

Physiological and pathophysiological implications of osteopontin and the diagnostic utility of the protein in kidney diseases

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Abstract

Introduction and objective. Osteopontin (OPN) is a protein playing many pleiotropic both physiological and pathophysiological roles in various organs. The paper briefly characterizes both the positive and negative roles of OPN, with particular emphasis on the role of the protein in kidney functioning.

Brief description of the state of knowledge. As its name suggests, OPN is a protein mainly regulating bone homeostasis, but it also participates in the pathogenesis of inflammation, neoplasm, atherosclerosis and vascular calcification. OPN is also an important protein involved in kidney physiology. Its main physiological function is to act as an inhibitor of the crystallization of urine mineral components and to prevent kidney stones formation. OPN is also overproduced in various kidney pathologies (tubulointerstitial fibrosis, crescenting glomerulonephritis, cyclosporine or diabetic nephropathy, renal ischemia reperfusion injury), and both experimental and clinical studies suggest that OPN may exert either a protective or detrimental effect on kidneys.

Conclusions. Considering the OPN role in kidneys, the protein is currently proposed as one of novel laboratory biomarkers for renal function. OPN, along with the other proteins found in the urine in various kidney diseases (neutrophil gelatinase-associated lipocalin-1, kidney injury molecule-1, and fatty acid binding protein) is a part of the laboratory panel that will be gradually introduced into common laboratory practice.

Key words

osteopontin, physiology, pathophysiology, kidney diseases

INTRODUCTION

Osteopontin (OPN) is a major sialoprotein found in the bone extracellular matrix. The name of the protein is associated with its most important function in the bone – it serves as a bridge (Latin “pons”) between cells and hydroxyapatite [1]. OPN was discovered and reported for the first time in the 1980s [2–5] and originally classified as “bone sialoprotein”. Bone tissue is composed of an organic fraction called the osteoid and a mineral phase. The bone osteoid matrix mostly contains type I collagen and other minor collagens (types III and V) and also some non-collagenous proteins, including osteocalcin, osteonectin, alkaline phosphatase, bone sialoproteins, thrombospondin, fibronectin, vitronectin, dental matrix protein 1 and OPN [6–8]. Therefore, initially, OPN was considered to be one of the noncollagenous proteins responsible for matrix organization, cell signaling metabolism and mineralization of bones. However, continuing progress in research on that compound has provided evidence for the presence of OPN also in other tissues and organs beyond bones, and revealed its pleiotropic, biological properties, also in kidneys.

OBJECTIVE

The aim of this short review was to discuss issues concerning the structure and biological functions of osteopontin, with particular attention drawn to the role of OPN in kidneys, and to give rationales for assessment of the protein as a novel laboratory marker of renal dysfunction. The discussion was based on a literature research, using the Ovid/Medline database, performed on June 16th 2017. The search results were limited to full-text in English, taking into account papers published between 2000 – 2017 and using searching terms “osteopontin” and “physiology” (396 records) and “osteopontin” and “pathophysiology” (88 records). Some of the obtained records were repeated in both search results. Finally, 58 items of synthetic reviews and original papers were chosen, which – according to the author – seemed to be the most valuable papers.

Osteopontin and bone. Osteopontin, is encoded by a gene located in the long arm of the chromosome 4 and is directly related to similar genes encoding bone sialoprotein (BSP), dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP). After synthesis, the resulting protein is subjected to a post-translational modification, including phosphorylation of serine and threonine residues, tyrosine sulphation, N- and O- glycosylation (therefore, the final molecule weight varies between 44–75 kDa) [1].

OPN expression increases in response to steroids, retinoic acid, and particularly to 1,25-dihydroxyvitamin D₃. OPN synthesis is also up-regulated by many growth factors

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(epidermal growth factor, platelet-derived, transforming growth factor β) and by proinflammatory cytokines [1]. On the other hand, OPN is down-regulated by bisphosphonates, and inhibition of OPN expression is one of the important element of their pharmacodynamics. The main physiological action of osteopontin is related to its regulation of mineral deposition in bone. OPN was demonstrated to inhibit hydroxyapatite crystal growth and OPN's ability to inhibit nucleation obligatory depends on phosphorylation of the protein [9–11]. The influence of OPN on bone mineral density is an element contributing to dynamic bone homeostasis. To sum up, osseous structures are constantly varying between bone resorption (performed by osteoclasts) and bone formation (carried out by osteoblasts). Those two opposing processes are controlled by numerous hormones and paracrine compounds, including osteopontin produced in bone. OPN is demonstrated to activate the osteoclasts functions. The cells form a bone resorption zone where osteoclasts locally acidify the bone tissue, leading to its decalcification and release of proteolytic enzymes modulating the bone matrix [12]. Osteoclasts are equipped with membrane integrins $\alpha_v\beta_3$ that are thought to play an essential role in the formation of the resorption zone [13, 14]. OPN has a characteristic RGD (arginine-glycine-aspartic acid) sequence [15, 16] and also a poly-Asp motif [17, 18]. Integrins $\alpha_v\beta_3$ are receptors for OPN facilitating OPN adhesion to osteoclasts while aspartic acid-rich sequences of OPN (similarly to glutamic acid-rich sequences of bone-sialoproteins) enable hydroxyapatite binding [15–18].

