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INVITED REVIEW

Biomolecular mechanism of urinary stone formation involving osteopontin

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Abstract Urinary stones consist of two phases—an inorganic (mineral) phase and an organic (matrix) phase. Studies on the organic components of kidney stones have been undertaken later than those on the inorganic components. After osteopontin was identified as one of the matrix components, the biomolecular mechanism of urinary stone formation became clearer. It also triggered the development of new preventive treatments. Osteopontin expression is sporadically observed in normal distal tubular cells and is markedly increased in stone-forming kidneys. Calcium oxalate crystals adhering to renal tubular cells are incorporated into cells by the involvement of osteopontin. Stimulation of crystal–cell adhesion impairs the opening of mitochondrial permeability transition pores (mPTP) in tubular cells and produces oxidative stress, apoptosis, and osteopontin expression. Macrophages phagocytose and digest a small amount of crystals, but many crystals aggregate into a mass containing osteopontin and epithelial cell debris and are excreted into the renal tubular lumen, becoming nuclei of urinary stones. This biomolecular mechanism is similar to atherosclerotic calcification. Based on these findings, new preventive treatments have been developed. Dietary control such as low-cholesterol intake and the ingestion of antioxidative foods and vegetables have successfully reduced the 5-year recurrence rate. Osteopontin antibodies and cyclosporine A, which blocks the opening of mPTP, have markedly inhibited the expression of osteopontin and urinary stone formation in animal models.

Keywords Osteopontin · Urinary stone · Calcium oxalate · Macrophage · Atherosclerosis · Mitochondria

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Three epoch-making events in the history of urinary stones

The most ancient urinary stone was found in the pelvic remains of a teenage Egyptian mummy in 4800 BC. The yellow stone, with a uric acid nucleus and concentric laminations of calcium oxalate, was found by Elliot Smith in 1901 [1].

In the long history of urinary stones, we enumerate three epoch-making events.

First, in the fourth century BC, Hippocrates recommended drinking a lot of water for the treatment of urinary stones [2], which, even now, is the most useful prophylaxis. Therefore, the greatness of Hippocrates can be understood.

Since an operation for bladder stones in those days was life-threatening, Hippocrates cautioned his pupils that cystolithotomy should be left to the experts [3]. In this way, Hippocrates is a person of historical note, even though he thought that the components of a urinary stone were bile and phlegm. Thus, the pathogenesis of urinary stones had not yet been elucidated.

Second, the inorganic components of urinary stones, such as calcium oxalate, cystine, uric acid, and magnesium ammonium phosphate were identified in the late 18th century [4]. Based on these discoveries, dissolution therapy for uric acid stones and cystine stones was developed in the early 19th century by alkalinization of urine. Dissolution

therapy is the most effective treatment for these stones even now.

Third, osteopontin (OPN) was identified as one of the organic (matrix) components of urinary calcium stones [5]. The presence of organic components has been known for 60 years [6, 7], but identification of these components was considered difficult because they accounted for only a few percent of the stone. Identification of the organic components radically changed the previous concept that 'urinary stones are formed when crystals oversaturate urine', leading to the elucidation of the biomolecular mechanism of urinary stone formation. It also triggered the development of new preventive treatments, changing previous treatments established for inorganic components, such as alkalinized dissolution therapy. This review mainly describes the biomolecular mechanism of urinary stone formation involving OPN and the latest information concerning preventive treatments.

Identification of osteopontin as a matrix component of urinary stones

Urinary stones consist of two phases—an inorganic (mineral) phase and an organic phase, termed the matrix. In terms of dry weight, organic components account for at least 5 % of the stone [8, 9].

The reason why studies on organic components were undertaken, later those on inorganic components, may be attributed to the lack of identification of components. To identify organic components, we hypothesized that these components were derived from renal tissue rather than urine, as was the previous assumption, because it is unlikely that crystals are formed in rapidly flowing urine in the renal tubular lumen. Therefore, we assumed that crystals were formed and grew while adhering to renal tissue. If this is correct, organic components are derived from renal tissue. We prepared renal tissue cDNA and a polyclonal antibody against organic components of the stone matrix and cloned matrix components, followed by determination of the DNA sequence and identification of OPN [5, 10].

Many researchers, including our group, have identified the components of urinary stone matrices. Among these, analysis of the following substances has progressed and their importance has been suggested: Tamm–Horsfall protein (THP), acidic mucopolysaccharides (glycosaminoglycans), such as hyaluronic acid and glycoproteins, calprotectin, nephrocalcin, and urinary prothrombin fragment 1 [11, 12]. Of these, OPN has the strongest effect on urinary stone formation [13].

Urinary stones are formed through four steps. The first three steps, 'nucleus formation, growth, and aggregation of crystals', occur in urine and in vitro experimental systems, in which inorganic components are mainly involved. On the other hand, the fourth step, 'concretion (progression to stones)', occurs in renal tissues and mainly involves organic components. Organic components harden inorganic crystals. When figuratively referring to a stone wall, inorganic components are the stones and organic components are the cement. Interesting data demonstrate this. Table 1 shows the five main inorganic components of urinary stones and the percentage of organic component contents in stones [8, 9]. Cystine stones containing organic components at the highest level are hard and not readily broken by lithotripsy, whereas infected stones (magnesium ammonium phosphate) containing organic components at the lowest level are readily broken. Avicenna stated 1000 years ago that 'stones formed by transparent urine are hard and those formed in turbid and infected urine are fragile' [2]. This keen discovery by Avicenna must have been obtained through careful observation of individual patients.

Table 1 Percentage of organic (matric) components and hardness in five kinds of inorganic components of urinary stone

Inorganic component	Percentage of organic component (%)	Hardness
Cystine	9	Hard
Calcium phosphate	3.2–6.0	↑
Calcium oxalate	2.0–3.2	↕
Uric acid	0.3–0.9	↕
Magnesium ammonium phosphate (Infected stone)	0.3–1.1	↓ Fragile

Osteopontin expression in kidneys in animal models of stone disease

The expression of OPN mRNA and protein has been sporadically observed in distal tubular cells in normal kidneys and is markedly increased in stone-forming kidneys induced with glyoxylic acid [5]. OPN expression is not detected in glomeruli, proximal tubules, or collecting ducts in normal kidneys. OPN is synthesized within renal tubular cells and is secreted into the urine by epithelial cells. This finding is the same as recent reports showing that interstitial crystal particles appear first in renal distal tubular cells and then in the tubular lumen [14, 15].

We recently examined microstructural changes in renal tubular cells and OPN expression in the early stage of urinary stone formation (Fig. 1). The internal structure of mitochondria underwent destruction, vacuolization, and

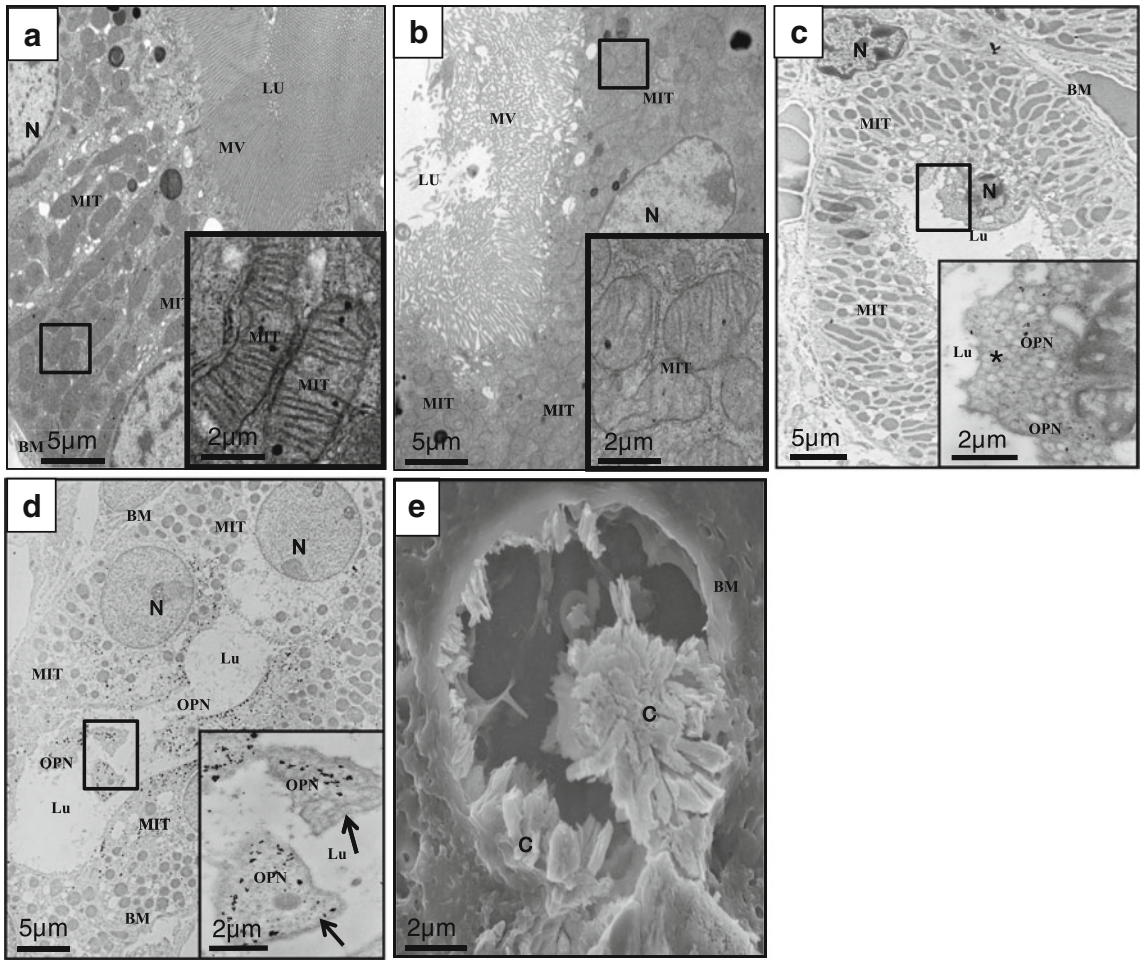

Fig. 1 Microstructural changes in renal tubular cells (RTC) and osteopontin (OPN) expression in the early stage of urinary stone formation induced with glyoxylic acid (GA). **a** The mitochondrial internal structure and double lumen were clearly observed in normal kidneys. **b** Microvilli shortened and decreased and the internal structure of mitochondria became indistinct after 6 h of GA administration. **c** OPN expression was observed on the luminal side of RTC with immuno-transmission electron microscopy (TEM) after 6 h. **d** OPN and collapsed mitochondria with cell debris were located (*arrows*) in the RCT lumen after 24 h. **e** Crystals adhering to epithelial cells and occupying the tubular lumen were observed at the early stage with a scanning electron microscope (SEM). *MIT* mitochondria, *LU* renal tubular lumen, *N* nucleus, *MV* microvilli, *BM* basement membrane, *C* crystal

