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PERSPECTIVES IN BASIC SCIENCE

Expression, roles, receptors, and regulation of osteopontin in the kidney

YUANSHENG XIE, MINORU SAKATSUME, SHINICHI NISHI, ICHIEI NARITA, MASAAKI ARAKAWA, and FUMITAKE GEJYO

Department of Medicine (II), Niigata University School of Medicine, Niigata, Japan

Expression, roles, receptors, and regulation of osteopontin in the kidney. Osteopontin (OPN) is a secreted glycoprotein in both phosphorylated and non-phosphorylated forms. It contains an Arg-Gly-Asp cell-binding sequence and a thrombin-cleavage site. OPN is mainly present in the loop of Henle and distal nephrons in normal kidneys in animals and humans. After renal damage, OPN expression may be significantly up-regulated in all tubule segments and glomeruli. Studies utilizing OPN gene-deficient mice, antisense-treated or anti-OPN-treated animals have demonstrated that OPN promotes accumulation of macrophages, and may play a role in macrophage-mediated renal injury, but that the effect may be mild and short-lived. On the other hand, OPN has some renoprotective actions in renal injury, such as increasing tolerance to acute ischemia, inhibiting inducible nitric oxide synthase and suppressing nitric oxide synthesis, reducing cell peroxide levels and promoting the survival of cells exposed to hypoxia, decreasing cell apoptosis and participating in the regeneration of cells. In addition, OPN is associated with renal stones, but whether it acts as a promoter or inhibitor of stone formation is controversial. It has been demonstrated that OPN receptors include two families: integrin and CD44. The OPN integrin receptors include $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_5$ and $\alpha_9\beta_1$, and $\alpha_4\beta_1$. In normal human kidneys, standard CD44 is expressed most dominantly. Different OPN functions are mediated via distinct receptors. Parathyroid hormone, vitamin D_3, calcium, phosphate and some cytokines increase OPN expression in vitro or in vivo, whereas female sex hormones and angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists decrease OPN expression in some renal damage states.

Osteopontin (OPN), a secreted glycoprotein, is present in normal kidneys, has up-regulated expression and many potential roles in abnormal kidneys. Although a review regarding the expression and role of OPN in renal disease has been published [1], there are few data from humans, or direct evidence that OPN plays a role in renal injury. In addition, reviews about receptors and regulation of OPN in the kidney are limited. This review introduces a generalization of OPN and describes the expression, roles, receptors and regulation of OPN in normal, abnormal, animal and human kidneys referring to typical reports in relation to "OPN and kidney" since 1986, the "birth" of OPN [2] to 2001, using evidence from humans, OPN gene-deficient, antisense-treated or anti-OPN antibody-treated animals.

Key words: osteopontin, macrophage, apoptosis, regeneration, integrin, CD44.

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GENERALIZATION OF OPN

Osteopontin also is known as a 44 kD bone phosphoprotein, sialoprotein I, secreted phosphoprotein I, 2ar, uropontin and early T-lymphocyte activation-1 (Eta-1) [2–6]. OPN cDNA clones initially isolated from a rat osteosarcoma (ROS 17/2.8) phage λgt11 library have a 1473-base-pair-long insert that encodes a protein with 317 amino acid residues. Because it is a product of cells in the osteoid matrix and can form a bridge (Latin pons) between cells and the mineral in the matrix, it was named "osteopontin" by Oldberg et al in 1986 in order to better reflect the potential function of this protein [2].

Osteopontin is synthesized at the highest levels in bone and epithelial tissues [7]. It also is expressed in an assortment of other cell types, including macrophages [8], activated T cells [9], smooth muscle cells [4] and endothelial cells [10]. OPN is synthesized by preosteoblasts, osteoblasts and osteocytes as a bone matrix protein, secreted into osteoids and incorporated into bone. In addition to bone cells, OPN is synthesized by extraosseous cells, as well as by odontoblasts, certain bone marrow cells and hypertrophic chondrocytes. Several types of fibroblasts and epithelial-derived cell lines in culture can secrete OPN [11].

Widely distributed in normal adult human tissues, OPN is abundant in the bone matrix, and is present in the kidney and epithelial cells of the gastrointestinal tract, gall bladder, pancreas, urinary and reproductive tracts, lungs, breasts, salivary glands, sweat glands, and inner ear,

brain, deciduum, placenta and arteries [4, 11, 12]. It also is present in body fluids such as urine and milk [7].

Osteopontin isolated from rat bone is a 44 kD glycosylated phosphoprotein. The OPN secreted by normal rat kidney (NRK) cells in vitro is a 69 kD glycoprotein in both phosphorylated (pp69) and non-phosphorylated (np69) forms. The pp69 forms a heat-stable complex with cell surface fibronectin, whereas the np69 contains N-linked carbohydrates and forms a heat-dissociable complex with plasma fibronectin, suggesting functional modulation of OPN by phosphorylation [13]. For example, dephosphorylation of purified, naturally produced OPN abolishes interleukin-12 (IL-12) stimulatory activity; phosphorylation of recombinant OPN at specific sites restores this activity [6]. Sequence analysis of this cDNA revealed the presence of a 52-nucleotide-long insert in the 5′-noncoding region, which was absent in OPN cDNA cloned from the cDNA library of ROS 17/2.8 rat osteosarcoma cells [14].

An important structural characteristic of OPN is that it contains a glycine-arginine-glycine-aspartate-serine (GRGDS or RGD) amino acid sequence, which is identical to the cell-binding sequence identified in fibronectin [2].

Another interesting feature of OPN's structure is the presence of a thrombin-cleavage site in close proximity to the RGD region (the RGD sequence in OPN is only six residues from the thrombin-cleavage site). OPN can be cleaved into two fragments by thrombin, an amino (N)-terminal fragment and a carboxyl (C)-terminal fragment. For all cell lines examined, thrombin-cleaved OPN promoted markedly greater cell attachment and spreading than uncleaved OPN [15]. The N-terminal fragment containing the RGD motif promoted enhanced adhesion of mouse and human fibroblasts 2.9- and 2.8-fold in comparison with full-length OPN, respectively. The enhanced adhesion of both cells mediated by the N-terminal fragment was significantly suppressed by addition of a C-terminal fragment lacking the RGD motif, which has less cell adhesive property than full-length OPN [16].

Osteopontin is associated with a number of functions involving regulation of osteoclast function during bone formation, renal stone formation, tumorigenesis and transformation, and accumulation of macrophages [7, 17]. In addition, it has immune functions [6–9], inhibits induction of inducible nitric oxide synthase (iNOS) [18], and protects cells from apoptosis [19] as a survival factor. Recently, we demonstrated that OPN is associated with cell proliferation and regeneration in the recovery process after renal injury [20].