OPN is a protein presenting pleiotropic properties, regulating the diverse biological processes outside of bones. Apart from osteoblasts and osteoclasts, the protein is secreted by other cells in gastrointestinal, respiratory, urogenital or cardiovascular systems [1, 18].

Expression of OPN was demonstrated in chondrocytes [19], fibroblasts [20], dendritic cells, macrophages and T-cells [21], hepatocytes [22], smooth muscle cells [23], skeletal muscle [24], endothelial cells [25], inner ear [26], brain [27], placenta and mammary glands [28] or kidney [29], and the protein may be assayed in some biological fluids, including blood, urine, milk and semen [1,18].

Osteopontin and inflammation. Osteopontin, besides its function as one of the major factor controlling bone homeostasis, also exerts a broad range of immune functions. OPN controls immune cell functions including monocyte adhesion, migration, differentiation, and phagocytosis, and acts as a pro-inflammatory mediator [1, 18, 30]. OPN is secreted into extracellular space by activated phagocytic cells and is a autocrine and paracrine chemoattractant of macrophages. OPN has also an opsonizing activity. The protein secreted by immune cells coats foreign particles and antigens, enabling their binding to superficial cell receptors located on immune cells, and subsequent phagocytosis. Moreover, OPN stimulates B-cells and production of antibodies [1]. On the other hand, OPN synthesized intracellularly, may contribute to decrease of the cytotoxic effect of macrophages due to inhibition of inducible nitrogen oxide synthase activity; therefore, OPN may act as an inhibitor of reactive nitrogen species [31, 32].

OPN plasma level is increased in many chronic inflammatory conditions [33]. OPN was also demonstrated to be involved in inflammatory-associated fibrosis [1, 34]. Therefore, the protein is one of the factors controlling wound healing. Some

experimental studies indicate impairment of the wound healing processes in the absence of OPN – formation of smaller and irregular collagen fibers and impaired removal of tissue debris have been reported. Healing was delayed with reduced presence of myofibroblasts and lower expression of transforming growth factor beta-1 [18, 35]. OPN maintains inflammation by sustaining macrophage activation, while a chronic inflammatory process is characterized by persistence of macrophages at sites of injury. OPN also modulates production of cytokines – stimulates secretion of interleukin-12 with simultaneous inhibition of interleukin-10 production [18, 21]. The rearrangement between pro-inflammatory (interleukin-12) and anti-inflammatory (interleukin-10) cytokines may contribute to shift of the dynamic balance towards the maintained inflammation [36, 37].

Osteopontin and cancer. Due to the fact that OPN is involved in the regulation of many cellular functions, alteration of OPN function is implicated in cancer pathology [38]. Cancer development results from the transformation of normal cells into cancer cells. The transformation process is conditioned by genetic and molecular abnormalities of the cellular apparatus. Because OPN is an agent exerting pleiotropic properties in the regulation of cellular functions, the protein is also regarded to be involved in the pathophysiology of cancer. OPN abnormalities were discovered in multiple malignancies, both at the stages of initiation, promotion and progression of a tumour [38].

Osteopontin and atherosclerosis. OPN is also highly expressed in atherosclerotic plaques; atherosclerosis is also a chronic inflammatory disease. In general, atherosclerosis starts with endothelial cells injury, the development of vascular lipid deposition and macrophage (foam cells) accumulation in the subendothelial space and vascular smooth muscle cells migration and proliferation [18]. The atherosclerosis plaque is susceptible to calcification (leading to increased total peripheral resistance) and may lead to rupture or aneurysm. OPN was found in atherosclerotic lesions, in association with macrophages, which suggest participation of the protein in atherosclerotic pathomechanisms. OPN expression is increased following an endothelial injury and it has been demonstrated that the protein reduces the re-endothelialization of a damaged endothelial lining by inhibition of the integrin-stimulated migration of vascular cells [18]. Moreover, OPN increases the macrophage accumulation and activation of vascular smooth muscle cells. In physiological conditions, the OPN level in intact arteries is low, and after a change of the phenotype of vascular smooth muscle cells into migration and proliferation, OPN expression is high [23]. On the other hand, however, OPN is one of the negative regulators of calcification of vascular smooth muscle cells. The protein inhibits that process binding to hydroxyapatite [18]. Therefore, OPN is regarded as a novel biomarker for atherosclerotic-associated cardiovascular disorders, including coronary artery disease. An increased OPN plasma level was observed in patients with that disease, and the protein is considered to be a laboratory marker for atherosclerosis [39].