calcification. Microvilli decreased, shortened, and disappeared [13]. After that, crystals adhering to epithelial cells and occupying the tubular lumen were detected in the renal cortex–medulla junction. OPN expression began to appear on the luminal side of renal tubular cells and was gradually detected in crystal nuclei in the tubular lumen with ultramicrostructural observations and immuno-transmission electron microscopy (TEM). Crystal nuclei contained collapsed mitochondria with cell debris (Fig. 1). In OPN knock-out mice, collapsed mitochondria were present, but no crystal formation was observed. Crystals in mice receiving green tea, one kind of antioxidative drink, were markedly decreased [14, 16].

The distribution of OPN on both calcium oxalate and calcium phosphate was examined in an immunohistochemical study. Core areas in the center showed stains for randomly aggregated OPN and peripheral layer stains were concentric circles in calcium oxalate stones. OPN distribution extended radically from a central area and this radical extension took the form of concentric circles as the matrix of calcium phosphate stones [17], (Fig. 2).

The inclusion of OPN in the culture medium increased the deposition of calcium oxalate crystals adhering to Madin–Darby canine kidney (MDCK) cells [18]. A reduction in crystal adhesion was observed in the cell line transfected with an OPN-antisense vector compared to cultures transfected with a null vector or cultures that were not transfected [19, 20]. We also transfected a vector expressing the antisense of OPN to rat normal epithelial cells (NRK cell) and established a permanent cell that suppresses OPN expression. The inhibition of OPN synthesis suppressed adhesion processes of calcium crystals

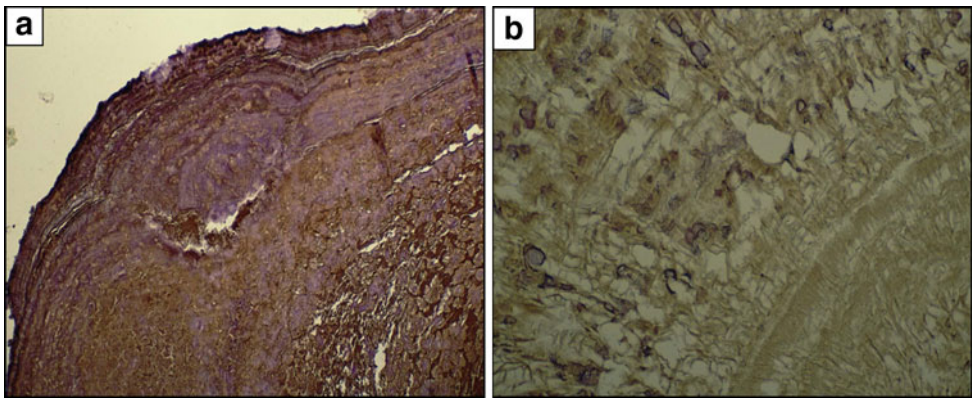

Fig. 2 **a** The distribution of OPN in calcium oxalate stones. Peripheral layers are strongly stained in concentric circles. **b** OPN distribution extends radically from a central area and forms concentric circles in calcium phosphate stones. Magnification: **a** ×100, **b** ×400

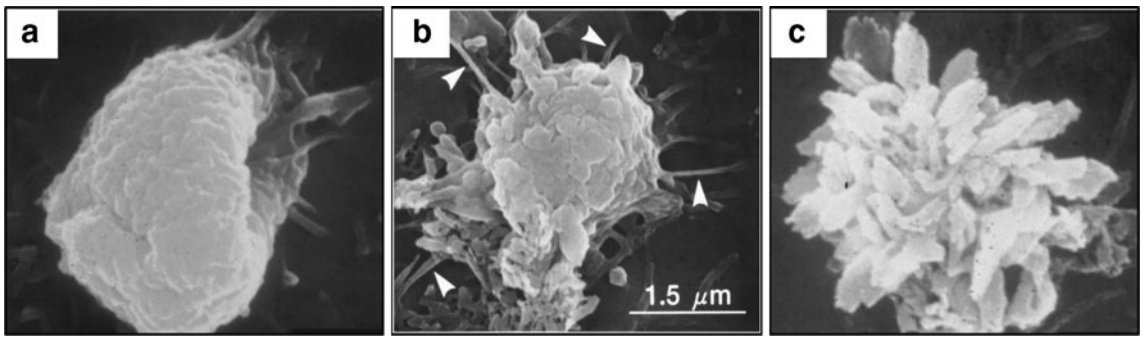

Fig. 3 **a** Scanning electron microscopy (SEM) images of calcium oxalate crystals demonstrated a plate-like shape in multilayers. **b** SEM findings show calcium oxalate crystals adhere to the surface of normal rat kidney (NRK) culture cells. *Arrowheads* indicate elongated microvilli toward the crystals. **c** In NRK culture cells transfected with antisense OPN expression vector, crystals do not adhere to cells and gather roughly

and crystals gathered roughly (Fig. 3). These findings suggest that OPN plays an important role in stimulating the deposition and adhesion of calcium crystals to renal epithelial cells in the stage of urinary stone formation [21, 22].

OPN expression was also increased in the renal tubules of rats with hydronephrosis, parathyroid hormone, vitamin D, and urinary tract infections, and was suppressed with estrogen, bisphosphonate, angiotensin-converting enzyme inhibitors, or angiotensin II receptor antagonists [23–26].

Renal epithelial cell injury and OPN expression in urinary stone formation

Renal tubular cell injury is regarded as a major risk factor for the initial formation of urinary stones [14, 27]. Exposure to calcium oxalate crystals induces oxidative stress, as shown by increased lipid peroxidation, decreased glutathione concentrations, increased free radical generation, and increases in arachidonic acid released by phospholipase A [28, 29]. We recently discovered that reducing oxidative stress with compounds such as green tea, vitamin E, and superoxide dismutase (SOD) was associated with decreased cellular injury and crystal deposition [30, 31]. Penta-galloyl-beta-glucose reduced the expression of OPN, renal crystallization, and oxidative stress, thereby preventing adhesion of crystals to kidney cells in a hyperoxaluric rat model [32].

Khan [33] has emphasized that reactive oxygen species are produced during interactions between crystals and renal cells and are responsible for various cellular responses. Exposure of renal epithelial cells to crystals results in the increased synthesis of osteopontin, bikunin, heparin sulfate, monocyte chemoattractant protein 1 (MCP-1), and prostaglandin (PG) E2, which are known to participate in inflammatory processes and in extracellular matrix production. Calcium oxalate crystal deposition in rat kidneys also activates the renin–angiotensin system.

Khan and Umekawa et al. [34] have shown that pre-treatment of renal epithelial cells in culture to diphenylene iodine chloride (DPI), an inhibitor of NADPH oxidase, leads not only to a reduction in the production of

reactive oxygen species, MCP-1, and OPN, but also to oxalate and calcium oxalate crystal-induced cell injury. They also investigated the influence of two free radical scavengers, citrate and vitamin E, on prevention of the shock wave-induced free radical surge [35]. These results point toward a great potential for the therapeutic application of antioxidants and free radical scavengers to reduce stone recurrence particularly after shock wave lithotripsy, which is itself known to generate ROS and cause renal damage [36]. Khan and co workers [37] reported that the kidneys of hyperoxaluric rats on the angiotensin II type I receptor blocker candesartan had fewer calcium oxalate crystal deposits, reduced OPN expression, and reduced malondialdehyde (MDA) than hyperoxaluric rats.

We recently examined the pathogenesis of the early phase of urinary stone formation in association with renal tubular cell injury (Fig. 4). Calcium oxalate crystals adhere to epithelial cells and NADPH oxidase generates superoxide, which activates cyclophilin D in mitochondria. Mitochondrial permeability transition pore (mPTP) opening and its associated mitochondrial collapse, oxidative stress, activation of the apoptotic pathway, and strong expression of OPN were observed in the initial process of renal calcium crystallization in both in vitro and in vivo experiments [38], (Fig. 4). Cyclosporine A successfully blocked the opening of mPTP, expression of OPN, and renal calcification [38].

Urinary stone formation mechanism based on the characteristics of OPN

We have found several stone matrix components including OPN [39]. Calprotectin is one of the developed matrix proteins [40]. Both calprotectin and OPN contain a 15–20 % aspartic acid residue content and mineral binding, which acts on crystals and produces a calcium-containing stone as a stone matrix [41].

These important biological activities of OPN can be attributed to its characteristic structure, which includes two calcium-binding domains, an Arg-Gly-Asp (RGD) sequence (amino acids 159 to 161 in mice) and a thrombin cleavage site [42–44], (Fig. 5). Calcium-binding domains include stretches of 10 aspartic acid residues (amino acids 86 to 95 in mice) and it has been suggested that these functional sites act complementarily in the regulation of calcification. OPN is considered to play a crucial role in the process of crystal adhesion to epithelial cells via the function of the RGD sequence [10, 40, 45, 46].