The effects of OPN are achieved by its receptors, which include integrin families and CD44 families [21, 22]. For example, the production of OPN by activated T cells is an essential proximal event that potentiates the macrophage IL-12 response through integrin engagement, and dampens the IL-10 response through CD44 engagement [6]. The thrombin cleavage of OPN allows greater accessibility of the RGD domain to cell surface receptors. The integrin $\alpha_9\beta_1$ binding site in OPN is cryptic, but is revealed after thrombin cleavage [23]. Thrombin cleavage enhances the accessibility of the binding motif for integrin $\alpha_V\beta_3$ on OPN [24].

Integrin-mediated cell survival involves regulation of nuclear factor-κB (NF-κB) activity. OPN and β_3 integrin ligation rapidly increased NF-κB activity. $\alpha_V\beta_3$ integrin was the most important for OPN-mediated NF-κB induction and survival, since adding a neutralizing anti-β_3 integrin antibody blocked NF-κB activity. NF-κB was required for OPN- and vitronectin-induced survival, as the inhibition of NF-κB activity with non-phosphorylatable I-κB completely blocked the protective effect of OPN and vitronectin. Activation of NF-κB by OPN depended on the small GTP-binding protein Ras and the tyrosine kinase Src, since Ras and Src dominant-negative mutants inhibited NF-κB reporter activity. These studies identify NF-κB as an important signaling molecule in $\alpha_V\beta_3$ integrin-mediated cell survival [25].

The expression of OPN is up-regulated in the injury and recovery processes of a lot of tissues and cells, including inflammation, fibrosis, mineralization and regeneration of bone, kidneys, heart, vessels and cultured cells [1, 4, 7, 10, 17–20, 26]. Its expression is regulated by many factors including hormones, growth factors, cytokines, vitamin D_3, calcium, phosphate and drugs (Table 1).

EXPRESSION OF OPN IN NORMALLY DEVELOPING AND ADULT KIDNEYS

Expression of OPN in normal kidneys depends on the type of animal (Fig. 1), and its age and gender.

In mice, OPN mRNA was detected in postnatal kidney tubules at 14.5 d p.c. during embryogensis by Northern blot analysis and in situ hybridization [27]. Both methods of in situ hybridization with a mouse cDNA probe, and immunohistochemical staining with three different antisera to mouse OPN, revealed that OPN expression in the normal mouse kidney is primarily restricted to the thick ascending limbs of the loop of Henle and distal convoluted tubules. The protein is detected predominantly at the apical surface of cells lining the lumen of a subset of tubules. OPN expression is not detected in healthy glomeruli, proximal tubules, thin limbs of the loop of Henle, collecting ducts, or interstitial fibroblasts. Relative to male mice, OPN expression is somewhat higher in female, pregnant and lactating mice and markedly increases in the parietal epithelium of glomeruli undergoing sclerosis in aging mice. OPN also is detected in the macula densa. These results suggest that OPN is synthesized and secreted into the tubule fluid by the luminal epithelia of distal portions of a subset of kidney nephrons [28].

Table 1. Generalization of osteopontin

Item	Contents	References
Name	Osteopontin, 44 kD bone phosphoprotein, sialoprotein I, 2ar, secreted phosphoprotein I, uropontin and early T-lymphocyte activation-1	[2–6]
Synthesis	Bone or extraosseous cells, epithelial or epithelial-derived cells, macrophages, activated T cells, smooth muscle cells and endothelial cells	[7–11]
Normal distribution	Bone tissue, epithelial tissue, kidney, artery, inner ear, brain, deciduum, placenta, urine and milk	[4, 7, 11, 12]
Modular volume	44 kD (isolated from rat bone); 69 kD (secreted by normal rat kidney cells)	[3, 13]
Structural characteristic	Arg-Gly-Asp sequence, thrombin-cleavage site, phosphorylated and non-phosphorylated forms	[2, 13, 15]
Roles	Adhesion, migration, signaling, biomineralization, immunity and survival action	[6–9, 17–21]
Receptors	Integrin, CD44	[21, 22]
Regulation	Increase: parathyroid hormone, vitamin D_3, calcium, phosphate, growth factor, cytokines	[32, 37, 42, 46, 52]
	Inhibit: female sex hormone, low salt, drugs	[94–105]

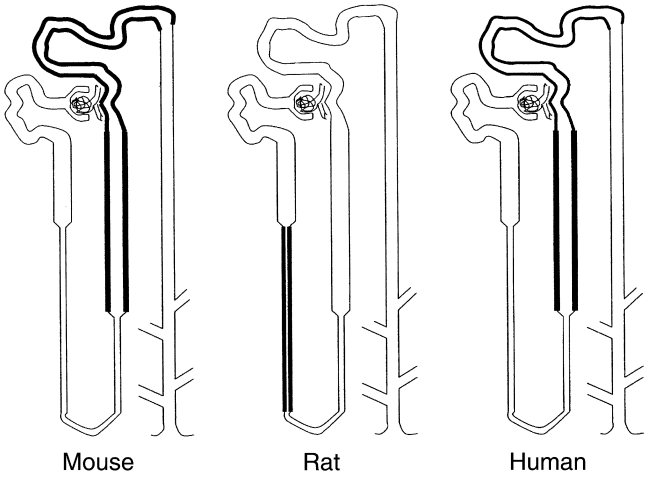

Fig. 1. Expression of osteopontin (OPN) in normal adult kidneys. OPN expression in normal mouse kidneys is primarily restricted to the thick ascending limbs of the loop of Henle and distal convoluted tubules. In normal rat kidneys, OPN is primarily present in the descending thin limbs of the loop of Henle in the outer medulla. In normal human kidneys, OPN is localized primarily to the distal nephrons and is strongly expressed by the thick ascending limbs of the loop of Henle.

