Osteopontin and Developing Kidney

Hyung Eun Yim, M.D. and Kee Hwan Yoo, M.D., Ph.D.

Department of Pediatrics, College of Medicine, Korea University, Seoul, Korea

= Abstract =

Osteopontin과 신장 발달

고려대학교 의과대학 소아과학교실

임 형 은 · 유 기 환

Osteopontin (OPN) is a glycosylated phosphoprotein which mediates cell adhesion and migration, and is produced by bone, macrophages, endothelial cells, and epithelial cells. The many regulatory functions of OPN include bone remodeling, tumor invasion, wound repair, and promotion of cell survival. It is produced by renal tubular epithelial cells, and expression is upregulated in glomerulonephritis, hypertension, ischemic acute renal failure, renal ablation, and UUO. In this review, we discuss about osteopontin in general aspect, expression, role on the development and pathologic condition of neonatal kidney. **(J Korean Soc Pediatr Nephrol 2006;10:1-7)**

Key Words : Osteopontin, UUO, Neonatal kidney

General aspect of osteopontin.

Osteopontin (OPN) is known as a 44 kD bone phosphoprotein, sialoprotein I, secreted phosphoprotein I, 2ar, uropontin and early T-lymphocyte activation-1 (Eta-1). 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. OPN cDNA clones initially isolated from a rat osteosarcoma, have a 1473-base-pair-long insert that encodes a protein with 317 amino acid residues (Fig. 1) [1].

OPN is synthesized at the highest levels in bone and epithelial tissues. It is expressed in an assortment of other cell types, including macrophages, activated T cells, smooth muscle cells and endothelial cells. OPN is also synthesized by preosteoblasts, osteoblasts and osteocytes as a bone matrix protein, secreted into osteoids and incorporated into bone. Several types of fibroblasts and epithelial-derived cell lines in culture can secrete OPN [2-5].

Widely distributed in normal adult human tissues, OPN is abundant in the bone matrix, and is present in the kidney, epithelial cells

접수 : 2006년 3월 30일, 승인 : 2006년 3월 31일
Correspondence : Kee Hwan Yoo, M.D., Ph.D.
#80, Guro-Dong, Guro-Gu, Seoul, Korea
Department of Pediatrics, Guro Hospital, Korea University Medical Center
Tel : 02)818-6735 Fax : 02)858-9396
E-mail : guroped @korea.ac.kr

of the gastrointestinal tract, gall bladder, pancreas, urinary and reproductive tracts, lungs, breasts, salivary glands, sweat glands, inner ear, brain, deciduum, placenta and arteries [5]. It is also present in body fluids such as urine and milk [2].

OPN 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 [6].

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 (Fig. 2) [1].

OPN 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]. In addition, it has immune functions, inhibits induction of inducible nitric oxide synthase (iNOS) [8], and protects cells from apoptosis [9] as a survival factor. Also OPN is associ-

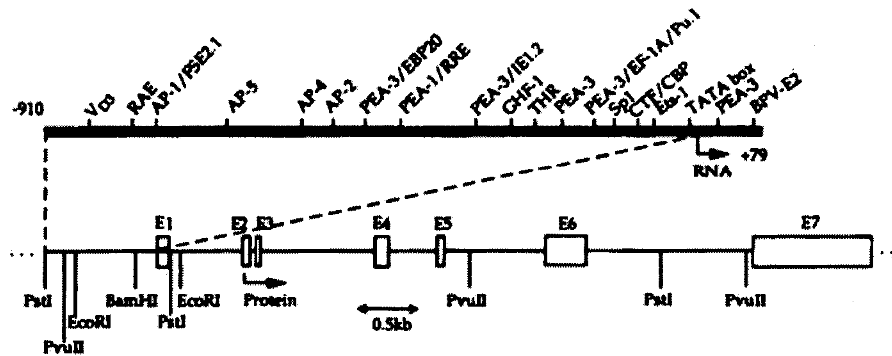

Fig. 1. Structure of mouse OPN gene.

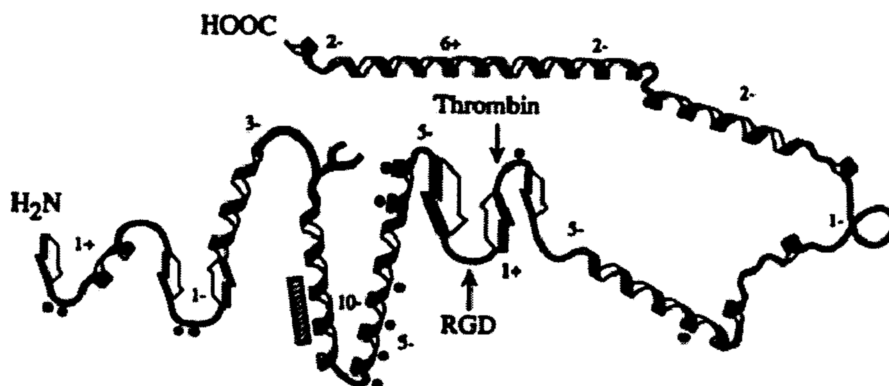

Fig. 2. Structure of mouse OPN protein.

ated with cell proliferation and regeneration in the recovery process after renal injury [10].