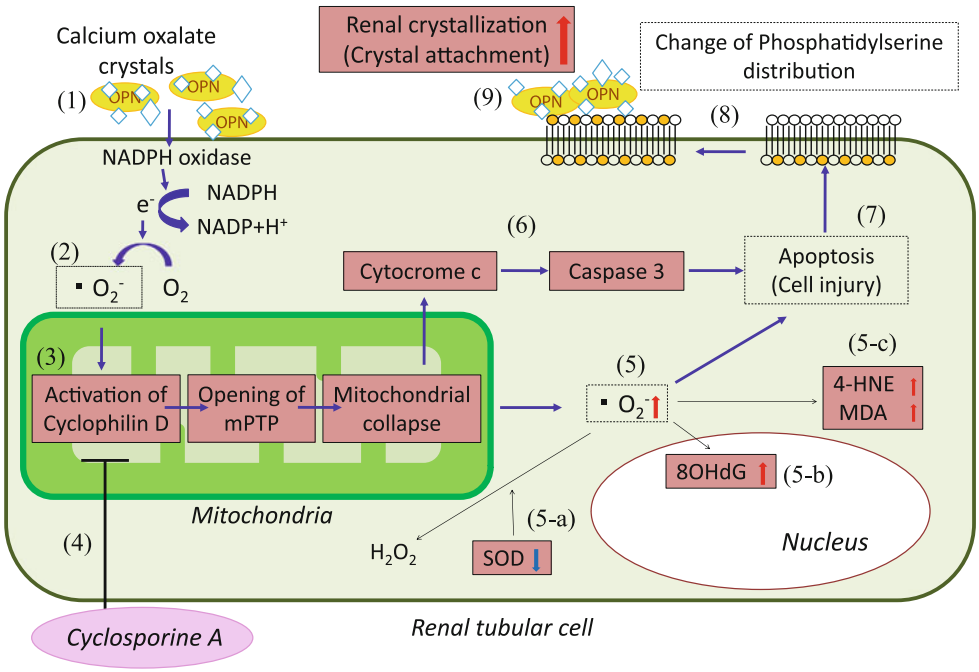

Fig. 4 The pathway depicted is proposed from the results of our studies and reference citations. Calcium oxalate crystals attach to renal tubular cell membranes by OPN involvement (*1*). NADPH oxidase generates superoxide (*2*), which activates cyclophilin D and leads to mPTP opening accompanied by mitochondria collapse (*3*). Cyclosporine A blocks mPTP opening by inactivating cyclophilin D (*4*). Superoxide released from mitochondria (*5*) decreases SOD (*5-a*) and increases 8-OHdG (*5-b*), 4-HNE, and MDA (*5-c*). Cytochrome c also released from mitochondria activates caspase 3 (*6*). These events activate apoptosis and cell injury (*7*) and alter phosphatidylserine distribution in renal tubular cell membranes (*8*), which increases crystal attachment and renal calcium crystallization (*9*)

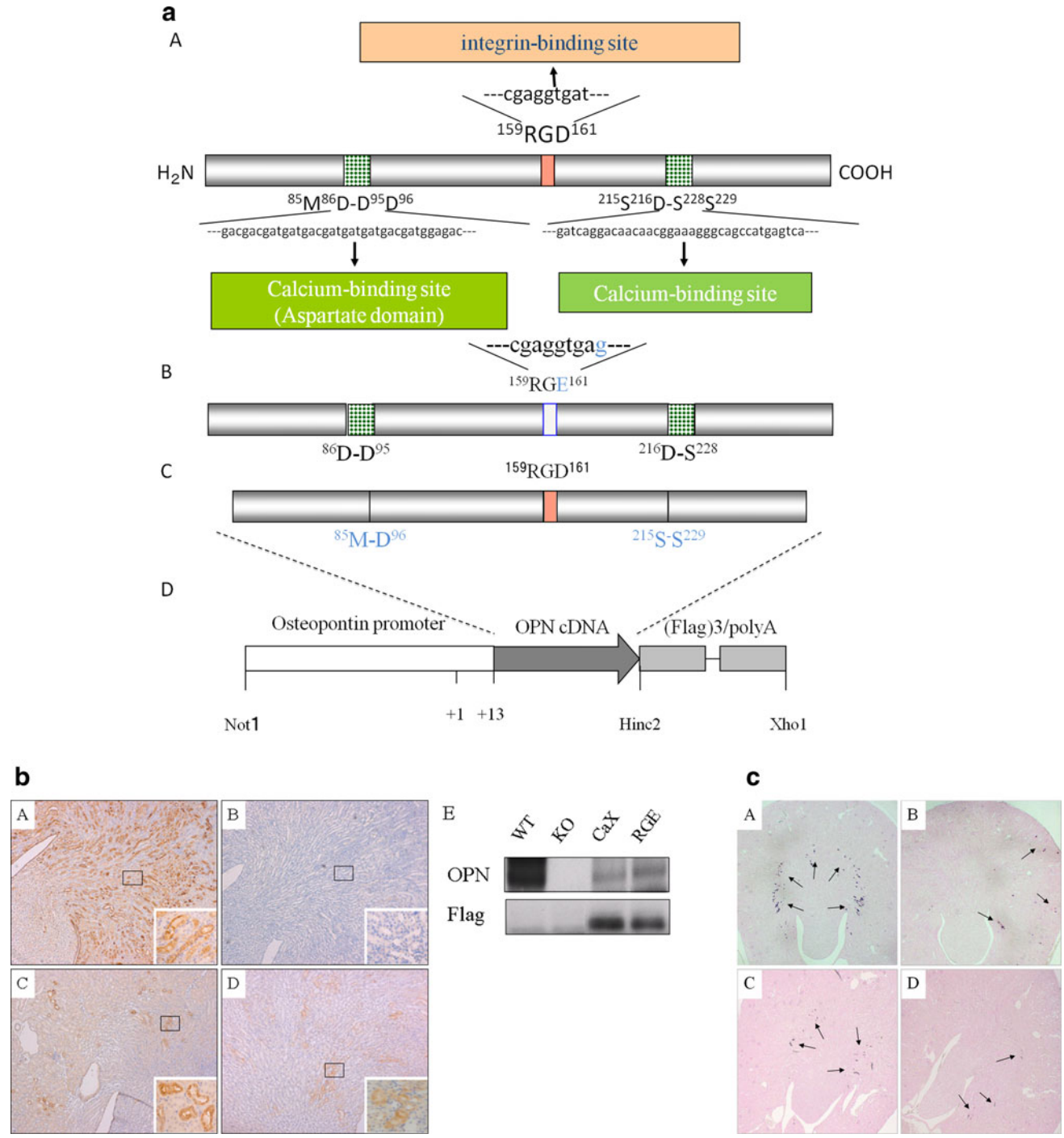

Fig. 5 **a** Putative motif in osteopontin (OPN) and construction of the OPN transgene in mice. (*A*) The locations of two calcium-binding sites and one integrin-binding site are shown. (*B*) Schematic of the RGE transgene; the 507-bpRGD sequence was modified from T to G. (*C*) Schematic of the CaX transgene; two parts of the amino acid sequences were spliced out. (*D*) Schematic of the transgene in which modified OPN cDNA was ligated to the OPN promoter (−5505 to +14), *D* aspartic acid; *R* arginine; *G* glycine; *S* serine; *E* glutamic acid; *M* methionine. **b** Immunohistochemical staining of OPN in the mouse kidney in WT (*A*), KO (*B*), CaX (*C*), and RGE (*D*) groups. OPN was visible in the tubular cells of WT, CaX, and RGE groups, but was not visible in cells of the KO group (×100). (*Inset*) (×400). (*E*) Expressions of endogenous OPN and Flag-tagged OPN were verified by Western blotting. **c** Localization of crystal deposits formed in the kidneys of each group of mice. WT (*A*), KO (*B*), CaX (*C*), and RGE (*D*) groups (×30). *Arrows* indicate crystal deposits

We investigated the effects of impaired domains of OPN, namely the RGD sequence and two calcium-binding sites, on stone formation. We used wild-type mice (WT group), OPN knockout mice (KO group), and OPN knock-out mice carrying either a transgene in which the RGD sequence had been modified to Arg-Gly-Glu (RGE

group) or whose two calcium-binding sites had been deleted (CaX group) (Fig. 5). As expected, kidney sections of the KO group showed no positive signal when immunostained for OPN. In both CaX and RGE groups, the OPN signal was less intense in tubular cells than that in the WT group. Crystal deposition was greatest in the WT group and least in the KO group. In the RGE group, the number of deposits was nearly equal to that in the KO group and nuclei exhibited a radical pattern similar to that in the WT group, in which a rosette petal-like radical pattern was observed. In the CaX group, crystal nuclei were stratified and occurred in a disordered pattern, which was dissimilar to that in the WT group [47, 48], (Fig. 5).

Thrombin cleavage of human OPN (Arg168-Ser169) exposes a C-terminal cryptic integrin-binding motif, 162SVVYGLR168 (Fig. 6). We evaluated the inhibitory effect of an antimurine OPN antibody that specifically reacts with the SLAYGLR domain. This antibody contributed to marked inhibition of early stage urinary stone formation by preventing tubular injury and crystal-cell attachment [49], (Fig. 6).

In in vitro experiments, OPN has an inhibitory effect. It was reported that phosphorylated versions of OPN (65–80) and OPN (220–235) are potent inhibitors of hydroxyapatite growth. The adsorption of acidic proteins to the calcium ion-rich crystal faces of biominerals is governed by electrostatics [50, 51].

These findings shed new light on the importance of crystal–cell interactions and crystal–crystal interactions, respectively, during stone formation, in which both cell-adhesion ligands and calcium-binding domains of OPN participate.

Genetic factors of urinary stones and polymorphism of OPN

Although individuals with recurrent urinary stones have a significantly higher incidence of a positive family history than that of controls, suggesting the involvement of genetic factors, the genes responsible have proved elusive [52–54]. Using SSCP analysis, we found a mutation of GCC to GCT encoding for amino acid position 250 (Ala-250) of OPN at a higher frequency in stone-forming individuals [55]. This mutation also appears to be inherited by the offspring of stone formers who themselves had developed stones.