In rats, OPN mRNA is present in kidneys obtained from embryos as early as embryonic day 13 (E13). Immunohistochemical staining of metanephroi obtained from E16 rat embryos, as well as metanephroi obtained from E13 embryos and cultured for three days in vitro, demonstrated that OPN is expressed both in developing nephrons and in the ureteric bud [29]. In an early immunohistochemical study in newborn rats, OPN was found only in the proximal tubules [3]. Because immunostaining is observed in components of the vacuolar-lysosomal system, it was concluded that OPN is absorbed from the tubule fluid. However, most later studies (including our study) do not support this result [20, 30–32]. Fluorescence, confocal and electron microscopy revealed OPN primarily in cells of the thin descending limb (TDL) of the loop of Henle in the outer medulla and in the papillary surface epithelium in the area of the calyceal fornix. In situ hybridization with labeled RNA made from cDNA containing the entire coding sequence for mouse OPN reveals messages at the same sites at which the protein is demonstrated by immunocytochemistry. Immunogold labeling is localized to a population of dense vesicles distinct from lysosomes and endosomes. To examine the turnover of OPN, rats were injected with the protein synthesis inhibitor, cyclohexamide, six hours prior to kidney fixation. These kidneys no longer demonstrated OPN in the TDL, and the immunofluorescence in the papillary surface was attenuated [30]. The results also suggest that OPN is synthesized and secreted into the tubule fluid by the luminal epithelia of distal portions of a subset of kidney nephrons. Occasionally, OPN immunostaining of individual cells is observed in the pars recta (S3 segment) of proximal tubules at the transition to the TDL. Weak immunostaining is observed in many thick ascending limb segments in the medulla. Transmission electron microscopy confirms the presence of strong OPN immunoreactivity in TDL cells and the papillary surface epithelium. In the TDL, labeling is located in the Golgi apparatus and in small cytoplasmic vesicles and small dense bodies with the appearance of secretory granules. A few proximal tubule cells show weak staining of lysosome-like structures [31]. Northern blot analysis shows that expression of OPN mRNA is stimulated powerfully in old rats. Dot-blot quantitation analysis showed that OPN mRNA increased 3.1- and 9.1-fold, respectively, in 12- and 24-month-old rats [32].

In human fetal, and normal mature, renal tissue, immunohistochemistry, immunoelectron microscopy, in situ hybridization and Northern blotting show that OPN protein and mRNA are expressed in the human embryonic renal tubular epithelium beginning on approximately day 75 to 80 of gestation. In the fetal kidney, OPN also can be occasionally seen expressed in the ureteric buds and in some interstitial cells. As localized at the protein and mRNA level, the tubular expression of OPN increases with increasing gestational age and persists into

Table 2. Expression of osteopontin in experimental models of renal injury

Reasons	Injury models	Animals	G	BC	PCT	PST	TL	DST	DCT	CD	CT	Methods	References
Glyoxylic acid	Renal stone	Rats	−		±	±	+	+	+	+		ISH, IHC, N	[36–38]
Angiotensin II	Tubulointerstitial nephritis	Rats		+				+	+	+		ISH, IHC, N, W	[39]
Puromycin, Thy-1	Glomerulonephritis	Rats			+	+		+	+	+		ISH, IHC	[5, 40]
Ischemia	Acute renal injury	Rats			+	+		+	+			ISH, IHC, N	[41–43]
Bovine albumin	Interstitial fibrosis	Rats									+	IHC, N	[44]
Ureteral obstruction	Hydronephrosis	Rats									+	ISH, IHC, N, W	[45, 46]
Cyclosporine	Nephropathy	Rats									+	IHC	[47, 48]
Hypercholesterol	Interstitial fibrosis	Rats									+	IHC, N	[49]
Anti-GBM serum	Crescent glomerulonephritis	Rats	+	+	+	+		+	+			ISH, IHC, N	[50]
5/6 nephrectomy	Chronic renal failure	Rats			+	+		+	+			IHC	[51, 52]
Streptozotocin	Diabetic nephropathy	Rats			+	+		+	+			IHC,	[53]
Autoimmune	Lupus nephritis	Mice			+	+						ISH, IHC, N	[54]
Phenylephrine	Hypertension	Rats			+	+		+	+			IHC	[55]
Gentamicin	Acute tubular necrosis	Rats	−	+	+	+	+	+	+			ISH, IHC, N	[20]

Abbreviations are: G, glomerular tuft; BC, Bowman's capsule; PCT, proximal convoluted tubule; PST, proximal straight tubule (thick descending limb); TL, thin limb of loop of Henle; DST, distal straight tubule (thick ascending limb); DCT, distal convoluted tubule; CD, collecting duct; CT, cortical tubule; GBM, glomerular basement membrane; ISH, in situ hybridization; IHC, immunohistochemistry; N, Northern blot analysis; W, Western blot analysis.

adulthood. In the normal adult kidney, OPN is localized primarily to the distal nephrons and is strongly expressed by the thick ascending limb of the loops of Henle. OPN expression also can be observed in some collecting duct epithelia. In cases that exhibit interstitial fibrosis foci and an associated influx of interstitial macrophages, OPN expression is significantly up-regulated in all tubule segments, including proximal tubules [33].

EXPRESSION OF OPN IN RENAL DAMAGE CONDITIONS

Osteopontin has been examined in various experimental models of renal injury (Table 2). Recently, expression of OPN in human renal carcinomas, essential hypertension, progressive idiopathic membranous nephropathy, crescentic glomerulonephritis, immunoglobulin A nephropathy (IgAN), diffuse proliferative lupus nephritis, minimal change nephrotic syndrome and human renal allografts has been reported.

Expression of OPN in experimental models of renal injury

When analyzing the chemical nature of urinary calcium oxalate stone protein as early as 1992, Kohri et al found that cDNA sequences of a protein in a urinary stone had exactly the same identity as human OPN, demonstrating that urinary stones consist of OPN protein [34]. A later study identified that the OPN protein is present around microcrystals of renal papillary stones on reflection-contrast light microscopical images with a high-resolution transmission-electron microscope [35]. In rat models of stone formation induced by glyoxylic acid, OPN mRNA and protein in distal tubular cells is markedly increased, but proximal tubular cells and glomeruli are negative with in situ hybridization and immunostaining. Northern blot analysis showed a significant increase in OPN mRNA in stone-forming rats in proportion to the dosage and duration of stone-inducing drugs [36, 37]. In other rat models of renal stones, OPN increased in all segments of renal tubules, including the proximal tubules (S3 > S2 > S1), distal tubules, limbs of Henle and collecting ducts, and the papillary surface epithelium [38]. These results suggest that OPN is associated with renal stones.

In angiotensin II-induced tubulointerstitial nephritis, preceding pathologic changes in tubular and interstitial cells, a dramatic increase in renal OPN protein and mRNA levels is observed primarily in epithelial cells of the distal tubules, collecting ducts and Bowman's capsule. Regions of the kidney expressing high OPN levels correlate with sites of monocyte/macrophage accumulation [39].

In experimental models of glomerulonephritis such as puromycin nephrosis, anti-Thy-1 nephritis and passive Heymann nephritis, OPN mRNA and protein levels are up-regulated in a portion of proximal and distal tubules. In all three models, the expression of OPN initially precedes histological evidence of tubular injury, but is correlated with subsequent sites of monocyte/macrophage accumulation and tubular damage. OPN expression also correlates with the severity of the tubulointerstitial injury, being greatest in amino-nucleoside nephrosis [5, 40].