The role of OPN in kidneys. Osteopontin was also found in developmental tissues, including kidneys. During embryogenesis, beginning on approximately day 75–80 of gestation, OPN was detected in human (and mouse) kidneys

Table 1. Osteopontin dysregulation in selected kidney diseases

Clinical entity	Short description and reference number
tubulo-interstitial fibrosis	The study revealed that wild-type rats with streptozocin-induced diabetes mellitus demonstrated increased kidney interstitial fibrosis compared to OPN-knockout ones [45]. The study investigated the expression of OPN in cisplatin-induced renal fibrosis in rats. After 35 days of cisplatin injection, a progressive renal interstitial fibrosis was observed. It was accompanied by increased mRNA level of the most potent fibrogenic factor – TGF- β 1 and increased OPN expression in the injured renal epithelial cells [46].
glomerulonephritis	Biopsies of human crescentic glomerulonephritis contained a significant number of cells that expressed osteopontin protein and mRNA, demonstrated by immunohistochemistry and in situ hybridization, respectively. The majority of the strongly osteopontin-positive cells were monocyte/macrophages [47]. Urinary OPN/creatinine ratio was assessed in four groups of children: I – with minimal change disease (MCD), II – with focal segmental glomerulosclerosis (FSGS), III – with IgA nephropathy (IgAN) and IV – in control group. A significantly higher urinary OPN/creatinine ratio was found in all the groups of children with glomerulonephritis, with the highest result in FSGS patients [48].
cyclosporine nephropathy	Animals with cyclosporine-induced nephropathy demonstrated increased tubular osteopontin expression that correlated with the infiltration of macrophages and interstitial fibrosis. OPN-knockout mice showed less cortical interstitial cell proliferation with reduction in cortical macrophage infiltration and reduction in interstitial collagen deposition [49]. Renal biopsy samples obtained before and after cyclosporine (CsA) treatment were evaluated in 18 children diagnosed with minimal change nephrotic syndrome. Interstitial fibrosis developed in two-thirds of patients after CsA treatment. OPN expression was significantly increased in the glomerular mesangium and tubules after CsA treatment, whereas increased tubular OPN expression and microvascular density was negatively related. The study demonstrated that increased OPN expression may be related to microvascular injury in human CsA nephrotoxicity [50].
diabetic nephropathy	OPN-knockout and OPN-wild-type mice were administered streptozocin to induce diabetes mellitus. OPN deletion decreased albuminuria, mesangial area, and glomerular collagen IV, fibronectin and transforming growth factor (TGF)-beta in the diabetic mice compared with their respective controls. The study showed that OPN expression in diabetic mice enhances glomerular damage, likely through the expression of TGF-beta [51]. 60 diabetic patients (divided into subgroups with diagnosed normoalbuminuria, microalbuminuria and nephritic syndrome), compared with healthy subjects were studied. It was found that diabetic microalbuminuria patients had a highly significant increase in urinary IgM, osteopontin, and podocyte levels as compared to other groups [52].
ischemia-reperfusion kidney injury	The study revealed that kidney tubular epithelial cells expressed a high level of osteopontin (OPN) in vitro and in vivo during renal ischemia and subsequent reperfusion. The kidneys of OPN-deficient mice were characterized by reduced NK cell infiltration with less tissue damage compared with wild-type mice after ischemia. [29]. Renal ischemia was induced in mice via bilateral clamping of renal pedicles for 35 min, followed by reperfusion for 24h. Anti-OPN antibody or normal saline were injected intravenously at the time of reperfusion. At 24h after renal ischemia, in anti-OPN antibody treated mice, serum levels of blood urea nitrogen and creatinine were decreased. Blockade of OPN also led to the reduced renal tissue inflammation [53].

in distal and convoluted tubules (at the apical surface of cells lining the lumen of the tubules), although there are some controversies and some studies reporting different localization. Tubular expression of the protein increases with gestational age and persists in adulthood. Most of the studies have not demonstrated the presence of OPN in proximal nephron (glomeruli, proximal tubules) [40]. However, the role of OPN in the process of nephrogenesis is ambiguous. Rogers et al. [41] removed developing kidneys from rat embryos and placed them in a culture medium which included antiserum to OPN. Under these conditions, the development of the kidney tubules was dramatically disturbed, suggesting a promotional role of OPN in tubulogenesis. On the other hand, in OPN-deficient mice, normal kidney development was observed [12]. The reason for the discrepancy in these studies is still unknown and the role of OPN in kidney tubulogenesis still requires further research.