Regarding OPN, in A9402G of exon 7 (a mutation from histidine to arginine) in another independent cases the genotype was G/G in 99.2 % and G/A in 0.8 % of healthy subjects, while it was G/A in 9.2 % of calcium stone patients, suggesting that the presence of an A allele at this position increases the risk of stones [56]. We also investigated SNP of the entire OPN sequence. On linkage disequilibrium analysis of all 61 SNP sites, haplotype tagging SNP (htSNP) was detected at four sites and haplotype specific for urinary stone patients and healthy subjects were identified at one of these sites [57].

Liu et al. [58] also reported three polymorphisms of the OPN gene and a strong association with urinary stone formation in Chinese patients. Gögebakan et al. [59] showed the existence of T-593A promoter polymorphism of the OPN gene, a significant association with the risk of developing nephrolithiasis, and marked associations between polymorphisms (C6982T and T-593A) of the OPN gene and stone-forming phenotypes. Polymorphism of the OPN gene could serve as a candidate genetic marker for evaluating the risk of calcium urolithiasis. In addition to environmental factors, genetic factors for the formation of urinary stones may be present.

Similarity of urinary stones to atherosclerosis and involvement of OPN in the formation mechanism

OPN is involved in both physiological and pathological processes in multiple organs and tissues including biomineralization, inflammation, and calcification [60–62]. We demonstrated that OPN was observed in tartar, otoliths, and tumor calcification [63–66]. OPN was also highly expressed at sites with atherosclerotic plaques, especially those associated with macrophages and foam cells [67, 68]. OPN may play a functional role in the pathogenesis of calcific aortic stenosis [69, 70]. OPN is involved in the calcification of multiple organs and we identified promoter regions involved in cell and developmental stages specific for OPN expression in bone, the kidney, and placenta using transgenic mice [71].

There are many common features between urinary stones and atherosclerosis. Macrophages and cytokines are involved as inducers, as described above. OPN, calcium, and phosphate acid are commonly regarded as the components of calcification [10, 45, 72]. Both diseases develop in middle-aged and elderly males and postmenopausal females at a high frequency [73–76]. Since the incidence of urinary stones is high in developed countries, westernized diets have been implicated as the main cause of stones [53], but we recently showed that administration of animal protein to rats did not cause urinary stones, although metabolic acidosis occurred and urinary calcium excretion increased.

Thus, we considered that excess ingestion of cholesterol, the main cause of atherosclerosis, could be the cause of urinary stones. When rats were fed a 3 % cholesterol-loaded diet, OPN mRNA levels in renal tubular cells and the kidney increased, followed by urinary stone formation [77], and administration of eicosapentaenoic acid, used to treat atherosclerosis, reduced OPN levels and inhibited stone

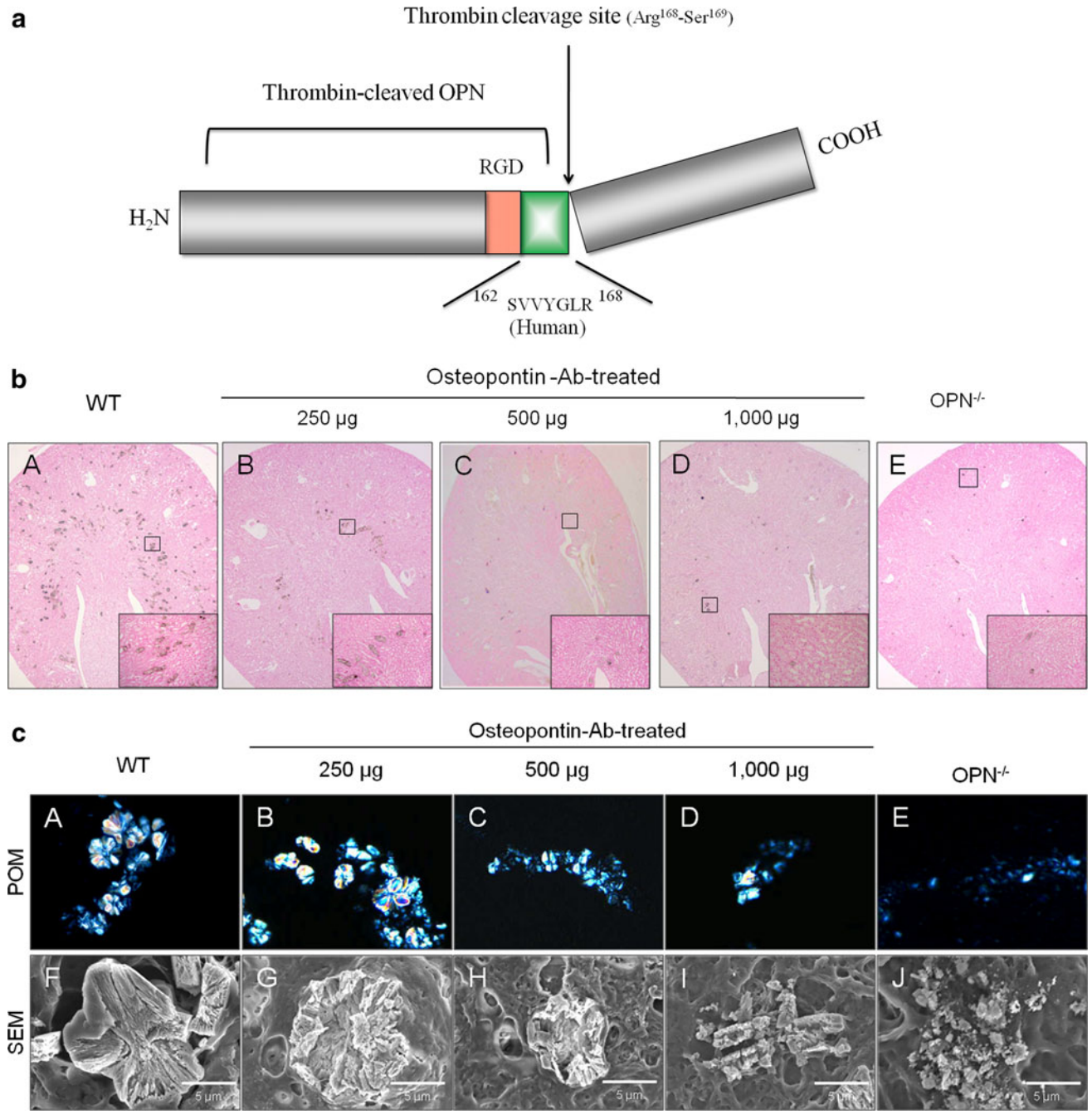

Fig. 6 **a** Structure of human osteopontin (OPN). The Arg-Gly-Asp (RGD) domain is located near the center of the OPN gene. The thrombin cleavage site is adjacent to the RGD sequence and thrombin-cleaved OPN exposes a cryptic integrin-binding motif, ^{162}SVVYGLR^{168}. **b** Renal crystal formation induced in WT, OPN-Ab-treated, and OPN$^{-/-}$ mice after glyoxylate administration. WT mice (*A*); mice treated with 250 (*B*), 500 (*C*), and 1000 μg OPN-Ab (*D*); OPN$^{-/-}$ mice (*E*). Original magnification: *A–E*, ×30 (*inset*: ×400). **c** Crystal structure analysis by POM and SEM from each group on day 7. WT mice (*A*, *F*); mice treated with 250 (*B*, *G*), 500 (*C*, *H*), and 1000 μg OPN-Ab (*D*, *I*); OPN$^{-/-}$ mice (*E*, *J*). In OPN-Ab-treated mice, crystals were small and aberrantly formed, and their density appeared to be low in contrast to those in WT mice. Original magnification: *A–E* ×800, *F–J* 6,000×. *Bar* = 5 μm

formation in both humans and rats [78–80]. The mechanism of this cholesterol load-induced OPN increase and subsequent stone formation has not been clarified. It has been assumed that excess cholesterol ingestion causes binding of intestinal bile acid to cholesterol, freeing oxalic acid and increasing its absorption from the intestine, resulting in an increase in urinary oxalic acid excretion. An increase in oxalic acid levels is considered to be one of the mechanisms of excess cholesterol ingestion-induced stone formation [77, 81, 82].

In clinical cases, abnormal lipid metabolism has already been observed in urinary stone patients [83, 84]. Serum cholesterol levels are significantly high. Calcification of the aortic wall on CT was observed in only 5 % of normal Japanese young subjects, but in about 40 % of stone patients [85].

Adipocytokines secreted by adipocytes play an important role in metabolic syndrome [86, 87]. We observed that adiponectin expression was decreased in renal tubular cells in stone formation model mice when OPN expression was enhanced, followed by urinary stone formation [88]. Mice with a knocked-out appetite center hormone, leptin, became obese and adiponectin decreased. OPN was markedly expressed in the kidneys of leptin-deficient mice and marked urinary stone formation was observed. Administration of adiponectin, an adipose-derived hormone, was demonstrated to inhibit urinary stone formation in these mice [88], (Fig. 7), as adiponectin prevents atherosclerotic vascular stenosis [86]. Our epidemiological study recently showed the impact of insulin resistance, insulin, and adiponectin on patients with urinary stones in the Japanese population [89].

OPN is highly up-regulated in adipose tissue in human and murine obesity and has been recently shown to be functionally involved in the pathogenesis of obesity-induced adipose tissue inflammation and associated insulin resistance in mice [68]. OPN stimulated inflammatory signaling pathways and the secretion of cytokines. Macrophages, cell-adhesion-substances (ICAM-1, VCAM-1), and cytokines (IL-1, IL-6, TNF, TGF) were observed 3 days after glyoxylic acid administration in stone-forming rats. OPN expression was increased at 7 days and the stone nucleus was detected in the renal distal tubular lumen at 14 days [90]. The urinary stone mechanism is similar to atherosclerotic calcification.

We have been performing dietary instruction for stone patients, where cholesterol intake is limited and the ingestion of vegetables at each meal is recommended. As a result, we succeeded in reducing the 5-year recurrence rate from about 40 % before dietary instruction to about 10 % after instruction [91, 92].

