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Osteopontin: a novel inflammatory mediator of cardiovascular disease

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1. ABSTRACT

Osteopontin, also called cytokine Eta-1, is a multifunctional protein containing Arg-Gly-Asp-Ser (RGDS) cell-binding sequence. It interacts with αvβ1, αvβ3 and αvβ5 integrins and CD44 receptors. OPN is suggested to play a role during inflammation via the recruitment and retention of macrophages and T-cells to inflamed sites. OPN regulates the production of inflammatory cytokines and nitric oxide in macrophages. In this review, we will discuss diverse roles of OPN related to cardiovascular diseases, including atherosclerosis, valvular stenosis, hypertrophy, myocardial infarction and heart failure.

2. INTRODUCTION

Osteopontin (OPN), meaning 'bone bridge', was initially identified in 1979 as a secreted protein associated with malignant transformation. Since then, OPN has been studied extensively and shown to be associated with a range of pathological processes (1). OPN is also known as bone sialoprotein 1, urinary stone protein, secreted phosphoprotein 1 (SPP-1), Early T lymphocyte activator -1 (ETA-1), nephropontin, and uropontin. OPN is expressed in various tissues and organs including the kidney, inner ear, placenta, decidua, brain and bone marrow. OPN is involved in diverse biological and pathophysiological

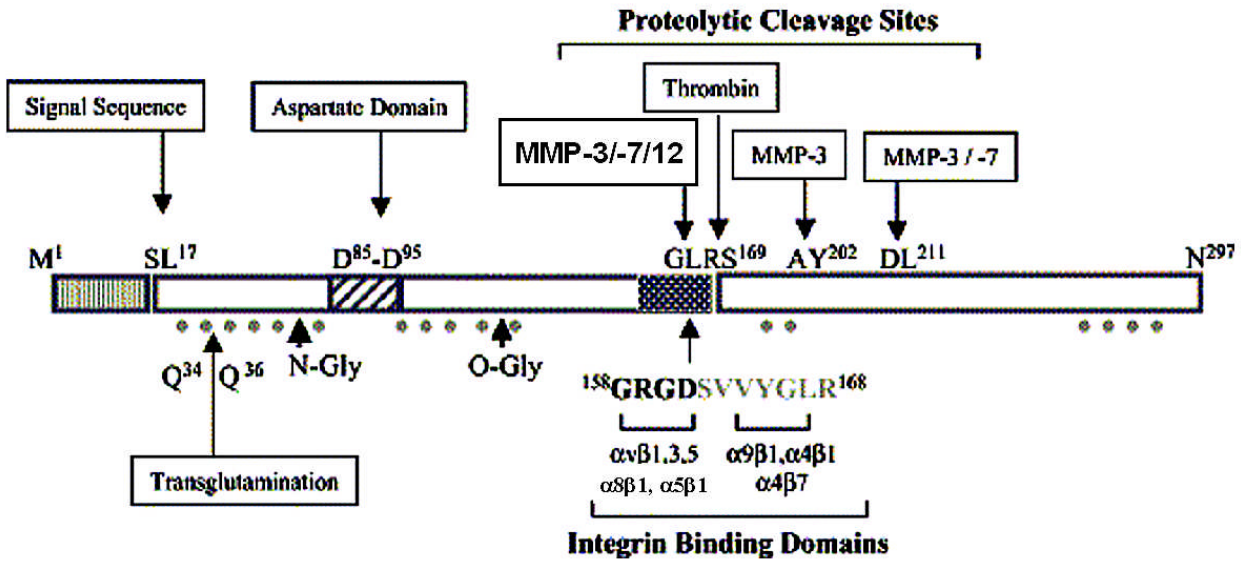

Figure 1. Schematic representation of OPN exhibiting cell-binding motifs, cleavage sites, and putative phosphorylation and glycosylation sites. *N*-gly-, *N*-glycosylation; MMP, matrix metalloproteinase; ●, phosphorylation sites. Reproduced with permission from Elsevier (1).

processes in multiple organs and tissues (2) . It is proposed as a key cytokine involved in immune cell recruitment and type-1 cytokine expression at sites of inflammation (1).