In a model of acute ischemic renal injury, levels of whole kidney OPN mRNA in rats rendered ischemic one day previously were elevated approximately 18-fold compared to levels measured in sham-operated controls, as determined by Northern analysis. In situ hybridization demonstrated that the elevated expression of OPN one day postinjury was localized predominantly to cells in the distal tubule and medullary thick ascending limb of Henle's loop. Immunostaining showed an identical localization for elevated protein expression. Five days postinjury, OPN peptide and mRNA were detected clearly

in regenerating proximal tubules, in addition to distal tubules and the medullary thick ascending limb [41]. In a rat model of ischemia-induced acute renal failure, Northern analysis of kidney cortical RNA showed a time-dependent increase in OPN mRNA expression that was significantly higher than in sham-operated rats [42]. After ischemia/reperfusion, all examined nephron segments showed a significant increase in OPN expression, but the time courses and staining pattern of OPN up-regulation were different in proximal and distal tubules. The distal tubules showed an early and persistent increase in OPN staining with a maximum 24 hours after reperfusion, followed by a slow decrease in the absence of major morphological injury, whereas OPN staining in the proximal tubules was delayed, with a maximum after five to seven days, and was mostly associated with morphological regeneration, higher in the outer stripe of the outer medulla than in the cortex [43].

In an interstitial inflammation and fibrosis model with significant proteinuria induced by daily injections of bovine serum albumin, whole kidney OPN mRNA levels were significantly elevated in the renal cortex from the fourth day. After two and three weeks, OPN mRNA levels increased and the proteins showed distinct tubular patterns of distribution, respectively [44].

In experimental hydronephrosis after unilateral ureteral obstruction (UUO), OPN mRNA levels in obstructed kidneys increased versus contralateral unobstructed specimens as early as four hours after UUO. OPN mRNA and protein increases reached fourfold after one day of UUO, and persisted at this level for the five-day duration of UUO. Focal labeling of OPN is noted in both the tubular and Bowman's capsular epithelium in obstructed kidneys, whereas in contralateral unobstructed specimens, there is only faint staining in Bowman's capsule. Paralleling the increments in renal cortical OPN mRNA levels, the number of macrophages was significantly elevated in the cortical renal interstitium [45, 46].

In a salt-depleted rat model of chronic cyclosporine nephropathy, increased cortical tubular staining for OPN correlated with a significant macrophage influx and interstitial fibrosis by day 35 [47, 48].

In a rat model of interstitial inflammation and fibrosis with diet-induced hypercholesterolemia, renal cortical OPN mRNA and protein levels increased significantly, accompanied by albuminuria and interstitial monocyte infiltration, after 12 weeks [49].

In a rat model of accelerated anti-glomerular basement membrane (GBM) glomerulonephritis, there was marked up-regulation of OPN mRNA and protein expression by glomerular visceral and parietal epithelial cells and tubular epithelial cells. Glomerular OPN expression preceded, and correlated with, macrophage infiltration in the development of hypercellularity, focal and segmental lesions and, notably, crescent formation; this also preceded, and correlated with, interstitial macrophage and T-cell infiltration. Both glomerular and tubular OPN expression correlated significantly with proteinuria and a reduction in creatinine clearance. In addition, strong OPN mRNA and protein expression are seen in macrophages and multinucleated giant cells [50].

In remnant kidneys, in a 5/6 renal mass ablation model, OPN was detectable in proximal tubules and distal tubular segments containing casts or showing signs of atrophy and thickened basement membranes [51]. In another study, following subtotal (5/6) nephrectomy (weeks 2 to 16), there was substantial up-regulation of OPN mRNA and protein expression in glomeruli and tubular epithelial cells. The up-regulation of OPN expression was associated with macrophage accumulation within the kidney, severe proteinuria, loss of renal function, and severe histologic damage, including tubulitis and tubulointerstitial fibrosis [52].

In a rat model of streptozotocin-induced diabetes, Northern blot analysis revealed a time-dependent up-regulation of renal cortical OPN expression reaching $138 \pm 6\%$ of the control levels after two weeks and $290 \pm 30\%$ after 12 weeks. During this period of time, diabetic nephropathy developed, as characterized by a reduced glomerular filtration rate and proteinuria. Immunostaining showed that increased OPN expression was present in the tubular epithelium of the renal cortex. Increased levels of OPN are not associated with accumulation of monocyte/macrophages identified by the cell type-specific monoclonal antibody, ED-1 [53].

In MRL-Fas (*lpr*) mice with lupus nephritis, immunofluorescence staining, Northern blot analysis, and in situ hybridization revealed prominent expression of OPN by proximal tubules in MRL-Fas (*lpr*) mice, but not in control mice. The enhanced OPN expression correlated with diffuse macrophage infiltration in the kidneys of MRL-Fas (*lpr*) [54].

In a rat model of hypertension induced by phenylephrine infusion, glomeruli were largely spared, but focal tubulointerstitial fibrosis was present, with the de novo expression of OPN by injured tubules, macrophages and myofibroblast accumulation [55].

In another rat model of gentamicin-induced acute tubular necrosis, OPN mRNA and protein were found markedly not only in proliferating cortical distal tubules, but also in regenerative proximal tubules by in situ hybridization and double immunohistochemical staining. The expression of OPN in the medullary tubules was stronger than that of the control [20].

Expression of OPN in human renal diseases

Osteopontin is overexpressed in the tissues of a variety of experimental malignancy models. In carcinoma of the human kidney, tumor cells and host macrophages strongly

express OPN mRNA. The presence of OPN mRNA in macrophages is particularly pronounced at the edge of tumors and in areas of tumor necrosis. Although in most cases tumor cells do not label detectably for OPN mRNA, both tumor cells and macrophages stain for OPN protein, suggesting that OPN secreted by macrophages may bind to tumor cells, possibly through the GRGDS cell-binding domain in OPN [56].

In renal biopsies from patients with essential hypertension with decompensated arteriolosclerosis, OPN mRNA and protein are increased in tubules in association with expression of α-smooth muscle actin by interstitial fibroblasts and increased type IV collagen deposition [57].

In human progressive idiopathic membranous nephropathy patients, a strong up-regulation of monocyte chemoattractant protein-1, regulated on activation normal T cell expressed and secreted (RANTES) chemokine and OPN was observed, mainly in tubular epithelial cells, with a significant major intensity. A strong correlation between OPN mRNA expression and the corresponding protein was noted. The presence of these chemokines is associated with interstitial cell infiltration [58].