Osteopontin involvement in urinary stones and osteoporosis

Urinary stones are similar to osteoporosis in many features. The components calcium, phosphate, and OPN are common [10, 93], as is age with a high incidence, middle-aged and elderly males and postmenopausal females [94], and both diseases are caused by a lack of calcium ingestion [91]. Bone mineral densities in patients with recurrent calcium stones are lower than that in patients with a single stone [95, 96].

As urinary stones have characteristics similar to those of osteoporosis in pathogenesis, it is thought bisphosphonate, an effective drug for osteoporosis is also useful for the prevention of urinary stones.

OPN expression in rats with hypercalcemia induced by parathyroid hormone was remarkably increased in renal tubular cells and was suppressed by the administration of bisphosphonate [24]. The formation of small calcium phosphate particles with strong OPN expression was observed in MDCK epithelial cell cultures at approximately 50 days and was suppressed with bisphosphonate [97]. We further showed that bisphosphonate reduced the excretion of risk factors for calcium phosphate stone formation in postmenopausal women with osteoporosis [98], as Liberman et al. [99] showed the effect of oral bisphosphonate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis.

It is well known that osteoporosis occurs in astronauts due to microgravity in space [100, 101]. We clarified that urinary stones are also formed under low-gravity conditions through a mechanism similar to that of osteoporosis [102, 103]. Based on this finding, we proposed the usefulness of bisphosphonate to prevent urinary stones in astronauts from international collaborative study results and this treatment is currently used.

Biomolecular mechanism of urinary stone formation

The biomolecular mechanism of urinary stone formation is schematically summarized based on knowledge obtained so far (Fig. 8). Urinary oxalate levels are normally about 5–50 μM, but may rise to about 20 times higher due to the influence of meals and dehydration [104], and produce nucleation, growth, and aggregation of calcium oxalate crystals. Crystals smaller than 10 μm are excreted from the body in most cases. Oxalate present at a high level forms crystals in the renal tubular lumen and adheres to tubular epithelial cells. OPN is closely involved in this adherence. Crystals adhering to tubular epithelial cells are incorporated into cells and are processed in lysosomes or released into the renal interstitium and degraded [105–107].

Stimulation by calcium oxalate crystals impairs mitochondrial permeability transition pore (mPTP) opening and its associated mitochondrial collapse, oxidative stress, activation of apoptosis, and strong expression of OPN has been observed in renal tubular cells [38]. The crystal–cell interaction promotes tubular epithelial cells to release chemokines, such as monocyte chemoattractant protein-1 (MCP-1) and OPN [108–110]. Macrophage chemotactic factor expression is also enhanced in tubular epithelial cells [111, 112]. Macrophages phagocytose and digest crystals, present antigens, and produce TGF-β. When crystals

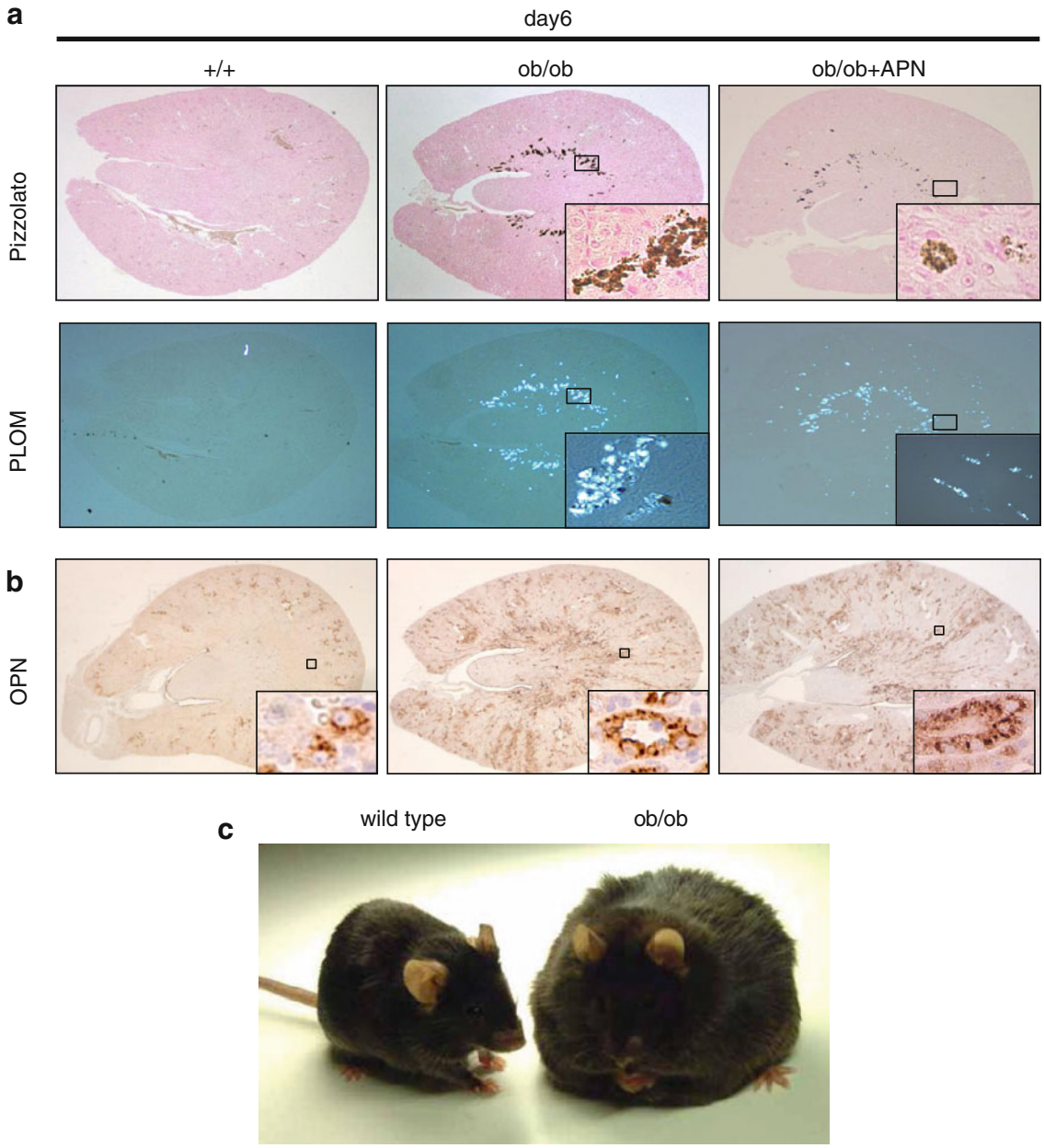

Fig. 7 Detection of calcium oxalate crystal formation and OPN expression in kidney sections of wild-type (+/+), obesity (ob/ob), and adiponectin-treated ob/ob (ob/ob + APN) mice at day 6 after glyoxylic acid and APN administration. **a** Upper images show calcium oxalate crystal deposits in Pizzolato-stained sections. Lower images show non-stained sections observed by polarized light optical microphotography (PLOM). **b** Immunohistochemical staining for OPN. Magnification is ×20 (*inset*:×400). **c** External appearance of the wild-type (+/+) mouse and leptin-deficient (ob/ob) mouse

increase to a level that macrophages cannot phagocytose, crystals aggregate into a mass and are excreted into the renal tubular lumen, becoming nuclei of urinary stones. The capability of phagocytosing crystals may lead to the disappearance of urinary stones [113, 114].

We consider that macrophages exhibit two different actions in urinary stone formation. We have recently established a stone-forming mouse model, which was previously difficult to establish [115]. Using this model, genetic studies, which could not be performed in rat models, rapidly progressed. In an interesting discovery, crystal formation was observed in renal tubular cells on day 3 of glyoxylic acid administration in the mouse model, peaked on day 6, and then mostly disappeared by day 15. This was a completely new phenomenon to be observed: calcium oxalate stones once formed in the kidney

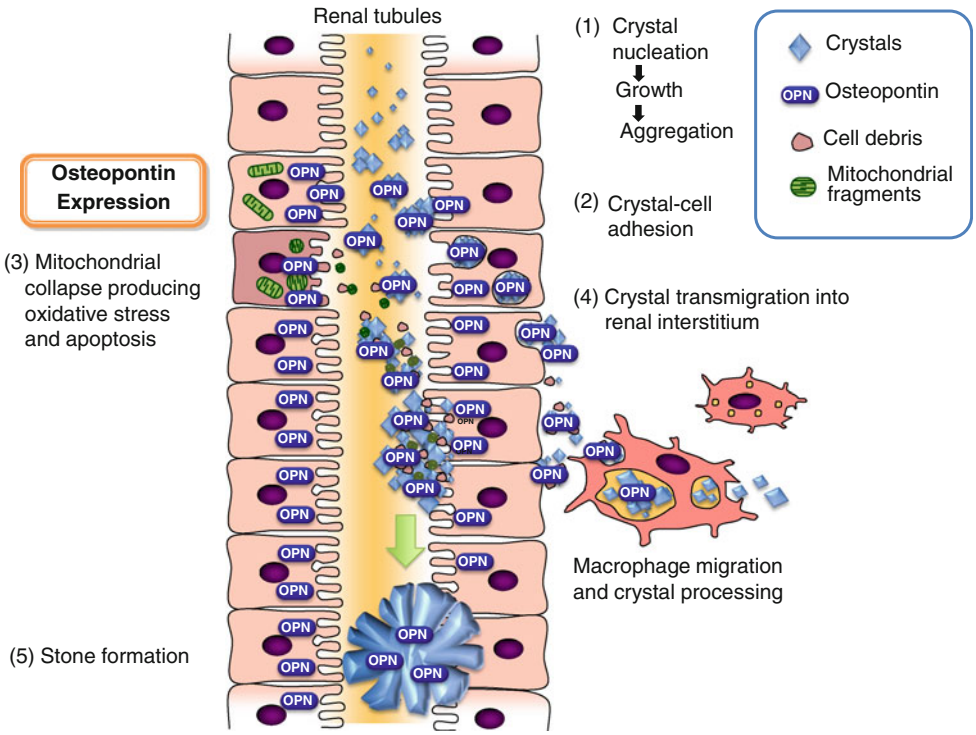

Fig. 8 Schema of the biomolecular mechanism of urinary stone formation. (*1*) Nucleation, growth, and aggregation of crystals. (*2*) Crystals adhere to tubular epithelial cells by the effect of OPN and are incorporated into cells. (*3*) Mitochondrial collapse produces oxidative stress, activation of apoptosis, and strong expression of OPN (as shown in Fig. 4). (*4*) Macrophages digest crystals. When crystals increase to high levels, crystals aggregate into a mass and are excreted into the tubular lumen as nuclei of the urinary stone. (*5*) Stone nuclei containing crystals, cell debris, and stone matrix substances, such as OPN, are produced in the tubular lumen

spontaneously disappear with time [113] and, OPN and macrophages were involved in this process. It appears that urinary stone formation is a purposive reaction to massively and rapidly excrete oxalate, which is harmful, from renal tissues to urine.