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 [11]. Its expression is regulated by many factors including hormones, growth factors, cytokines, vitamin D_3, calcium, phosphate, and drugs.

Expression of OPN in normally developing kidneys (Fig. 3) [12]

In mice, OPN mRNA was detected in postnatal kidney tubules by Northern blot analysis and in situ hybridization at 14.5 d p.c. during embryogensis [13]. 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.

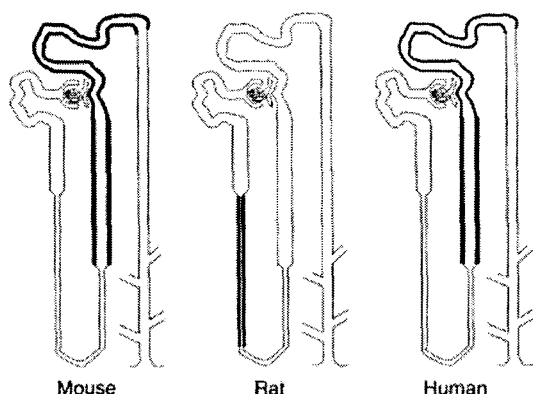

Fig. 3. Expression of OPN in normal kidney (12).

In rats, OPN mRNA is present in kidneys obtained from embryos as early as embryonic day 13 (E13). Immunohistochemical staining of metanephroi obtained from rat embryos and cultured for three days in vitro demonstrated that OPN is expressed both in developing nephrons and in the ureteric bud. In an immunohistochemical study in early newborn rats, OPN was found only in the proximal tubules. Because immunostaining is observed in components of the vacuolar-lysosomal system, it was concluded that OPN is absorbed from the tubular fluid. However, most later studies do not support this result.

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 [14-17].

In human fetal and 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 kid-

ney, 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 adulthood.

Role of OPN during renal development and in normal kidneys

OPN 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 [7], although the addition of anti-OPN antibodies to metanephric organ cultures results in failure of the metanephric blastema to undergo normal tubulogenesis [14].

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 and from studies on the expression of OPN protein in the normal kidney [7]. The OPN protein is present at very low levels in the normal kidney tissue, and is undetectable by Western blotting techniques [18].

However, OPN is found at high levels in urine. Daily urinary OPN excretion in 13 normal young adult human volunteers was 3,805±1,805 μg/24 h (mean±1 SD) and the mean urinary levels were 1.9 μg/mL. Gang et al reported that mean±SD of urinary OPN in 20 normal humans was 21.4±6:2 mg/g creatinine. Apparently, the majority of OPN made in the kidney is destined for export to the urine. Therefore, it likely functions as an inhibitor of calcium oxalate formation, helping to prevent mineral precipitation in this supersaturated fluid. However, this viewpoint is still controversial [19, 20].

Role of OPN in neonatal ureteral obstruction

Congenital obstructive nephropathy is a major cause of renal insufficiency in children [21]. In contrast to the effects of urinary tract obstruction on renal hemodynamics and renal cellular function in the mature kidney, the renal response to urinary tract obstruction in early development reflects the unique characteristics of the immature kidney. Most forms of clinical obstructive nephropathy develop in fetal life, during the period of nephrogenesis. For this reason, we have developed a model of unilateral ureteral obstruction (UUO) in the neonatal rat and mouse, since in these species nephrogenesis is only 10% complete at birth and continues throughout the first ten postnatal days [22, 23].

Chronic UUO in the neonatal mouse leads to progressive tubular apoptosis and nephron loss, as well as interstitial inflammation and interstitial fibrosis [23].

OPN is a glycosylated phosphoprotein which mediates cell adhesion and migration, and is produced by bone, macrophages, endothelial cells, and epithelial cells [12]. The many regulatory functions of OPN include bone remodeling, tumor invasion, wound repair, and promotion of cell survival [24]. It is produced by renal tubular epithelial cells, and

the expression is upregulated in glomerulonephritis, hypertension, ischemic acute renal failure, renal ablation, and UUO [12].