3. OSTEOPONTIN STRUCTURE AND CELL BINDING

OPN is located on human chromosome 4q13 and has 314 amino acid residues including an RGDS cell binding sequence, a calcium binding site and two heparin binding domains. OPN exhibits a high amino acid homology among different mammalian species such as mouse, rat, human, and pig (3;4) . Post-translational modifications of OPN include glycosylation and phosphorylation (Figure 1). Post-translational modifications of OPN affect its biochemical and physiological properties (5). OPN contains consensus sites for phosphorylation by casein kinases. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification (6).

OPN interacts with □v□1, □v□3, □v□5 integrins and CD-44 family of receptors in an RGD-dependent manner (1;7). The □4□1 integrins, expressed on leukocytes, are shown to interact with OPN in a dose-dependent manner through a cryptic site that becomes functional following thrombin cleavage of OPN. Other integrins such as □5□1 and □9□1 are also shown to act as OPN receptors. However, the interaction of OPN with □5□1 and □9□1 integrins occurs in a non-RGD-dependent manner.

4. ROLE IN VASCULAR DISEASE

Blood vessels express low levels of OPN under normal conditions. OPN levels increase significantly in the aorta and carotid arteries in response to balloon angioplasty and with age (8). At 4 days after carotid artery ligation, OPN-null mice exhibited diminished leukocyte adhesion/invasion. At 14 days after ligation, OPN null mice had smaller neointimal lesions but greater constrictive remodeling compared with wild-type mice, resulting in similar lumen areas. Continued remodeling resulted in a similar morphological phenotype in both groups at 28 days. Thus endogenous OPN contributes to the vascular remodeling response by regulating vascular compliance and the inflammatory response (9).

4.1. Role in angiogenesis

The process of angiogenesis involves proteolysis of the ECM. Studies from non-cardiac cells demonstrate that OPN plays a critical role in tissue remodeling by modulating angiogenesis and ECM organization. Integrins, αvβ3, are shown to play an important role in tumor formation and angiogenesis (10-12). Blockade of αvβ3 integrins by either antibodies or peptide antagonists induces apoptosis in angiogenic blood vessels (11). OPN is one of the ligands for αvβ3 integrins. Anti-OPN antibodies inhibited vascular endothelial growth factor (VEGF)-induced tube formation, and coexpression of OPN and VEGF was closely associated with angiogenesis and poor prognosis in Stage I lung adenocarcinoma (13). Implantation of millipore chambers containing OPN-transfected cells to the dorsal air sac of mice significantly induced neovascularization (14). In addition, quantitation of vessels around the bone discs in subcutaneous implantation experiments revealed impairment of angiogenesis in the absence of OPN (15). OPN-mediated recruitment of proangiogenic monocytes is also demonstrated to be a mechanism of amplification of FGF2-induced neovascularization during inflammation, wound healing, and tumor growth (16).

4.2. Role in inflammation

The development of vascular inflammation as well as macrophage activation is essential to the

development of atherosclerosis (17;18). OPN, a cell-secreted protein with pleiotropic functions, has been implicated in tissue repair, remodeling, and inflammation (19). The significance of OPN in vascular inflammation has not been entirely elucidated. It is becoming evident that OPN may be an important mediator of the inflammatory response. OPN-induced angiogenesis is paralleled by the recruitment of a massive mononuclear infiltration, thus indicating that OPN contributes to vascular inflammation (16). OPN is shown to be upregulated at the sites of inflammation due to an increase in *de novo* expression by macrophages, T cells, fibroblasts and other cell types responding to injury (reviewed in (1)). Purified osteopontin induced macrophage accumulation after injection in rat dermis and facilitated adhesion and migration of cultured macrophage-like cells in vitro, suggesting that osteopontin acts as a critical mediator of inflammation in specific disease and injury states (20).