OPN is reported to have two different functions depending on the condition. First, OPN is a strong inhibitor of nucleation, growth, and aggregation of calcium oxalate crystals in in vitro experiments and in urine. Free OPN in the tubular lumen acts toward the inhibition of crystal development. However, OPN in this state may be rare in the body because OPN has strong binding activity to calcium rather than oxalic acid and phosphate due to its strong negative ionic action. Shiraga et al. [116] identified OPN as uropontin in potent crystal-inhibitory substances in urine, which was due to this action of OPN.

The second action of OPN is to promote aggregation, and growth of crystals and to adhere crystals to tubular epithelial cells. Wesson [117] reported OPN-deficient mice given ethylene glycol, an oxalate precursor, demonstrated significant intratubular deposits of calcium oxalate crystals, whereas wild-type mice were completely unaffected, and OPN plays a critical renoprotective role in vivo as an inhibitor of crystal formation. However, we carefully observed that crystal formation in established OPN knockout mice with glyoxylic acid administration was less than that of stone-forming mice [47, 48, 115].

This promoting action may be important for urinary stone formation. Two completely different actions of OPN have been reported, but these were due to differences in experimental conditions. It is important to be aware of this difference to fully understand the reports on OPN.

Osteopontin as a target for urinary stone prevention

OPN is one of the most important components in the urinary calcium stone matrix and is up-regulated in renal tubules in experimental stone-forming animal models. Therefore, it is thought that some factors or agents decreasing OPN expression inhibit urinary stone formation, and will be useful, preventive therapies in the future.

First of all, antioxidants and free radical scavengers including green tea, citrate, DPI, vitamin E, candesartan, and eicosapentaenoic acid reduce OPN expression and calcium crystal deposition in animal models.

Cyclosporine A blocks the opening of mPTP in mitochondria of renal tubular cells, and inhibits cell injury, OPN expression, and calcium crystal formation in mouse stone-forming kidneys. The derivative of cyclosporine A also protected stone formation (in preparation of contribution).

OPN antibody, which specifically reacts with thrombin cleavage of human OPN, the ^{162}SVVYGLR^{168} domain, contributed to the inhibition of early stage stone formation by preventing both crystal-renal epithelial cell attachment and calcium adhesion.

Excess ingestion of cholesterol increased OPN expression and renal crystal formation. Eicosapentaenoic acid reduced OPN levels and inhibited stone formation in both

humans and rats. Recently, we reported the inhibitory effect of pioglitazone, a peroxisome proliferator-activated receptor γ agonist, on calcium depositions through renal tubular cell protection, and its antioxidative and anti-inflammatory effects in a stone model mouse [118].

OPN expression is increased in renal tubular cells by parathyroid hormone in rats, and reduced by bisphosphonate administration. Bisphosphonate prevents urinary stones in astronauts and postmenopausal women with osteoporosis.

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References

1. Desnos E (1972) The history of urology to the nineteenth century. In Murphy LJT The history of urology. Charles C Thomas, Springfield
2. Griffith DP (1978) Struvite stones. Kidney Int 13:372–382
3. Chadwick MA, Mann WN (1950) The medical works of Hippocrates. Charles C. Thomas, Springfield
4. Lingeman JE, Smith LH, Woods JR, Newman DM (1989) Hippocrates to the modern era, Urinary calculi: ESWL endourology, and medical therapy. Lea & Febiger, Philadelphi
5. Kohri K, Suzuki Y, Yoshida K, Yamamoto K, Amasaki N, Yamate T, Iguchi M, Shinohara H, Kurita T (1992) Molecular cloning and sequencing of cDNA encoding urinary stone protein, which is identical to osteopontin. Biochem Biophys Res Commun 184:859–864
6. Boyce WH, Sulkin NM (1956) Biocolloids of urine in health and in calculous disease. III. The mucoprotein matrix of urinary calculi. J Clin Invest 35:1067
7. Boyce WH, Garvey FK (1956) The amount and nature of the organic matrix in urinary calculi. Rev J Urol 76:213
8. Boyce WH (1968) Organic matrix of human urinary concretions. Am J Med 45:683
9. Roberts SD, Resnick MI (1986) Glycosaminoglycans content of stone matrix. J Urol 135:1078–1083
10. Kohri K, Nomura S, Kitamura Y, Nagata T, Yoshioka K, Iguchi M, Yamate T, Umekawa T, Suzuki Y, Shinohara H, Kurita T (1993) Structure and expression of the mRNA encoding urinary stone protein (osteopontin). J Biol Chem 268:15180–15184
11. Fleming DE, van Riessen A, Chauvet MC, Grover PK, Hunter B, van Bronswijk W, Ryall RL (2003) Intracrystalline proteins and urolithiasis: a synchrotron X-ray diffraction study of calcium oxalate monohydrate. J Bone Miner Res 18:1282–1291
12. Williams JC, Zarse CA, Jackson ME, Witsmann FA, McAteer JA (2006) Variability of protein content in calcium oxalate monohydrate (COM) stones. J Endourol 20:560–564
13. McKee MD, Nanci A, Khan SR (1995) Ultrastructural immunodetection of osteopontin and osteocalcin as major matrix components of renal calculi. J Bone Miner Res 10:1913–1929
14. Hirose M, Yasui T, Okada A, Hamamoto S, Shimizu H, Itoh Y, Tozawa K, Kohri K (2010) Renal tubular epithelial cell injury and oxidative stress induce calcium oxalate crystal formation in mouse kidney. Int J Urol 17:83–93
15. Coe FL, Evan AP, Worcester EM, Lingeman JE (2010) Three pathways for human kidney stone formation. Urol Res 38:147–160
16. Hirose M, Tozawa K, Okada A, Hamamoto S, Higashibata Y, Gao B, Hayashi Y, Shimizu H, Kubota Y, Yasui T, Kohri K. (2011) Role of osteopontin in early phase of renal crystal formation: immunohistochemical and microstructural comparisons with osteopontin knock-out mice. Urol Res (Epub ahead of print)
17. Tawada T, Fujita K, Sakakura T, Shibutani T, Nagata T, Iguchi M, Kohri K (1999) Distribution of osteopontin and calprotectin as matrix protein in calcium-containing stone. Urol Res 27:238–242
18. Yamate T, Kohri K, Umekawa T, Iguchi M, Kurita T (1999) Interaction between osteopontin on Madin Darby canine kidney cell membrane and calcium oxalate crystal. Urol Int 62:81–86
19. Yamate T, Kohri K, Umekawa T, Amasaki N, Ishikawa Y, Kurita T (1996) The effect of osteopontin on the adhesion of calcium oxalate crystals to Madin–Darby canine kidney cells. Eur Urol 30:388–393
20. Yamate T, Kohri K, Umekawa T, Iguchi M, Kurita K (1998) Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin–Darby canine kidney cell. J Urol 160:1506–1512
21. Yasui T, Fujita K, Asai K, Kohri K (2002) Osteopontin regulates adhesion of calcium oxalate crystals to renal epithelial cells. Int J Urol 9:100–108
22. Yasui T, Fujita K, Tozawa K, Asai K, Soji T, Kato T, Kohri K (2000) Calcium oxalate crystal attachment to cultured rat kidney epithelial cell, NRK-52E. Urol Int 67:73–76
23. Iguchi M, Takamura C, Umekawa T, Kohri K, Kurita T (1999) Inhibitory effects of female sex hormones on urinary stone formation in rats. Kidney Int 56:479–485
24. Yasui T, Fujita K, Sasaki S, Iguchi M, Hirota S, Nomura S, Azuma Y, Ohta T, Kohri K (1998) Alendronate inhibits osteopontin expression enhanced by parathyroid hormone-related peptide (PTHrP) in the rat kidney. Urol Res 26:355–360
25. Liang CT, Barnes J (1995) Renal expression of osteopontin and alkaline phosphatase correlates with BUN levels in aged rats. Am J Physiol 269:F398–F404
26. Umekawa T, Kohri K, Kurita T (1995) Expression of osteopontin messenger RNA in the rat kidney on experimental model of renal stone. Biochem Mol Int 35:223–230
27. Asselman M, Verhulst A, De Broe ME, Verkoelen CF (2003) Calcium oxalate crystal adherence to hyaluronan-, osteopontin-, and CD44-expressing injured/regenerating tubular epithelial cells in rat kidneys. J Am Soc Nephrol 14:3155–3166
28. Thamilselvan S, Byer KJ, Hackett RL, Khan SR (2000) Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. J Urol 164:224–229
29. Kojimoto Y, Kennington L, Scheid CR, Honeyman TW (1999) Role of phospholipase A2 in the cytotoxic effects of oxalate in cultured renal epithelial cells. Kidney Int 56:1432–1441
30. Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol 173:271–275
31. Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) Examination of the anti-oxidative effect in renal tubular cells and apoptosis by oxidative stress. Urol Res 33:261–266
32. Lee HJ, Jeong SJ, Lee HJ, Lee EO, Bae H, Lieske JC, Kim SH (2011) 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose reduces renal crystallization and oxidative stress in a hyperoxaluric rat model. Kidney Int 79:538–545