The role of OPN in modulating renal injury is unclear, with evidence for both inflammatory and anti-inflammatory actions [24]. Chronic UUO in the adult rat results in an early upregulation of renal OPN, associated with the accumulation of interstitial macrophages [25]. Stimulation of renal OPN production by UUO may result at least in part from increased intrarenal angiotensin generated by mechanical stretch of dilated tubules [26]. Compared to the adult, UUO in the neonatal rat produces significantly greater stimulation of the renin-angiotensin system as well as a greater renal apoptotic response [22, 27]. Moreover, in the neonatal mouse subjected to UUO, tubular apoptosis is directly related to the severity of tubular dilatation [23], and the severity of interstitial fibrosis is directly dependent on the number of functional copies of the angiotensinogen gene [28]. The stimulation of OPN by angiotensin is mediated by AT1 and AT2 receptors [29], both of which are upregulated by UUO in the neonatal rat [30]. These considerations suggest that, compared to the condition in the adult, UUO in the neonatal mouse may produce significantly different consequences for the hydronephrotic kidney.

As in human, the expression of OPN in the adult mouse is specifically located in thick ascending limbs of the loop of Henle and distal convoluted tubules [12]. While the expression of renal OPN in the mouse has been detected at embryonic day 14.5 [13], targeted deletion of the OPN gene does not interfere with normal renal development [31, 32].

We therefore decided to investigate the contribution of OPN to the progressive renal injury resulting from UUO in neonatal mice. Mice lacking functional OPN (-/-) [31] were compared to wild-type mice (+/+) subjected to complete UUO or sham operation within the first two days of life, and studied 7 or 21 days later. The results show that OPN plays a significant role in the renal response to UUO in early development, and has differing effects on the tubules and interstitium. At 7 and 21 days of age, fibroblasts (FSP-1), myofibroblasts (α-SMA), and macrophages were identified by immunohistochemical staining. Apoptotic cells were detected by TUNEL technique and interstitial collagen by Masson-trichrome or picrosirius red stain. Compared to sham-operated or contralateral kidneys, obstructed kidneys showed increases in all parameters by 7 d, with further increases by 21 d. After 21 d UUO, there was an increase in tubular and interstitial apoptosis in OPN -/- mice as compared to +/+ animals. However, FSP-1 and α-SMA positive cells and collagen in the obstructed kidney were decreased in OPN -/- compared to +/+ mice, while the interstitial macrophage population did not differ between groups. So, OPN plays a significant role in the recruitment and activation of interstitial fibroblasts to myofibroblasts in the progression of interstitial fibrosis in the developing hydronephrotic kidney. However, OPN also suppresses apoptosis.

We conclude that endogenous OPN upregulates the accumulation of interstitial myo-

fibroblasts in the developing hydronephrotic kidney, thereby constituting an injurious response. However, compared to the adult [9], the relative contribution of OPN to interstitial fibrosis in the neonate is severalfold lower, suggesting that therapeutic inhibition of OPN in congenital obstructive nephropathy may be less effective than in the adult. New therapeutic approaches for managing progression of obstructive nephropathy must therefore take into account the characteristics of the developing kidney as well as the differing effects of individual compounds on each renal compartment.

References

1) Oldberg A, Franzen A, Heinegard D. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. Proc Natl Acad Sci USA 1986;83:8819-23.
2) Denhardt DT, Guo X. Osteopontin : A protein with diverse functions. FASEB J 1993;7:1475-82.
3) Singh RP, Patarca R, Schwartz J, Singh P, Cantor H. Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages in vitro and its effect upon macrophages in vivo. J Exp Med 1990;171:1931-42.
4) Weber GF, Cantor H. The immunology of Eta-1/osteopontin. Cytokine Growth Factor Rev 1996;7:241-8.
5) Liaw L, Lindner V, Schwartz SM, Chambers AF, Giachelli CM. Osteopontin and beta 3 integrin are coordinately expressed in regenerating endothelium in vivo and stimulate Arg-Gly-Asp-dependent endothelial migration in vitro. Circ Res 1995;77:665-72.
6) Singh K, DeVouge MW, Mukherjee BB. Physiological properties and differential glycosylation of phosphorylated and nonphosphorylated forms of osteopontin secreted by normal rat kidney cells. J Biol Chem 1990;265:18696-701.
7) Rittling SR, Denhardt DT. Osteopontin function in pathology : Lessons from osteopontin-deficient mice. Exp Nephrol 1999;7:103-13.
8) Rollo EE, Laskin DL, Denhardt DT. Osteopontin inhibits nitric oxide production and cytotoxicity by activated RAW264.7 macrophages. J Leukoc Biol 1996;60:397-404.
9) Ophascharoensuk V, Giachelli CM, Gordon K, Hughes J, Pichler R, Brown P, et al. Obstructive uropathy in the mouse : Role of osteopontin in interstitial fibrosis and apoptosis. Kidney Int 1999;56:571-80.
10) Xie Y, Nishi S, Iguchi S, Imai N, Sakatsume M, Saito A, et al. Expression of osteopontin in gentamicin-induced acute tubular necrosis and its recovery process. Kidney Int 2001;59:959-74.
11) McKee MD, Nanci A. Osteopontin : an interfacial extracellular matrix protein in mineralized tissues. Connect Tissue Res 1996;35:197-205.
12) Xie Y, Sakatsume M, Nishi S, Narita I, Arakawa M, Gejyo F : Expression, roles, receptors, and regulation of osteopontin in the kidney. Kidney International 2001;60:1645-57.
13) Nomura S, Wills AJ, Edwards DR, Heath JK, Hogan BL. Developmental expression of 2ar (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridization. J Cell Biol 1988;106:441-50.
14) Rogers SA, Padanilam BJ, Hruska KA, Giachelli CM, Hammerman MR. Metanephric osteopontin regulates nephrogenesis in vitro. Am J Physiol 1997;272:F469-76.
15) Kleinman JG, Beshensky A, Worcester EM, Brown D. Expression of osteopontin, a urinary inhibitor of stone mineral crystal growth, in rat kidney. Kidney Int 1995;47:1585-96.
16) Madsen KM, Zhang L, Abu Shamat AR, Siegfried S, Cha JH. Ultrastructural loca-