As an anti-inflammatory mediator, OPN is identified as an inhibitor of cytokine (inteleukin-10) production, inducible nitric oxide synthase (iNOS) gene expression, and nitric oxide production in macrophages (21;22). In cardiac microvascular endothelial cells, OPN is shown to inhibit iNOS gene expression (23). It is interesting to note that nitric oxide stimulates expression of OPN in macrophages (24). Increased OPN expression may in turn inhibit iNOS transcription and decrease NO production, therefore providing a negative feedback mechanism.

4.3. Role in calcification

Dystrophic calcification of vascular tissue is a frequent complication in aging, diabetes, renal failure, atherosclerosis, aortic stenosis, and prosthetic valve replacement (25-27). Purified OPN binds calcium (28). In human carotid arteries from endarterectomy samples, OPN was found to be associated with calcium deposits in 50% of type V (fibroatheroma) and 94% of type VI lesions (36). In bovine aortic SMC cultures, purified OPN dose dependently inhibited calcification while vitronectin and fibronectin had no effect (25).

Calcium deposition is associated with the atherosclerotic process and with increased incidence of adverse events such as myocardial infarction. In vitro studies have shown that vascular tissue contains cells that can differentiate into osteoblast-like cells. Areas of cartilage are demonstrated in the myocardium of oophorectomized, aged female rats (29). Histological analysis of the heart revealed staining for several markers consistent with cartilage and bone tissue: acid phosphatase and bone matrix proteins, osteocalcin, OPN, osteonectin, and bone sialoprotein. Presence of cartilage types II, X and procollagen type I in the vascular tissue suggests endochondral calcification as a possible mechanism by which coronary calcification occurs (29). Plasma OPN levels were found to be higher in patients with calcification than in those without calcification. The increased plasma OPN levels correlated with the number of calcified segments (30), although only the phosphorylated form of OPN is shown to inhibit calcification of SMC in vitro (6).

4.4. Aneurysms/Dissections

The aorta is the vessel through which blood is ejected from the LV and delivered to systemic arterial circulation. Due to the constant exposure to high pulsatile pressure and stress, the aorta is particularly prone to injury and structural damage which may result in conditions such as aortic aneurysms or dissection of the aorta. During the formation of aneurysms, dissection of the thoracic aorta, or aortic dilatation associated with valvular disease, the transition of SMCs from the contractile to the synthetic type is important (31). Synthetic phase is characterized by increased production of substances involved in the remodeling of the vascular wall, such as components of ECM, growth factors and proteases. OPN mRNA and protein expression are shown to be increased in the synthetic phase of SMC (31). Thus, increased OPN protein levels provide a useful marker to indicate the transition of SMC from the contractile to the synthetic phenotype. Further studies are needed to examine the role of OPN in aortic aneurysms or dissection of the aorta.

5. ROLE IN HEART DISEASE

5.1. Osteopontin and valvular pathology

Calcification in vascular and valvular tissue is an actively regulated process rather than a degenerative one (27;32). Studies carried out on human aortic valves along with animal models, and *in vitro* studies demonstrated that degenerative aortic valvular stenosis is the result of active bone formation in the aortic valve (33). Presence of OPN has been detected in calcified human and bio-prosthetic aortic, and mitral valves (26;27;32;34;35). OPN and other noncollagenous proteins (osteonectin, osteocalcin) are found at higher levels in calcified valves as compared to noncalcified valves (27). OPN has been found to be strongly associated with macrophage and calcium aggregation in human mitral valves (26;34). In vivo studies using OPN null mice suggest that OPN is a potent inhibitor of ectopic calcification. Subcutaneous implantation of glutaraldehyde-fixed aortic valve leaflets indicated accelerated calcification in OPN null mice as compared to wild-type mice (37).

Calcific aortic stenosis has become the most common indication of surgical valve replacement. Recent epidemiological analysis of the risk factors leading to aortic valve disease has identified causative factors, similar to atherosclerotic vascular disease, such as smoking, sex, hypertension, and elevated cholesterol levels. Using an experimental hypercholesteremic rabbit model, Rajamannan et al., (2002) showed increased macrophages, proliferation cell nuclear antigen (PCNA) levels and bone matrix proteins including OPN in the aortic valve during the experimental hypercholesterolemia (38). These findings provide evidence of a proliferative atherosclerosis-like process in the aortic valve associated with the transformation to an osteoblast like phenotype (33;38).