33. Khan SR (2004) Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. Clin Exp Nephrol 8:75–88
34. Umekawa T, Byer K, Uemura H, Khan SR (2005) Diphenileneiodium (DPI) reduces oxalate ion- and calcium oxalate monohydrate and brushite crystal-induced upregulation of MCP-1 in NRK 52E cells. Nephrol Dial Transplant 20:870–878
35. Delvecchio FC, Brizuela RM, Khan SR, Byer K, Li Z, Zhong P, Preminger GM (2005) Citrate and vitamin E blunt the shock wave-induced free radical surge in an in vitro cell culture model. Urol Res 33:448–452
36. Khan SR (2005) Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res 33:349–357
37. Umekawa T, Hatanaka Y, Kurita T, Khan SR (2004) Effect of angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in rat kidneys. J Am Soc Nephrol 15:635–644
38. Niimi K, Yasui T, Hirose M, Hamamoto S, Itoh Y, Okada A, Kubota Y, Kojima Y, Tozawa K, Sasaki S, Hayashi Y, Kohri K (2012) Mitochondrial permeability transition pore (mPTP) opening induces the initial process of renal calcium crystallization. Free Radical Bio Med 52:1207–1217
39. Kohri K, Fujita K, Itoh T, Sakakura T, Umekawa T, Kurita T (1996) Purification and characterization of urinary stone protein. Akt Urol 27:27–29
40. Kido J, Nishikawa H, Ishida H, Yamashita K, Kitamura S, Kohri K, Nagata T (1997) Identification of calprotectin, a calcium binding leukocyte protein, in human dental calculus matrix. J Periodont Res 32:355–361
41. Verhulst A, Asselman M, Persy VP, Schepers MS, Helbert MF, Verkoelen CF, De Broe ME (2003) Crystal retention capacity of cells in the human nephron: involvement of CD44 and its ligands hyaluronic acid and osteopontin in the transition of a crystal binding- into a nonadherent epithelium. J Am Soc Nephrol 14:107–115
42. Oldberg A, Franzén A, Heinegård D (1986) Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell binding sequence. Proc Natl Acad Sci USA 83:8819–8823
43. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, Rittling SR, Denhardt DT, Glimcher MJ, Cantor H (2000) Eta-1(osteopontin): an early component of type-1(cell-mediated) immunity. Science 287:860–864
44. Pepinsky RB, Mumford RA, Chen LL, Leone D, Amo SE, Riper GV, Whitty A, Dolinski B, Lobb RR, Dean DC, Chang LL, Raab CE, Si Q, Hagmann WK, Lingham RB (2004) Comparative assessment of the ligand and metal ion binding properties of integrins alpha9beta1 and alpha4beta1. Biochemistry 41: 7125–7141
45. Kohri K, Kodama M, Ishikawa Y, Katayama K, Takada M, Katoh Y, Kurita T, Iguchi M (1990) Inhibitory effect of glutamic acid and aspartic acid on calcium oxalate crystal formation. Eur Urol 17:173–177
46. Denhardt DT, Guo X (1993) Osteopontin: a protein with diverse functions. FASEB J 7:1475–1482
47. Hamamoto S, Nomura S, Yasui T, Okada A, Hirose M, Shimizu H, Itoh Y, Tozawa K, Kohri K (2010) Effects of impaired functional domains of osteopontin on renal crystal formation: analyses of OPN transgenic and OPN knockout mice. J Bone Miner Res 25:2712–2723
48. Okada A, Nomura S, Saeki Y, Higashibata Y, Hamamoto S, Hirose M, Itoh Y, Yasui T, Tozawa K, Kohri K (2008) Morphological conversion of calcium oxalate crystals into stones is regulated by osteopontin in mouse kidney. J Bone Miner Res 23:1629–1637
49. Hamamoto S, Yasui T, Okada A, Hirose M, Matsui Y, Kon S, Sakai F, Kojima Y, Hayashi Y, Tozawa K, Uede T, Kohri K (2011) Crucial role of the cryptic epitope SLAYGLR within osteopontin in renal crystal formation of mice. J Bone Miner Res 26:2967–2977
50. Chien YC, Masica DL, Gray JJ, Nguyen S, Vali H, McKee MD (2009) Modulation of calcium oxalate dihydrate growth by selective crystal-face binding of phosphorylated osteopontin and polyaspartate peptide showing occlusion by sectoral (compositional) zoning. J Biol Chem 284:23491–23501
51. Thurgood LA, Cook AF, Sørensen ES, Ryall RL (2010) Face-specific incorporation of osteopontin into urinary and inorganic calcium oxalate monohydrate and dehydrate crystals. Urol Res 38:357–376
52. Ljunghall S (1979) Family history of renal stones in a population study of stone-formers and health subjects. Br J Urol 51:249–252
53. Yasui T, Iguchi M, Suzuki S, Kohri K (2008) Prevalence and epidemiological characteristics of urolithiasis in Japan; national trends between 1965 and 2005. Urology 71:209–213
54. Yasui T, Iguchi M, Suzuki S, Okada A, Itoh Y, Tozawa K, Kohri K (2008) Prevalence and epidemiologic characteristics of lower urinary tract stones in Japan. Urology 72:1001–1005
55. Yamate T, Tsuji H, Amasaki N, Iguchi M, Kurita T, Kohri K (1999) Analysis of osteopontin DNA in the urine of healthy and stone-forming men. Urol Res 27:225–230
56. Gao B, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) A polymorphism of the osteopontin gene is related to urinary calcium stones. J Urol 174:1472–1476
57. Gao B, Yasui T, Itoh Y, Li Z, Okada A, Tozawa K, Hayashi Y, Kohri K (2007) Association of osteopontin gene haplotypes with nephrolithiasis. Kidney Int 72:592–598
58. Liu CC, Huang SP, Tsai LY, Wu WJ, Juo SH, Chou YH, Huang CH, Wu MT (2010) The impact of osteopontin promoter polymorphisms on the risk of calcium urolithiasis. Clin Chim Acta 411:739–743
59. Gögebakan B, Igci YZ, Arslan A, Igci M, Erturhan S, Oztuzcu S, Sen H, Demiryürek S, Arikoglu H, Cengiz B, Bayraktar R, Yurtseven C, Sarıca K, Demiryürek AT (2010) Association between the T-593A and C6982T polymorphisms of the osteopontin gene and risk of developing nephrolithiasis. Arch Med Res 41:442–448
60. Nagata T, Todescan R, Goldberg HA, Zhang Q, Sodek J (1989) Sulphation of secreted phosphoprotein 1(SPP1, osteopontin) is associated with mineralized tissue formation. Biochem Biophys Res Commun 165:234–240
61. Giachelli CM, Pichler R, Lombardi D, Denhardt DT, Alpers CE, Schwartz SM, Johnson RJ (1994) Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. Kidney Int 45:515–524
62. Kleinman JG, Worcester EM, Beshensky AM, Sheridan AM, Bonventre JV, Brown D (1995) Upregulation of osteopontin expression by ischemia in rat kidney. Ann NY Acad Sci. 760:321–323
63. Kido J, Kasahara C, Ohishi K, Nishikawa S, Ishida H, Yamashita K, Kitamura S, Kohri K, Nagata T (1995) Identification of osteopontin in human dental calculus matrix. Archs Oral Biol 40:967–972
64. Hirota S, Ito A, Nagoshi J, Takeda M, Kurata A, Takatsuka Y, Kohri K, Nomura S, Kitamura Y (1995) Expression of bone matrix protein messenger ribonucleic acids in human breast cancers, Possible involvement of osteopontin in development of calcifying foci. Lab Invest 72:64–69
65. Hirota S, Asada H, Kohri K, Tsukamoto Y, Ito A, Yoshikawa K, Xu Z, Nomura S, Kitamura Y (1995) Possible role of