lization of osteopontin in the kidney: Induction by lipopolysaccharide. J Am Soc Nephrol 1997;8:1043-53.

17) Liang CT, Barnes J. Renal expression of osteopontin and alkaline phosphatase correlates with BUN levels in aged rats. Am J Physiol 1995;269:F398-404.

18) Rittling SR, Feng F. Detection of mouse osteopontin by Western blotting. Biochem Biophys Res Commun 1998;250:287-92.

19) Gang X, Ueki K, Kon S, Maeda M, Naruse T, Nojima Y. Reduced urinary excretion of intact osteopontin in patients with IgA nephropathy. Am J Kidney Dis 2001;37:374-9.

20) Min W, Shiraga H, Chalko C, Goldfarb S, Krishna GG, Hoyer JR. Quantitative studies of human urinary excretion of uropontin. Kidney Int 1998;53:189-93.

21) Seikaly MG, Ho PL, Emmett L, Fine RN, Tejani A. Chronic renal insufficiency in children. The 2001 annual report of the NAPRTCS. Pediatr Nephrol 2003;18:796-804.

22) Chevalier RL, Chung KH, Smith CD, Ficenec M, Gomez RA. Renal apoptosis and clusterin following ureteral obstruction: the role of maturation. J Urol 1996;156:1474-79.

23) Cachat F, Lange-Sperandio B, Chang AY, Kiley SC, Thornhill BA, Forbes MS, et al. Ureteral obstruction in neonatal mice elicits segment-specific tubular cell responses leading to nephron loss. Kidney Int 2003;63:564-75.

24) Denhardt DT, Giachelli CM, Rittling SR. Role of osteopontin in cellular signaling and toxicant injury. Annu Rev Pharmacol Toxicol 2001;41:723-49.

25) Diamond JR, Kees-Folts D, Ricardo SD, Pruznak A, Eufemio M. Early and persistent up-regulated expression of renal cortical osteopontin in experimental hydronephrosis. Am J Pathol 1995;146:1455-66.

26) Diamond JR, Kreisberg R, Evans R, Nguyen TA, Ricardo SD. Regulation of proximal tubular osteopontin in experimental hydronephrosis in the rat. Kidney Int 1998;54:1501-9.

27) Chevalier RL, Cachat F. Role of angiotensin II in chronic ureteral obstruction, in The Renin-Angiotensin System and Progression of Renal Diseases., 1 ed., edited by Wolf G, Basel, Karger, 2001:14:250-60.

28) Fern RJ, Yesko CM, Thornhill BA, Kim HS, Smithies O, Chevalier RL. Reduced angioteninogen expression attenuates renal interstitial fibrosis in obstructive nephropathy in mice. J Clin Invest 1999;103:39-46.

29) Cao Z, Kelly DJ, Cox A, Casley D, Forbes JM, Martinello P, et al. Angiotensin type 2 receptor is expressed in the adult rat kidney and promotes cellular proliferation and apoptosis. Kidney Int 2000;58:2437-51.

30) Yoo KH, Norwood VF, el-Dahr SS, Yosipiv I, Chevalier RL. Regulation of angiotensin II AT1 and AT2 receptors in neonatal ureteral obstruction. Am J Physiol 1997;273:R503-9.

31) Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. Altered wound healing in mice lacking a functional osteopontin gene (spp1). J Clin Invest 1998;101:1468-78.

32) Rittling SR, Matsumoto HN, McKee MD, Nanci A, An XR, Novick KE, et al. Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. J Bone Miner Res 1998;13:1101-11.