Osteopontin in Vascular Disease: Friend or Foe?

Zoe Shin Yee Lok, MBBS¹,² and Alicia N. Lyle, PhD¹,#

¹Department of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, GA
²Department of Surgery, School of Clinical Sciences, Monash Health, Clayton, Australia

Abstract

Inflammatory cytokines are necessary for an acute response to injury and the progressive healing process. However, when this acute response does not resolve and becomes chronic, the same proteins that once promoted healing then contribute to chronic inflammatory pathologies, such as atherosclerosis. Osteopontin (OPN) is a secreted matricellular cytokine that signals through integrin and CD44 receptors, is highly upregulated in acute and chronic inflammatory settings, and has been implicated in physiologic and pathophysiologic processes. Evidence from the literature suggests that OPN may fit within the “Goldilocks” paradigm with respect to cardiovascular disease, where acute increases are protective, attenuate vascular calcification, and promote post-ischemic neovascularization. In contrast, chronic increases in OPN are clinically associated with an increased risk for a major adverse cardiovascular event and OPN expression is a strong predictor of cardiovascular disease independent of traditional risk factors. With the recent finding that humans express multiple OPN isoforms as the result of alternative splicing and that these isoforms have distinct biologic functions, future studies are required to determine what OPN isoform(s) are expressed in the setting of vascular disease and what role each of these isoforms plays in vascular disease progression. This review aims to discuss our current understanding of the role(s) of OPN in vascular disease pathologies using evidence from in vitro, animal and clinical studies. Where possible, we discuss what is known about OPN isoform expression and our understanding of OPN isoform contributions to cardiovascular disease pathologies.

Keywords

Vascular Disease; Extracellular Matrix; Inflammation; Osteopontin; OPNa; OPNb; OPNc; Peripheral Vascular Disease; Atherosclerosis; Biomarkers; Inflammation

1. Introduction

Osteopontin (OPN) is a secreted multifunctional glyco-phosphoprotein that plays important roles in physiological and pathophysiological processes. As the name implies, OPN is produced by cells involved in bone morphogenesis and one major physiological role of OPN...
is in the control of biomineralization and calcification. In this brief review, we aim to examine OPN functions specifically in the vasculature and additional physiological functions of OPN have been reviewed previously. Under physiologic conditions, circulating and tissue OPN expression levels in the vasculature are quite low, but are important for normal arterial physiology. Indeed, these low OPN expression levels were shown to be necessary for normal arterial mechanics and it is also known that OPN acts as a physiological inhibitor of vascular calcification. In response to injury, OPN is acutely upregulated and promotes cell adhesion, proliferation, migration and survival to aid in the healing process, but expression typically resolves over time. Vascular cell types that upregulate and secrete OPN include endothelial cells, vascular smooth muscle cells (VSMCs), and macrophages. In contrast, OPN expression levels remain elevated in several disease pathologies with a chronic inflammatory component including Crohn’s disease, multiple sclerosis and other autoimmune disorders, wound healing, various cancer types and cardiovascular disease (CVD) pathologies.

More recently, there has been considerable interest in OPN as a biomarker for various pathological conditions. Peripheral blood and cerebrospinal fluid concentrations of OPN are elevated in multiple sclerosis patients and neurodegenerative diseases like Alzheimer’s. There is also interest in OPN as a prognostic and diagnostic marker for diseases including, but not limited to, multiple sclerosis, coronary artery disease, and several cancer types with a recent specific focus on individual OPN isoforms for this purpose. Increased OPN expression is also a strong predictor of outcomes in patients with calcific aortic valve disease and ischemic vascular pathologies including stroke, myocardial infarction, and peripheral artery disease. While interest in OPN as a potential biomarker increases, there is a large body of evidence that clearly establishes that OPN is specific driver of cellular functions that impact physiologic and pathophysiologic processes in the vascular setting, as discussed in more detail below, and ultimately point to OPN as a potential therapeutic target. In this review, we will examine our current understanding of OPN protein structure and function, regulation of OPN expression, and role(s) of OPN in vascular disease pathologies while introducing what is currently understood about OPN isoform contributions.

2. Osteopontin Structure and Function

Osteopontin was first described in 1985 by Franzen, et al. as one of two sialoproteins derived from bovine bone matrix. Osteopontin was previously identified as secreted phosphoprotein 1 (SPP1), bone sialoprotein 1 (BSP-1), and early T-lymphocyte activation-1 (Eta-1). This plurality of names reflects the range of functions attributable to OPN. As a matricellular protein, OPN differs from the structural extracellular matrix proteins, such as collagen, in that it does not serve a primary structural role. Matricellular proteins do, however, function as modulators of cell-matrix interactions, often achieved by binding to cell-surface receptors, growth factors, proteases, and structural matrix proteins, making them important components of the extracellular matrix environment. Indeed, matricellular proteins like OPN are often induced during tissue remodeling and repair, as well as in disease states. The original members of the matricellular protein family included secreted.
protein acidic and rich in cysteine (SPARC), thrombospondin 1 (TSP-1), and tenascin-C and has expanded to include CCN proteins (Cyr61, CCN2, CCN3) and OPN.23