In human crescentic glomerulonephritis, macrophages, parietal epithelial cells, CD_3-positive T cells, or α-smooth muscle actin-positive myofibroblasts present in the glomerular crescent expressed OPN protein and mRNA at a high level. Interstitial monocyte/macrophages did not express OPN, except when located in a periglomerular inflammatory infiltrate [59].

In patients with IgA nephritis (IgAN) and diffuse proliferative lupus nephritis, OPN expression was up-regulated in the cytoplasm of the proximal and distal tubular epithelium parallel to the degree of interstitial mononuclear cell infiltration. CD68(+) monocyte infiltration significantly correlated with the degree of OPN expression in the tubular epithelium. Conversely, there was no apparent induction of OPN in the proximal and distal tubular epithelium of patients with myeloperoxidase-antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis, despite marked monocyte infiltration. These data suggest that inducible expression of OPN in the tubular epithelium seems to be associated with interstitial monocyte infiltration and subsequent tubulointerstitial changes in some forms of human renal diseases [60].

Additionally, compared with normal controls, the mean ± SD of urinary OPN in IgAN patients was decreased significantly (21.4 ± 6.2 vs. 11.6 ± 9.6 mg/g creatinine, $P < 0.001$). In contrast, the levels of urinary OPN in patients with minimal-change nephrotic syndrome (MCNS) or membranous nephropathy (MN) did not differ significantly from normal values. Immunoblot analysis showed that OPN is present as a 55 to 60 kD molecule in normal urine. A 34 kD fragment of OPN was the major immunoreactive band in samples from IgAN patients. This fragment also was detectable in the urine from some patients with MCNS or MN but was absent in normal subjects. Thrombin treatment of the urine from normal controls resulted in 34 kD OPN fragments. Although the underlying mechanisms remain to be determined, these data provide evidence that secretion or processing (or both) of urinary OPN is altered in patients with IgAN [61].

In human renal allografts, strong OPN expression by the proximal tubular epithelium (PTE) was observed in the majority of pre-transplant donor biopsies, as well as early post-transplant biopsies. In patients that did not experience episodes of rejection, OPN expression by PTE decreased substantially, as seen in protocol biopsies taken one year post-transplant. In biopsies with either cellular or vascular rejection, OPN expression by PTE was widespread and intense, and correlated with macrophage infiltration. In biopsies with cyclosporine toxicity, OPN expression by PTE was moderate compared with rejection biopsies, but was equally widespread and without demonstrable association with macrophage infiltration. These data suggest that OPN expression by PTE is up-regulated in human allografts most likely in response to ischemia (pre-transplant and cyclosporine toxicity biopsies), and that further up-regulation of OPN in rejection may contribute to the influx of monocytes/macrophages into the tubulointerstitium (abstract; Hudkins et al, *J Am Soc Nephrol* 11:A3497, 2000).

ROLES OF OPN IN RENAL PATHOLOGY AND PATHOPHYSIOLOGY

Osteopontin has a number of diverse biological functions, including cell adhesion, migration, signaling, biomineralization, immunity, and survival action. In the kidney, OPN may be associated with renal stones and accumulations of monocytes/macrophages in injured tissue. In some settings, OPN may act as a survival factor for renal cells (Table 3).

Role of OPN during renal development and in normal kidneys

Osteopontin may not play an essential role during renal development because in OPN-deficient mice, kidney development is entirely normal, and histologically the kidney has no evidence of abnormalities [17], although the addition of anti-OPN antibodies to metanephric organ cultures results in failure of the metanephric blastema to undergo normal tubulogenesis [29].

In addition, it appears that OPN does not play an essential role in normal kidney cell function per se. This conclusion derives both from the normal morphology of the OPN-deficient kidneys, as well as from studies on the expression of OPN protein in the normal kidney [17]. The OPN protein is present at very low levels in the normal kidney tissue, and is undetectable by Western

Table 3. Roles of osteopontin (OPN) in the kidneys

Angles	Possible roles	References
Renal development	No essential role	[17]
Normal kidney	No essential role	[17]
	Inhibiting calcium oxalate formation?	[17]
Renal stone formation	Inhibiting crystal growth, adherence, aggregation and nucleation	[64–69]
	Increasing crystal deposition and adhesion	[70–72]
Renal injury	Mediating early interstitial macrophage influx and interstitial fibrosis	[5, 19, 54]
	Contributing to glomerular crescent formation or not	[74–76]
Renal protection	Inhibiting iNOS levels and suppressing NO synthesis	[77, 79]
	Reducing cell peroxide levels	[80]
	Decreasing interstitial and tubular cell apoptosis	[19]
	Participating in regeneration and repair of tubular cells	[20, 41, 43, 81]

blotting techniques [62]. Our study also demonstrated that OPN was present at very low levels in normal kidney tissue, especially in the renal cortex [20].

However, OPN is found at high levels in urine. Min et al demonstrated that daily urinary OPN excretion in 13 normal young adult human volunteers was 3805 ± 1805 μg/24 h (mean ± 1 SD) and the mean urinary levels were 1.9 μg/mL [63]. Gang et al reported that mean ± SD of urinary OPN in 20 normal humans was 21.4 ± 6.2 mg/g creatinine [61]. Apparently, the majority of OPN made in the kidney is destined for export to the urine. There, it likely functions as an inhibitor of calcium oxalate formation, helping to prevent mineral precipitation in this supersaturated fluid. However, this viewpoint is controversial (detailed in the next section).

OPN roles in inhibiting or promoting renal stone formation

Osteopontin is one of the most important components in the renal calcium stone matrix, and is up-regulated in renal tubules, especially distal tubules, in experimental models and renal stone patients. However, whether it acts as an inhibitor or promoter of stone formation is also controversial.

Inhibiting renal stone formation. The majority of human urinary stones are primarily composed of calcium salts. Although normal urine is frequently supersaturated with respect to calcium oxalate, most individuals do not form stones. This suggests that there are some factors inhibiting urinary stone formation. A potent crystal growth inhibitor is present in the conditioned media from primary cultures of mouse kidney cortical cells. Conditioned media incubated with the kidney cells for 6 to 72 hours, resulted in the inhibitory activity on crystal growth increasing 15-fold within 24 hours. The sequence of the N-terminal 21 amino acids of this inhibitor matches that of OPN [64].