Valve replacement is the second most common open-heart surgery procedure performed in the US. Within 10 yrs of valve replacement, 30-55% of patients die due to prosthetic valve complications and one-third of

bioprosthetic valves must be replaced. A revolutionary alternative to existing prosthetic valves is the tissue-engineered valve - a 'living' valve made *in vitro* from individual cellular components grown on a biodegradable architectural support. Studies have shown that aortic valve interstitial cells (VIC), which are the most prevalent valve leaflet cells, interact with OPN through integrin $\alpha_9\beta_1$ through SVVYGLR (serine-valine-valine-tyrosine-glycine-leucine-arginine) motif. Although this interaction did not stimulate migration or proliferation of VIC, it appeared to be involved in the regulation of anchorage (39). Stable endothelialization of tissue-engineered heart valve is essential for proper valve function. However, it is interesting to note that aortic valve endothelial cell (VEC) cultures adhered preferentially to fibronectin, collagen type I and IV over laminin and OPN (40).

5.2. Osteopontin and heart failure

Heart failure is one of the major public health problems in industrialized nations and is a leading cause of morbidity and mortality. In the United States, heart failure is responsible for almost 1 million hospital admissions and 40,000 deaths every year. The main etiologies of heart failure are myocardial infarction (MI), ischemia, valvular and congenital heart diseases (41).

The basal expression of OPN is low in normal adult rat heart (23). However, OPN expression has been shown to increase under various pathophysiological conditions of the heart. Increased OPN expression is observed in the myocardium of Syrian hamsters with heritable cardiomyopathy, a condition of chronic injury and repair. The source of OPN appeared to be macrophage-like cells at the sites of inflammation (42). Marked increase in OPN expression co-incident with the development of heart failure was observed in spontaneously hypertensive and aortic banded rats (43). This increase in OPN expression was inhibited by ACE inhibitor, captopril. In situ hybridyzation and immunohistochemical analyses showed increased OPN expression associated with interstitial cells and focal areas of myocyte injury. Cardiac myocytes are also suggested to be a source of OPN in the heart especially during pressure overload hypertrophy (44;45), a condition not related to inflammation and the appearance of macrophages. A significant correlation of increased OPN immunoreactivity was observed in cardiac myocytes of patients with impaired left ventricular function (46). These studies suggest that increased OPN expression is associated with cardiac cell injury and hypertrophy.

Dramatic increase in OPN and decorin gene expression was observed in desmin-null myocardium. Immunohistochemical analysis showed OPN localization in the desmin-null myocardium in areas with massive myocyte death, as well as in hypercellular regions with variable degrees of calcification and fibrosis. Osteopontin is consistently co-localized with calcified deposits, which progressively are transformed to psammoma bodies surrounded by decorin, especially in the right ventricle (47). OPN is shown to inhibit vascular calcification (25). In desmin-null heart, OPN co-localized with the areas of calcification. These studies indicate that the calcification in desmin null mice progressed either overriding the inhibitory action of OPN or OPN is in a form that cannot act as an inhibitor of calcification. The second hypothesis seems to be a rational explanation, as high-molecular weight complexes of OPN were observed in desmin null mice. The high molecular weight complexes of OPN are suggested to have increased ability to bind to collagen (48). This increased binding of OPN to collagen may serve in adhering together calcified deposits, the surrounding cells and adhesive matrix (47).

5.3. Role of osteopontin in myocardial infarction

OPN interacts with extracellular matrix (ECM) proteins such as fibronectin and collagen (48;49). The dynamic process of synthesis and breakdown of ECM proteins is suggested to play an important role in myocardial remodeling. Using myocardial infarction (MI) as a model of myocardial remodeling, marked increases in OPN expression were observed in the areas of infarct and non-infarct 3 days post MI (50). The findings in the infarct area are similar to the trans-diaphragmatic freeze-thaw injury response (51). The most important findings using MI as a model and OPN null mice were that lack of OPN resulted in greater LV chamber dilation and reduced fibrosis. An important aspect of early healing is the deposition of collagen, which stabilizes the damaged myocardium. Type I collagen content and collagen I(α1) mRNA levels were increased in wild type hearts but not in OPN null hearts (50). These data suggest that increased OPN expression after MI protects the heart against LV dilation by promoting collagen synthesis and deposition, thus playing a crucial role in the regulation of post-MI remodeling.