osteopontin in deposition of calcium phosphate in human pilomatricomas. J Invest Dermatol 105:138–142
66. Hirota S, Nakajima Y, Yoshimine T, Kohri K, Nomura S, Taneda M, Hayakawa T, Kitamura Y (1995) Expression of bone-related protein messenger RNA in human meningiomas : possible involvement of osteopontin in development of psammoma bodies. J Neuropathol Exp Neurol 54:698–703
67. Hirota S, Imakita M, Kohri K, Ito A, Morii E, Adachi S, Kim HM, Kitamura Y, Yutani C, Nomura S (1993) Expression of osteopontin messenger RNA by macrophages in atherosclerotic plaques-A possible association with calcification. Am J Pathol 134:1003–1008
68. Zeyda M, Gollinger K, Todoric J, Kiefer FW, Keck M, Aszmann O, Prager G, Zlabinger GJ, Petzelbauer P, Stulnig TM (2011) Osteopontin is an activator of human adipose tissue macrophages and directly affects adipocyte function. Endocrinology 152:2219–2227
69. Yu PJ, Skolnick A, Ferrari G, Heretis K, Mignatti P, Pintucci G, Rosenzweig B, Diaz-Cartelle J, Kronzon I, Perk G, Pass HI, Galloway AC, Grossi EA, Grau JB (2009) Correlation between plasma osteopontin levels and aortic valve calcification: potential insights into the pathogenesis of aortic valve calcification and stenosis. J Thorac Cardiovasc Surg 138:196–199
70. Cho HJ, Cho HJ, Kim HS (2009) Osteopontin: a multifunctional protein at the crossroads of inflammation, atherosclerosis, and vascular calcification. Curr Atheroscler Rep 11:206–213
71. Higasibata Y, Sakuma T, Kawahata H, Fujihara S, Moriyama K, Okada A, Yasui T, Kohri K, Kitamura Y, Nomura S (2004) Identification of promoter regions involved in cell- and developmental stage-specific osteopontin expression in bone, kidney, placenta, and mammary gland: an analysis of transgenic mice. J Bone Miner Res 19:78–88
72. Scatena M, Liaw L, Giachelli CM (2007) Osteopontin a multifunctional molecule regulating chronic inflammation and vascular disease. Arterioscler Thromb Vasc Biol 27:2302–2309
73. Nordin BE, Need AG, Morris HA, Horowitz M, Robertson WG (1991) Evidence for a renal calcium leak in postmenopausal women. J Clin Endocrinol Metab 72:401–407
74. Heller HJ, Sakhaee K, Moe OW, Pak CY (2002) Etiological role of estrogen status in renal stone formation. J Urol 168: 1923–1927
75. Johnson RC, Leopold JA, Loscalzo J (2006) Vascular calcification : pathobiological mechanisms and clinical implications. Circ Res 99:1044–1059
76. Osako MK, Nakagami H, Koibuchi N, Shimizu H, Nakagami F, Koriyama H, Shimamura M, Miyake T, Rakugi H, Morishita R (2010) Estrogen inhibits vascular calcification via vascular RANKL system: common mechanism of osteoporosis and vascular calcification. Circ Res 107:466–475
77. Itoh Y, Yoshimura M, Niimi K, Usami M, Hamamoto S, Kobayashi T, Hirose M, Okada A, Yasui T, Tozawa K, Kohri K (2011) The role of long-term loading of cholesterol in renal crystal formation. Arch Ital Urol Androl 83:23–25
78. Buck AC, Davies RL, Harrison T (1991) The protective role of eicosapentaenoic acid in the pathogenesis of nephrolithiasis. J Urol 146:188–194
79. Yasui T, Suzuki S, Itoh Y, Tozawa K, Tokudome S, Kohri K (2008) Eicosapentaenoic acid has a preventive effect on the recurrence of nephrolithiasis. Urol Int 81:135–138
80. Yasui T, Ito Y, Okada A, Hirose M, Tozawa K, Kohri K (2005) Eicosapentaenoic acid (EPA) medically prevents the recurrence of urinary calculus. Urol Res 33:150–151
81. Schmiedl A, Schwille PO, Bonucci E, Erben RG, Grayczyk A, Sharma V (2000) Nephrocalcinosis and hyperlipidemia in rats fed a cholesterol- and fat-rich diet: association with hyperoxaluria, altered kidney and bone minerals, and renal tissue phospholipid-calcium interaction. Urol Res 28:404–415
82. Khan SR, Glenton PA, Backov G, Talham DR (2002) Presence of lipids in urine, crystals and stones: implications for the formation of kidney stones. Kidney Int 62:2062–2072
83. Taylor EN, Stampfer MJ, Curhan GC (2005) Obesity, weight gain, and the risk of kidney stones. JAMA 293:455–462
84. Taylor EN, Stampfer MJ, Curhan GC (2005) Diabetes mellitus and the risk of nephrolithiasis. Kidney Int 68:1230–1235
85. Yasui T, Itoh Y, Bing G, Okada A, Tozawa K, Kohri K (2007) Aortic calcification in urolithiasis patients. Scand J Urol Nephrol 41:419–421
86. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kihshida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y (2002) Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem 277:37487–37491
87. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. Endocr Rev 26:439–451
88. Fujii Y, Okada A, Yasui T, Niimi K, Hamamoto S, Hirose M, Kubota Y, Kojima Y, Tozawa K, Hayashi Y, Kohri K. (2012) Adiponectin ameliorates kidney stone formation in metabolic syndrome model mice by inhibition of inflammation and apoptosis (submitted)
89. Ando R, Suzuki S, Nagaya T, Yamada T, Okada A, Yasui T, Tozawa K, Tokudome S, Kohri K (2011) Impact of insulin resistance, insulin and adiponectin on kidney stones in the Japanese population. Int J Urol 18:131–138
90. Ito T, Kohri K (1996) Significance of macrophage and cytokines in expression of stone matrix. Jpn J Urol 87:865–874
91. Iguchi M, Ishikawa Y, Katayama K, Kodama M, Takada M, Katoh Y, Kataoka K, Kohri K, Kurita T, Umekawa T (1990) Dietary intake and habits of Japanese renal patients. J Urol 143:1093–1095
92. Iguchi M, Katayama K, Takada M, Katoh Y, Kataoka K, Kohri K, Kurita T (1990) Clinical effects of prophylactic dietary treatment on renal stones. J Urol 144:229–232
93. Caudarella R, Vescini F, Buffa A, La Manna G, Stefoni S (2004) Osteoporosis and urolithiasis. Urol Int 72:17–19
94. Kohri K, Ishikawa Y, Katayama Y, Takada M, Katoh Y, Kataoka K, Iguchi M, Kurita T (1991) Relationship between metabolic acidosis and calcium phosphate stone formation in women. Int Urol Nephrol 23:307–316
95. Jaeger P, Lippuner K, Casez JP, Hess B, Ackerman D, Hug C (1994) Low bone mass in idiopathic renal stone formers: magnitude and significance. J Bone Miner Res 9:1525–1532
96. Tugcu V, Ozbek E, Aras B, Ozbay B, Islim F, Tasci AI (2007) Bone mineral density measurement in patients with recurrent normocalciuric calcium stone disease. Urol Res 35:29–34
97. Senzaki H, Yasui T, Okada A, Ito Y, Tozawa K, Kohri K (2004) Alendronate inhibits urinary calcium microlith formation in a three-dimensional culture model. Urol Res 32:223–228
98. Yasui T, Itoh Y, Okada A, Hamamoto S, Hirose M, Kobayashi T, Tozawa K, Kohri K (2009) Alendronate reduces the excretion of risk factors for calcium phosphate stone formation in postmenopausal women with osteoporosis. Urol Int 83:226–229
99. Liberman UA, Weiss SR, Bröll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW, Dequeker J, Favus M (1995) Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 333:1437–1443
100. LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, Voronin L (2000) Bone mineral and lean tissue loss after long duration space flight. J Musculoskelet Neuronal Interact 1:157–160

101. Smith SM, Heer M (2002) Calcium and bone metabolism during space flight. Nutrition 18:849–852
102. Watanabe Y, Ohshima H, Mizuno K, Sekiguchi C, Fukunaga M, Kohri K, Rittweger J, Felsenberg D, Matsumoto T, Nakamura T (2004) Intravenous pamidronate prevents femoral bone loss and renal stone formation during 90-day bed rest. J Bone Miner Res 19:1771–1778
103. Okada A, Oshima H, Itoh Y, Yasui T, Tozawa K, Kohri K (2008) Risk of renal stone formation induced by long-term bed rest could be decreased by premedication with bisphosphonate and increased by resistive exercise. Int J Urol 15:630–635
104. Koul HK, Koul S, Fu S, Santosham V, Seikhon A, Menon M (1999) Oxalate: from crystal formation to crystal retention. J Am Soc Nephrol 14:417–421
105. Wüthrich Rudolf P (1998) The complex role of osteopontin in renal disease. Nephrol Dial Transplant 13:2448–2450
106. Huang HS, Ma MC, Chen CF, Chen J (2003) Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology 62:1123–1128
107. Khan SR, Johnson JM, Peck AB, Cornelius JG, Glenton PA (2002) Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. J Urol 168:1173–1181
108. Kohri K, Takada M, Imanishi M, Katoh Y, Kataoka K, Kurita T, Iguchi M (1991) Immunofluorescent study on the interaction between collagen and calcium oxalate crystals in renal tubules. Eur Urol 19:249–252
109. Azzopardi PV, O'Young J, Lajoie G, Karttunen M, Goldberg HA, Hunter GK (2010) Roles of electrostatics and conformation in protein-crystal interactions. PLoS One 19:e9330
110. Taller A, Grohe B, Rogers KA, Goldberg HA, Hunter GK (2007) Specific adsorption of osteopontin and synthetic polypeptides to calcium oxalate monohydrate crystals. Biophys J 93:1768–1777
111. Kumar V, Peňa de la Vega L, Farell G, Lieske JC (2005) Urinary macromolecular inhibition of crystal adhesion to renal epithelial cells is impaired in male stone formers. Kidney Int 68:1784–1792
112. Hirose M, Tozawa K, Okada A, Hamamoto S, Shimizu H, Kubota Y, Itoh Y, Yasui T, Kohri K (2008) Glyoxylate induces renal tubular cell injury and microstructural changes in experimental mouse. Urol Res 36:139–147
113. Okada A, Yasui T, Hamamoto S, Hirose M, Kubota Y, Itoh Y, Tozawa K, Hayashi Y, Kohri K (2009) Genome-wide analysis of genes related to kidney stone formation and elimination in the calcium oxalate nephrolithiasis model mouse: detection of stone-preventive factors and involvement of macrophage activity. J Bone Miner Res 24:908–924
114. Okada A, Yasui T, Fujii Y, Niimi K, Hamamoto S, Hirose M, Kojima Y, Itoh Y, Tozawa K, Hayashi Y, Kohri K (2010) Renal macrophage migration and crystal phagocytosis via inflammatory-related gene expression during kidney stone formation and elimination in mice: detection by association analysis of stone-related gene expression and microstructural observation. J Bone Miner Res 25:2701–2711
115. Okada A, Nomura S, Higashibata Y, Hirose M, Gao B, Yoshimura M, Itoh Y, Yasui T, Tozawa K, Kohri K (2007) Successful formation of calcium oxalate crystal deposition in mouse kidney by intraabdominal glyoxylate injection. Urol Res 35:89–99
116. Shiraga H, Min W, VanDusen WJ, Clayman MD, Miner D, Terrell CH, Sherbotie JR, Foreman JW, Przysiecki C, Neilson EG et al (1992) 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
117. Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, Liaw L, Alpers CE, Couser WG, Kleinman JG, Hughes J (2003) Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. J Am Soc Nephrol 14:139–147
118. Taguchi K, Okada A, Yasui T, Kobayashi T, Ando R, Tozawa K, Kohri K. (2012) Pioglitazone, a peroxisome proliferator activated receptor γ agonist, decreases renal crystal deposition, oxidative stress and inflammation in hyperoxaluric rats. J Urol 188:1002–1011