Uropontin, the urinary form of OPN, has been shown previously to reduce growth [65] and aggregation [66] of calcium oxalate crystals, and block the binding of the crystals to renal epithelial cells [67]. Uropontin inhibits calcium oxalate monohydrate (COM) aggregation at concentrations similar to those that inhibit COM growth, with a mean concentration of 28 ± 4 nmol/L required for a 50% reduction in aggregation and 16 ± 2 nmol/L required for a 50% reduction in growth. The concentrations of uropontin in normal urine (131 ± 13 nmol/L) exceed the levels that substantially decrease calcium oxalate crystal growth and aggregation. In contrast, the 50% inhibition of COM nucleation using a seeded nucleation assay occurred at a concentration of human uropontin (200 nmol/L) that was roughly tenfold higher than the concentrations required for the equivalent inhibition of crystal growth, aggregation or adherence to renal epithelial cells. These studies using a seeded nucleation assay suggest that uropontin only modestly contributes to nucleation inhibition. However, studies using an unseeded nucleation assay showed inhibition of nucleation at concentrations of mouse OPN as low as ~10 nmol/L [68]. These latter studies indicate a much greater capacity for inhibition of nucleation.

In addition, OPN also favors formation of calcium oxalate dihydrate (COD) over COM crystals in vitro. COD is less (50%) adherent to renal epithelial cells than COM, suggesting that OPN may prevent calcium oxalate nephrolithiasis by inducing COD formation [69]. In a model of calcium oxalate formation in OPN knockout, heterozygote, and wild-type mice by exposing them to 1% ethylene glycol, COD crystals were detected in the urine from all mice, but, importantly, were enriched in the urine of wild-type mice. Numerous intratubular COM crystals were seen in thin sections from knockout mice kidneys after four weeks of exposure to ethylene glycol. These results suggesting that OPN directed formation of COD in preference to COM may represent an important and novel mechanism to protect the kidney from calcium oxalate crystal deposition (abstract; Beshensky et al, *J Am Soc Nephrol* 11:A2948, 2000).

Promoting renal stone formation. Some reports suggest that OPN plays an important role in the process of stone formation. In vitro, the ^{45}Ca concentration in calcium oxalate crystals adhering to Madin-Darby canine kidney

(MDCK) cells was about 1.4 times higher in MDCK cells incubated with OPN than in a control group, and was about one half that in MDCK cells incubated with thrombin than in the control group [70].

With the aid of the lipofection reagent, DOTAP, Yamate et al introduced antisense and sense oligonucleotides corresponding to an appropriate part of the coding sequence for OPN into MDCK cells grown in a confluent monolayer. After further incubation, inhibition of OPN expression in the cells was assessed by immunofluorescence photomicrography, while formation of calcium oxalate crystals was quantitated by incorporation of ^{45}Ca into the stone and visualized via scanning electron microscopy. As a result, antisense oligonucleotides at concentrations higher than 20 μmol/L inhibited synthesis of OPN. Incorporation of ^{45}Ca into a calculus stone was inhibited by the addition of an oligonucleotide in a concentration-dependent manner in a range above 20 μmol/L. More than 90% of incorporation was inhibited at 50 μmol/L as compared to a control. This suggests that OPN plays an important role in stimulating deposition and adhesion of calculus crystals to cells in the early stages of urolithiasis [71].

To determine the degree to which this increased deposition of calcium oxalate crystals on the surface of MDCK cells is caused by OPN, MDCK cells (2×10^6 cells/well) were cultured to a confluent state, and the binding of OPN to the cellular surface was then inhibited by adding an anti-OPN polyclonal antibody. The cells were cultured for 24 hours. The degree of calcium oxalate crystal deposition was inhibited for 80% or more in the antibody-treated group, and for 50 to 80% in the thrombin-treated group [72]. These results suggest that intact OPN in the extracellular matrix may be the cause of calcium oxalate crystal deposition on the surface of MDCK cells, whereas thrombin-cleaved OPN may inhibit calcium oxalate crystal deposition.

Additionally, OPN had no effect on the binding of uric acid crystals to the surface of monkey kidney epithelial cells in vitro [73].

OPN roles in renal injury

As in the prior descriptions, after some renal injuries, regions of OPN up-regulated expression are usually correlated with sites of subsequent or synchronous monocyte/macrophage accumulation [5, 40, 41, 54]. Macrophage influx was less in OPN−/− mice compared to OPN+/+ mice on day 4 (threefold, $P < 0.02$), day 7 (fivefold, $P < 0.02$), but not on day 14. Interstitial deposition of types I and IV collagens were also two- to fourfold less in obstructed OPN−/− kidneys ($P < 0.02$). These results suggest that OPN may mediate early interstitial macrophage influx and interstitial fibrosis in unilateral ureteral obstruction and that the effect of OPN may be mild and short-lived in mediating macrophage accumulation [19].

In accelerated crescentic glomerulonephritis, up-regulated OPN expression precedes and correlates with macrophage infiltration in the development of hypercellularity and crescent formation; this also precedes and correlates with interstitial macrophage and T cell infiltration. Anti-OPN treatment significantly reduced glomerular injury (urinary protein excretion) and prevented loss of renal function (creatinine clearance) during the induction of disease. This was accompanied by a significant reduction in renal macrophage and T-cell accumulation, T-cell activation, and histological injury (glomerular hypercellularity, segmental lesions, crescents, and tubulointerstitial lesions). An important finding was that anti-OPN treatment of established crescentic glomerulonephritis led to a significant reduction in glomerular injury and recovery of renal function in association with inhibition of macrophage and T-cell accumulation, T-cell activation, and histological damage [74]. Intravenously injecting an OPN antisense oligodeoxynucleotide into Goodpasture syndrome rats, in parallel with blockade of tubular OPN expression, significantly attenuated monocyte infiltration and preserved renal plasma flow, compared with sense oligodeoxynucleotide-treated and untreated Goodpasture syndrome rats [75]. These results suggest that OPN plays a functional role in macrophage and T-cell accumulation and renal damage in both the induction and progression of a rat model of crescentic glomerulonephritis.

However, there is some contrary evidence. For example, no significant changes were observed in the histopathology of the glomeruli after OPN antisense or sense oligodeoxynucleotide treatment in Goodpasture syndrome rats [75]. Additionally, in rapidly progressive glomerulonephritis in OPN knockout mice, proteinuria was also similar, compared with OPN wild-type mice. Glomerular crescent formation was not different in OPN+/+ and OPN−/− groups (26 ± 6% vs. 29 ± 7%). Tubulointerstitial infiltration was assessed qualitatively and showed no significant difference between the two genotypes. Formation of thrombi in the glomerular capillaries on a scale from 0 to 3 also showed no significant difference (1.3 ± 0.3 for OPN+/+ and 1.4 ± 0.3 for OPN−/− mice). It is concluded that the known up-regulation of OPN in murine anti-GBM nephritis does not significantly contribute to the glomerular and tubulointerstitial mononuclear cell infiltration in this model [76].