Increased expression of OPN is also observed in rat MI model as early as 1 day post MI. It is interesting to note that the expression of osteonectin, also a cell-secreted protein, was increased 7 days post MI and almost paralleled with that of type 1 collagen mRNA. These data suggest involvement of OPN in the early phase of repair while osteonectin may contribute to the tissue remodeling during the fibrotic stage (i.e., healing stage) (52). A time course analysis of gene expression in rat experimental autoimmune myocarditis (EAM) model showed dramatic increase in OPN mRNA during the early EAM phase (53). The EAM model in rat is similar to giant cell myocarditis in humans, recurrent forms of which lead to dilated cardiomyopathy and heart failure. These data are consistent with the findings of increased OPN expression soon after MI (52). Taken together, these studies indicate that OPN plays an important role in the early inflammatory phase by enhancing the proliferation and migration of cells, including macrophages and fibroblasts. It may also play a role in the clearance of necrotic tissue and lays the ground work before the collagen deposition and repair process begins.

Association of OPN with post-MI LV remodeling in patients is shown by the observation that plasma OPN concentrations were significantly higher in the coronary sinus than in the aortic root (54). The transcardiac gradient

of plasma OPN concentration correlated negatively with LV ejection fraction and positively with LV end-diastolic and end-systolic volume indexes suggesting that OPN is released from the heart into the coronary circulation in patients with a previous anterior wall MI in proportion to the LV systolic function and volumes.

5.4. Possible mechanisms mediating action in the heart

Myocardial fibrosis occurs as a consequence of the remodeling process initiated by pathophysiological events associated with hypertension, cardiac hypertrophy and ischemic injury. Nitric oxide (NO) is generally considered to have an important role in maintaining vascular tone and blood flow to different organs including heart. In the heart, NO is suggested to prevent fibrosis (55). OPN is demonstrated to inhibit the expression of inducible nitric oxide synthase gene in cardiac cells (23). Thus, OPN may contribute to cardiac fibrosis by modulating NO production.

Matrix metalloproteinases (MMPs), a large family of zinc-containing endoproteinases, are involved in the degradation of collagens and other ECM proteins. The expression and activity of MMPs increase in the heart post MI (56). Proinflammatory cytokines, IL-1β and TNF-α, are also increased in the heart post MI (57;58). Both IL-1β and TNF-α increase expression and activities of MMP-2 and MMP-9 in cardiac fibroblasts *in vitro* (59;60). Purified OPN protein alone does not affect expression and activity of MMPs. However, it reduces IL-1β-stimulated increases in activity and expression of MMP-2 and MMP-9 (59). Analysis of intracellular signaling pathways indicated that OPN inhibits IL-1β-stimulated increases in phosphorylation and translocation of protein kinase-C-ζ (PKC-ζ) from cytosolic to membrane fractions. These data suggest that OPN plays an important role in collagen accumulation following MI, at least in part, by inhibiting cytokine-stimulated increases in expression and activity of MMPs (MMP-2 and -9) via the inhibition of PKC-ζ. OPN is also shown to be a substrate for MMP-3 (stromelysin-1) and MMP-7 (matrilysin). The cleavage product of OPN potentiated the function of OPN as an adhesive and migratory stimulus in vitro through cell surface integrins, suggesting that interaction of MMPs with OPN may be a mechanism of regulation of OPN bioactivity (61). MMP-12 has also been suggested to cleave OPN (62).