Despite the relatively high level of mRNA of OPN in kidney tubules, concentration of the protein in the renal tissue is very low but it is detectable in higher levels in urine. Therefore, it can be concluded that OPN produced in renal tubules is exported to urine [12]. The finding is closely related to the proposed OPN physiological function as an agent preventing mineral precipitation in the supersaturated urine [12, 40]. Consistent with the above-mentioned OPN structure, the protein is regarded to inhibit formation of both calcium oxalate and calcium phosphate crystals using the poly-Asp sequence, thereby diminishing the concentration of mineral compounds in urine [42, 43]. In that light, similarly to the Tamm-Horsfall protein (uromodulin), OPN may be a potent inhibitor of crystallization and a preventive factor of nucleation, growth, and aggregation of most common crystals found in urine and in kidney stones [44].

Besides the role of OPN as a physiological modulator of nephrolithiasis, there is evidence that the protein is also involved in numerous kidney pathologies. Examples of both experimental studies and clinical entities characterized by OPN abnormalities are presented in Table 1.

Considering the broad spectrum of renal diseases in which OPN abnormalities were demonstrated, there is no a single theory explaining the role of the protein in the pathogenesis of the above-mentioned diseases. First of all, it should be mentioned that two conflicting concepts are currently considered, assuming both positive (protective) and negative (supporting development of a particular disorder) roles of the protein.

According to the first theory, OPN acts as a protective agent in some pathologies, especially those associated with high production of reactive nitrogen intermediates, which are highly reactive and tissue damaging compounds. This would explain the high level of OPN demonstrated in post-reperfusion kidney injury. The nephroprotective feature of OPN is a consequence of its ability to inhibit nitric oxide synthase, and thus to lower the nitric oxide (NO) level. NO is a source of reactive nitrogen species. Along with free oxygen species they are essential pro-inflammatory mediators [31, 32]. However, mechanisms of the beneficial effect of OPN in reducing the degree of kidney damage include also some complex anti-inflammatory influences. In the experimental study by Wolak et al. [54], the OPN-knockout mice with angiotensin II-mediated kidney injury, demonstrated no relevant macrophage infiltration and increased expression of α -smooth muscle actin, fibronectin, and transforming growth factor- β . Moreover, knockout mice demonstrated increased expression of monocyte chemoattractant protein-1; NADPH oxidase subunits such as NOX2, and

plasminogen activator inhibitor-1 compared to the wild type animals [54]. Therefore, it should be concluded that OPN seems to play an important anti-inflammatory role in some kidney pathologies, promoting the survival of cells exposed to prolonged hypoxia, decreasing the apoptosis and contributing to the regeneration of the damaged cells. On the other hand, however, the protein is also regarded as exerting a damaging effect. One of the important biological OPN features is that it is a strong chemo-attractant for macrophages, as mentioned above. Thus, OPN promotes macrophage-mediated kidney injury, and contributes to the development of chronic inflammation. Therefore, there are also reports suggesting that OPN contributes to kidney interstitial fibrosis and increases the severity of kidney damage [55–57]. The assessment of the role of osteopontin in the pathophysiology of various kidney disease requires further studies to clarify current controversies and doubts.

CONCLUSIONS

Osteopontin is a protein that is currently a subject of numerous experimental and clinical studies. The protein is involved in bone homeostasis and exerts a co-regulating effect on inflammation, carcinogenesis and atherosclerosis. OPN also plays an important, diverse role in the kidneys. In many kidney diseases of various pathogenesis, the increased intrarenal synthesis of the protein is demonstrated, which may reflect its either nephro-protective or nephro-damaging properties. The increased urinary excretion of osteopontin in the course of many renal diseases makes it a candidate for novel biomarker of kidney injury, along with other protein biomarkers, such as neutrophil gelatinase-associated lipocalin-1 (NGAL-1), kidney injury molecule-1 (KIM-1), and fatty acid binding protein (FABP) [58]. Those biomarkers are characterized by improved sensitivity and specificity, compared to the classical laboratory parameters estimating renal function (e.g. urea, electrolytes, creatinine); therefore, the introduction of the compounds for common laboratory practice in diagnosing kidney damage could be expected in the near future.

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