Role for OPN as a renoprotective factor

In an experimental model of renal ischemia, mice with a disrupted OPN gene exhibited ischemia-induced renal dysfunction that was twice as pronounced as that observed in mice with intact OPN. In addition, the structural damage caused by ischemia of kidneys obtained

from OPN−/− mice was more pronounced than that observed in similarly treated OPN+/+ mice. This could be associated with the augmented expression of inducible nitric oxide synthase (iNOS) in kidneys from OPN−/− mice versus their OPN+/+ counterparts [77]. OPN inhibits iNOS mRNA and protein levels and suppresses nitric oxide (NO) synthesis induced by the inflammatory mediators γ-interferon and lipopolysaccharide in primary mouse kidney proximal tubule epithelial cells. This process is blocked by anti-OPN antiserum and by the peptide GRGDS, but not GRGES. NO is believed to control blood flow through the glomerulus, regulating salt and water balance [78]. In vitro, NO synthesis was found to be significantly reduced when rat mesangial cells were plated on OPN-coated dishes compared to type I or IV collagen-coated dishes. Furthermore, anti-OPN antibodies increased NO synthesis in mesangial cells. iNOS mRNA levels were increased by tumor necrosis factor-α, and abruptly diminished after OPN mRNA was significantly induced [79]. These results suggest that OPN may be an important regulator of the NO signaling pathway and NO-mediated cytotoxic processes.

Osteopontin also can reduce cell peroxide levels, and promote the survival of cells exposed to hypoxia [80]. In vitro studies with proximal tubular cells subjected to hypoxia resulted in cytoprotection in the presence of OPN, but not OPN with a deleted RGD domain [77].

In addition, obstructed kidneys from OPN−/− mice have more interstitial and tubular apoptotic cells (TUNEL assay) compared to those from OPN+/+ mice. The ability of OPN to act as a cell survival factor also was documented by showing that the apoptosis of serum-starved NRK52E renal epithelial cells was markedly enhanced in the presence of a neutralizing anti-OPN antibody [19].

Recently, OPN was reported to be associated with regeneration and repair in the kidney after renal ischemia/reperfusion injury. In a model of renal ischemia, the OPN peptide and mRNA were clearly detected in regenerating proximal tubules, in addition to distal tubule and medullary thick ascending limb [41]. In rats given left renal ischemia for 60 minutes and a right nephrectomy, up-regulated expression of OPN was predominantly associated with morphological regeneration in proximal tubules. The expression reached a maximum after five to seven days, and was higher in the outer stripe of the outer medulla than in the cortex [43]. In another model, OPN mRNA was expressed in regenerating tubules three days after induction of acute ischemic injury. An immunoreactive OPN peptide continued to be localized in those tubules up to seven days after the injury [81]. In our model of gentamicin-induced acute tubular necrosis, OPN mRNA and protein were expressed only in proliferating cell nuclear antigen (PCNA)-positive cortical distal tubules, but not in necrotic proximal tubules, until day 6 after the first administration, but were found markedly in PCNA-positive regenerative proximal and distal tubules on days 10, 15 and 30. Double staining for OPN and PCNA indicated that the PCNA-positive cells were almost always accompanied by the up-regulated expression of OPN. This result suggested that OPN is related to the proliferation and regeneration of tubular epithelial cells in the recovery process following tubular damage [20].

These studies, especially the comparative analysis of functional and morphological sequelae in OPN+/+ and OPN−/− mice, provide strong evidence for the renoprotective action of OPN in renal injury.

OPN RECEPTORS IN THE KIDNEY

It has been demonstrated that OPN receptors include two families of integrin [21] and CD44 [22], which are ubiquitous multistructural and multifunctional transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions.

The OPN integrin receptors include $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_5$ [21, 82] and $\alpha_9\beta_1$ [83], and $\alpha_4\beta_1$ [84]. In human kidneys, $\alpha_v\beta_3$ is expressed in glomerular epithelial cells, Bowman's capsule, vascular endothelium, and weakly in tubular epithelial cells. $\alpha_v\beta_5$ has a similar distribution, except for minimal expression on the vascular endothelium. β1 expression is seen in glomerular epithelial cells, Bowman's capsule, the vascular epithelium and tubular epithelial cells [85]. Using neutralizing antibodies, all integrin-type OPN receptors, $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_5$ and $\alpha_9\beta_1$, and $\alpha_4\beta_1$ were found to support cellular adhesion to OPN. In contrast, only cells containing $\alpha_v\beta_3$ integrin migrated towards an OPN gradient, demonstrating that different functions of OPN are mediated via distinct receptors [83, 84, 86].

The CD44 family of cell surface glycoprotein receptors is widely expressed in embryonic, normal adult, and neoplasms. CD44 has at least 20 multiple isoforms of different molecular sizes (85 to 230 kD), such as standard CD44, which is also known as hemopoietic CD44, variant CD44 and epithelial CD44 [87]. In normal human kidneys, gene expression of the CD44 standard isoform and the CD44 variant isoforms 8 to 10 are detectable, and among them, the CD44 standard isoform is expressed most dominantly [88], whereas the CD44 peptide is barely detectable in distal tubule using immunohistochemistry [89, 90]. In normal rat kidneys, CD44 has been reported to be detectable in resident glomerular macrophages, parietal epithelial cells of Bowman's capsule, medullary tubules, and occasional cortical tubules (thick ascending limb of Henle's loop and distal tubules) [91, 92]. In renal damage states, such as rat anti-Thy-1 glomerulonephritis [91], rat crescentic glomerulonephritis [50], tubulointerstitial nephritis in kdkd mice [93] and acute ischemic renal injury [81], CD44 is up-regulated in areas of renal injury accompanying its ligand OPN or

hyaluronan. In rat anti-Thy-1 nephritis, the expression of CD44 mRNA and its protein for the 90 kD isoform on the surface of rat mesangial cells is restricted to the transient period of mesangial cell proliferation, as shown by double-staining with an antibody against the proliferating cell nuclear antigen (PCNA). In models of crescentic glomerulonephritis and tubulointerstitial nephritis, double staining showed coexpression of OPN and CD44 in intrinsic renal cells in a crescent formation, such as visceral and parietal epithelial cells, tubular epithelial cells, infiltrating macrophages and T cells. Anti-OPN treatment significantly inhibited the up-regulation of OPN and CD44 [74]. In the recovery process following ischemic or toxic injury, CD44 and OPN are expressed in regenerating tubules [20, 81]. These data suggest that the interaction of OPN and its receptor CD44 may participate in the process of inflammation and recovery after renal injury.