Ang II, a member of the renin-angiotensin-aldosterone system, plays a vital role in the cardiac remodeling process which involves hypertrophy, fibroblast proliferation, extracellular matrix production, and progressive interstitial fibrosis (63;64). Ang II stimulates norepinephrine from cardiac nerve endings and endothelin from endocardial and endothelial cells. Norepinephrine and endothelin are potent hypertrophic factors for cardiac myocytes (63). Ang II increases OPN expression in cardiac fibroblasts (23;64). It is interesting to note that proinflammatory cytokines (IL-1β and TNF-α) act synergistically with Ang II to increase OPN mRNA levels in cardiac fibroblasts (65). OPN modulates Ang-II-induced collagen gel contraction in cardiac fibroblasts (64). Ang II-mediated increases in DNA synthesis and collagen gel contraction by cardiac fibroblasts were inhibited by antibodies against OPN, β_3 integrins or the RGD-peptide. These data suggest that OPN is an important mediator of Ang II-stimulated regulation of cardiac fibroblast behavior in the cardiac remodeling process.

Recent studies show that selective aldosterone blocker, eplerenone, or aldosterone abalation by adrenalectomy attenuate Ang II-induced myocardial necrosis in nitric oxide deficient rats, indicating a role for aldosterone in Ang II-induced myocardial injury (66). Aldosterone combined with a high salt diet in uninephrectomized rats induces severe coronary inflammatory lesions, which are characterized by monocyte/macrophage infiltration resulting in focal ischemic and necrotic changes. Aldosterone infusion increases expression of cyclooxygenase-2 (COX-2), proinflammatory cytokines, macrophage chemoattractant protein-1, and OPN (67;68) . The aldosterone antagonist, eplerenone, decreased the expression of these proinflammatory molecules in the heart and subsequent vascular and myocardial damage. Further studies on Ang II-induced hypertension in rats reinforced the view that aldosterone plays a vital role in vascular inflammation in the heart involving OPN and COX-2 as potential mediators. In OPN null mice, chronic infusion of Ang II resulted in reduced cardiac fibrosis, impairment of cardiac systolic function and subsequent left ventricular dilation (69;70). Aldosterone infusion also increased LV chamber diameter with reduced fibrosis and contractile function in mice lacking OPN (71).

Collectively, the above observations suggest that increased OPN expression in the heart protects against LV dilation possibly by promoting fibrosis. However, ACE inhibition, and β-adrenergic receptor blockade reduce fibrosis and improve survival, suggesting that increased collagen deposition and fibrosis are detrimental for the heart. The observations in OPN null mice that lack of OPN leads to reduced fibrosis and LV dilation are similar to mice overexpressing MMP-1. Overexprssion of MMP-1 in the heart induces loss of cardiac interstitial collagen coincident with marked deterioration of LV systolic and diastolic function (72) These studies indicate that the relationship between cardiac function and fibrosis may not be that simple. It may depend on the level and types of ECM proteins involved in cardiac fibrosis.

6. CLINICAL RELEVANCE/SIGNIFICANCE

OPN may have multiple clinical implications in the cardiovascular system. As previously mentioned, inhibition of OPN has been shown to lead to an increase in vascular compliance which in turn may lead to decreased afterload on the heart (9). Plasma OPN levels are associated with the presence and extent of coronary artery disease suggesting that OPN plays a critical role in the development of atherosclerotic plaques (30). Of note, Ang II-accelerated atherosclerosis, and aneurysm formation is attenuated in mice lacking OPN (18). The most frequent indication for valvular replacement surgery is calcific aortic stenosis. OPN may help in slowing valvular stenosis due to

calcification and possibly reversing the calcification and thereby preventing the late complications of aortic stenosis (33;37;38). In heart failure, OPN levels may lead to a new marker in evaluating the level of left ventricular function (46). MI is one of the leading causes of death. As discussed, role of OPN in the repair process after MI may lead to a better understanding of when post-MI medications should be started so as not to disturb the natural protective mechanisms of the body.

7. CONCLUSIONS

OPN has a vast number of functions ranging from an extracellular matrix protein involved in fibrosis, calcification, and wound repair to a cytokine involved in mediating immune cell recruitment such as leukocytes and macrophages at sites of inflammation. OPN has been shown to be involved in multiple disease states of the cardiovascular system, such as atherosclerosis, valvular stenosis, and heart failure. The precise role of OPN in these disease states is still unclear.

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