteinases (MMPs) in renal fibrosis. *Kidney Int* (Suppl 54):S61–S63, 1996
43. Lelongt B, Trugnan G, Murphy G, Ronco PM: 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* 136:1363–1373, 1997
44. Nielsen S, Frokiaer J, Marples D, *et al*: Aquaporins in the kidney: From molecules to medicine. *Physiol Rev* 82:205–244, 2002
45. Verhulst A, Persy VP, Van Rompay AR, *et al*: Osteopontin synthesis and localization along the human nephron. *J Am Soc Nephrol* 13:1210–1218, 2002
46. Carome MA, Striker LJ, Peten EP, *et al*: Assessment of 72-kilodalton gelatinase and TIMP-1 gene expression in normal and sclerotic murine glomeruli. *J Am Soc Nephrol* 5:1391–1399, 1994
47. Kallakury BV, Karikehalli S, Haholu A, *et al*: Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. *Clin Cancer Res* 7:3113–3119, 2001
48. McMillan JI, Riordan JW, Couser WG, *et al*: Characterization of a glomerular epithelial cell metalloproteinase as matrix metalloproteinase-9 with enhanced expression in a model of membranous nephropathy. *J Clin Invest* 97:1094–1101, 1996
49. Suzuki D, Miyazaki M, Jinde K, *et al*: In situ hybridization studies of matrix metalloproteinase-3, tissue inhibitor of metalloproteinase-1 and type IV collagen in diabetic nephropathy. *Kidney Int* 52:111–119, 1997
50. Brown LF, Berse B, Van de Water L, *et al*: Expression and distribution of osteopontin in human tissues: Widespread association with luminal epithelial surfaces. *Mol Biol Cell* 3:1169–1180, 1992
51. Hampel DJ, Sansome C, Romanov VI, *et al*: Osteopontin traffic in hypoxic renal epithelial cells. *Nephron Exp Nephrol* 94:e66–e76, 2003
52. Gang X, Ueki K, Kon S, *et al*: Reduced urinary excretion of intact osteopontin in patients with IgA nephropathy. *Am J Kidney Dis* 37:374–379, 2001
53. Knowlden J, Martin J, Davies M, Williams JD: Metalloproteinase generation by human glomerular epithelial cells. *Kidney Int* 47:1682–1689, 1995
54. Thrailkill KM, Kumar S, Rosenberg CK, *et al*: Characterization of matrix metalloproteinases in human urine: Alterations during adolescence. *Pediatr Nephrol* 13:223–229, 1999
55. Karadag A, Ogbureke KU, Fedarko NS, Fisher LW: Bone sialoprotein, matrix metalloproteinase 2, and alpha(v)beta3 integrin in osteotropic cancer cell invasion. *J Natl Cancer Inst* 96:956–965, 2004