REGULATION OF OPN EXPRESSION IN THE KIDNEY

Factors increasing OPN expression

Osteopontin is a secreted phosphoprotein initially isolated from bone. Therefore, parathyroid hormone (PTH), vitamin D_3, calcium and phosphate, which are strongly associated with bone development and stone formation, increase OPN expression. In the kidneys of young rats infused with PTH, OPN mRNA increased 15.3-fold compared with control rats [32]. In hypercalcemic rats induced by treatment with a PTH-related peptide, OPN mRNA and protein were enhanced in distal tubular cells [94]. Monkey renal epithelial cells (BSC-1 line) in a monolayer culture constitutively secreted OPN into the culture medium. After addition of calcium oxalate monohydrate crystals to the medium, the net OPN content of the cultures increased by 18% after 24 hours [95]. OPN is not induced by 1,25-$(OH)_2D_3$ in the kidneys of D-deficient rats. However, in vitamin D-replete rats given 1,25-$(OH)_2D_3$ for five days, OPN induction in the kidneys significantly increased by 6.0-fold [96]. In addition, there is a specific link between OPN induction and the enzymatic activity of alkaline phosphatase, which results in the generation of free phosphate. Increased free phosphate levels can directly induce strong and specific induction of OPN mRNA and protein in the culture medium [97].

Many cytokines increase OPN expression. Tumor necrosis factor-α (TNF-α) and platelet-derived growth factor (PDGF) increase OPN gene and protein expression in serum-starved cultured rat mesangial cells [98, 99]. In cultured NRK52E cells, a rat renal epithelial cell line, transforming growth factor-β1 (TGF-β1) and epidermal growth factor (EGF) were potent inducers of OPN mRNA and protein in those cells. The results from OPN mRNA stability analysis and nuclear run-on assays suggest that induction of OPN expression by TGF-β1 and EGF is via increased transcription of the OPN gene [100]. Data from an in vitro study indicated that endothelin-1 caused a modest, but reproducible, increase in OPN mRNA in a rat mesangial cell-line [42]. In rat anti-GBM glomerulonephritis, interleukin-1 (IL-1) receptor antagonist treatment significantly reduced OPN mRNA and protein expression in glomeruli and tubules during either induction, or the established phase of the disease. In vitro, the addition of IL-1 to the normal rat tubular epithelial cell line, NRK52E, up-regulated OPN mRNA and protein levels. The effect was dose-dependent and inhibited by the addition of an IL-1 receptor antagonist, thus demonstrating that IL-1 directly up-regulates renal OPN expression [101]. In a rat model of acute ischemic renal injury, pretreatment with insulin-like growth factor-I (IGF-I) enhanced expression of OPN at an earlier period (12 hours, 1 day, and 5 days) after ischemia [41].

In addition, high protein and high cholesterol diets induced an increase in renal OPN mRNA [102]. A high salt concentration or salt crystals also enhanced OPN expression in intact kidneys or cultured renal cells [103].

Factors inhibiting OPN expression

As described above, OPN expression up-regulates during stone formation and the inflammatory process in the kidney. Therefore, some factors or drugs inhibiting stone formation and inflammatory responses can decrease OPN expression. In a renal stone formation model administered glyoxylic acid and 1,25-$(OH)_2D_3$, the expression of OPN mRNA was markedly inhibited by concomitant administration of the female sex hormones, estrogen, estradiol and/or progesterone [37, 104]. OPN expression was enhanced in the distal tubular cells of hypercalcemic rats treated with a PTH-related peptide and was decreased by alendronate, a bisphosphonate derivative [94]. In UUO in rats, OPN mRNA and protein were significantly increased, whereas enalapril, an angiotensin-converting enzyme (ACE) inhibitor, treatment had no effect on the increase in OPN mRNA, but significantly attenuated the increase in protein in tubular cells compared to untreated animals [46]. Enalapril and/or verapamil also blunted cyclosporine-induced OPN mRNA expression significantly [105]. In subtotal (5/6) nephrectomy rats, treatment with either valsartan, an angiotensin II type I receptor antagonist, or ramipril, an ACE inhibitor completely abrogated the up-regulation of OPN mRNA and protein expression in glomeruli and tubules [52].

Since a high salt concentration or salt crystals are known to enhance OPN expression in intact kidneys or cultured renal cells, it is clear that a low salt condition decreases OPN expression in the kidneys. In rats fed a sodium-deficient diet for one week, there was a marked decrease in OPN protein immunoreactivity in the descending thin limbs of Henle's loop, but no changes in

OPN mRNA expression by in situ hybridization, indicating that decreased OPN protein expression is a result of transcriptional regulation. As expected, rats fed a sodium-deficient diet are associated with increased immunoreactivity for Na,K-ATPase and renin compatible with activation of the renin-angiotensin system. These results suggest that dietary sodium is involved in the regulation of OPN expression in the descending thin limbs of Henle's loop in the rat kidney [103].

PERSPECTIVES

Osteopontin is a molecule that has multistructural forms and complex functions. The structural differences in OPN, such as whether it contains the RGD sequence or not, whether it is an intact molecule or a thrombin-cleaved molecule, an N-terminal fragment or C-terminal fragment, or is in a phosphorylated or nonphosphorylated state, may result in different or even contrary functions. In addition, the differences in OPN expression in different damage states or distinct damage stages, in animals or humans in vitro or in vivo, may be associated with its distinct roles in the kidney or cultured renal cells. Moreover, the roles of OPN also may depend on the target cell receptors it joins.

Reprint requests to Fumitake Gejyo, M.D., Department of Medicine, (II), Niigata University School of Medicine, 1- 757 Asahimachi-dori, Niigata 951- 8510, Japan.
E-mail: gejyo@med.niigata-u.ac.jp

APPENDIX

Abbreviations used in this article are: ACE, angiotensin-converting enzyme; COD, calcium oxalate dihydrate; COM, calcium oxalate monohydrate; Eta-1, early T-lymphocyte activation-1; GBM, glomerular basement membrane; GRGDS or RGD, glycine-arginine-glycine-aspartate-serine; IgAN, immunoglobulin A nephropathy; IL, interleukin; iNOS, inducible nitric oxide synthase; MCNS, minimal-change nephrotic syndrome; MDCK, Madin-Darby canine kidney; MN, membranous nephropathy; NF-κB, nuclear factor-κB; NO, nitric oxide; NRK, normal rat kidney; OPN, osteopontin; PCNA, proliferating cell nuclear antigen; PTE, proximal tubular epithelium; PTH, parathyroid hormone; TDL, thin descending limb of the loop of Henle; UUO, unilateral ureteral obstruction.

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