**Osteopontin Receptor Binding Domains**

OPN contains several functional domains that allow for receptor binding to promote various biological functions and include: 1) Arg-Gly-Asp (RGD) binding domain that allows interaction with integrin receptors including: αvβ1, αvβ3, αvβ5, αvβ6, αvβ1,25 2) SVVYGLR domain that interacts with α9β1, α4β1, and α4β; 3) ELVTDFTDLPAT domain reported to bind to α4β1,26 4) calcium binding domain (aa 216–228) and 5) heparin binding domain. Furthermore, OPN has been shown to interact with several splice variants of the hyaluronic acid receptor, known as CD44, via the C-terminal calcium binding domain including: CD44v3 and CD44v6–7. Katagiri et al. suggested that multiple CD44 binding domains are present in the N- and C-terminal regions of OPN.27 This same study proposed a potential complex between CD44 variants and integrin receptors, since the binding of CD44 variants to OPN was inhibited by anti-β1 antibodies.27 OPN has primarily been described as a secreted protein; however, an intracellular form of OPN, made possible by a non-AUG alternative start codon that omits the N-terminal secretion peptide,28 has been reported in rodents and localizes to the cell membrane where it binds to CD44 to regulate cell migration.29,30

**Post-translational Modifications of Osteopontin**

OPN is a complex protein that is aspartic-acid rich, contains long stretches of negatively charged sequences that bind calcium, and can be cleaved by thrombin and matrix metalloproteinases (MMPs). OPN is subjected to numerous post-translational modifications (PTMs) including serine/threonine phosphorylation, glycosylation, and tyrosine sulfation, which increase the monomeric molecular weight from the predicted ~35 kDa to 41–75 kDa.31 A polymeric form of OPN with a mass of >200 kDa can also be generated upon protein transglutamination.32 Importantly, it has been shown that many of these PTMs regulate and alter OPN function.

**Phosphorylation**—The OPN sequence contains 36 predicted phosphorylation sites that include serine, threonine and tyrosine residues.33 The serine/threonine phosphorylation of OPN has been primarily attributed to a golgi apparatus casein kinase over casein kinases-1 and −2,34 identified recently as FAM20C, which phosphorylates numerous secreted proteins with S-x-E motifs.33,35 The distinct functional properties of OPN are dependent, in part, on the nature and extent of phosphorylation. Interestingly, Christensen et al. demonstrated that the degree of OPN phosphorylation can be cell-type specific, where they showed a osteoblast cell line (MC3T3-E1) added 21 phosphates, while only ~4 phosphates were added by a ras-transformed fibroblast cell line (275–3-2).36 This same study demonstrated that fibroblast-derived OPN (low phosphorylation) mediated greater adherence of human breast cancer cells than the highly phosphorylated osteoblast-derived OPN.36 Therefore, OPN generated by different cell types and, thus, differentially phosphorylated may exhibit different biologic effects. Indeed, phosphorylated OPN can inhibit calcification in cultured human VSMCs, which was not observed with recombinant bacterial OPN that lacks PTMs.37 Additionally, OPN phosphorylation is necessary for interleukin-12 expression in.
macrophages, while dephosphorylation abolishes this effect. The differences in PTMs made by vascular wall cell types, such as endothelial cells and VSMCs, remains to be established. Furthermore, there is a lack of understanding with respect to how OPN phosphorylation patterns change in physiologic vs. pathophysiologic settings and the few studies that have explored this in the setting of CVD are discussed below.

**Glycosylation**—Exon 6 of human OPN contains five O-glycosylation sites. Glycosylation influences the folding structure, proteolytic cleavage and, subsequently, the functional properties of OPN. Indeed, deletion of multiple O-glycosylation sites in OPN affects cell adhesion activity and phosphorylation state. Similarly, lung cancer cells that stably expressed an OPN mutant lacking three O-glycosylation sites exhibit a reduction in cell growth and migration. To our knowledge, N-glycosylation has so far only been reported in rat and human bone OPN and no apparent N-linked oligosaccharides were found in human milk OPN, suggesting that this form of glycosylation may be tissue specific.

**Transglutamination**—Osteopontin can serve as a substrate for transglutaminase 2 (TG2). TG2 is a ubiquitously expressed calcium-dependent enzyme that catalyzes cross-linking of glutamine and lysine residues. TG2 cross-linking of OPN can be both inter- and intra-molecular. TG2-mediated OPN polymerization alters both conformation and function, which is attributed to the exposure a new integrin binding site that allows polymeric OPN to bind the α9β1 receptor independent of the SVVYGLR sequence. Other *in vitro* studies show that polymeric OPN displays increased collagen type I binding affinity and promotes enhanced cell adhesion and migration compared to monomeric OPN. The significance of OPN polymerization *in vivo* was highlighted by Nishimichi et al., who demonstrated OPN polymerization is required for neutrophil recruitment. Originally, two TG2 reactive glutamine (Gln) residues were described in bovine exon 4, Gln34 and Gln36, which correspond to Gln50 and Gln52 in OPNa when designating the initial methionine (Met) residue as aa 1; however, OPN was recently reported to have 12 TG2 reactive residues, with Gln34, Gln42, Gln193 and Gln248 (corresponding to Gln50, Gln58, Gln209, and Gln264 in OPNa) exhibiting the highest reactivity. This could explain why polymerization of OPN-c, a splice variant lacking exon 4 and two glutamine residues, could still be observed with high TG2 concentrations.

**Cleavage**—In addition to PTMs, OPN can also undergo proteolytic cleavage by thrombin and MMPs. A conserved thrombin cleavage site at Arg-Ser can be found seven amino acids downstream of the RGD integrin receptor binding site. OPN function is altered when cleaved by thrombin, which generates N-terminal and C-terminal fragments. The cryptic SVVYGLR motif, just C-terminal to the RGD site, also allows OPN to interact integrin receptors, including: αvβ1, αvβ1 and αvβ3 integrin receptors. Recently, it was shown that phosphorylation of the C-terminal fragment could inhibit OPN binding to αvβ3. This could potentially explain the conflicting findings by various studies suggesting that thrombin cleavage was not a prerequisite for adhesion to αvβ3, αvβ5, and αvβ6 as they could bind

---

*Note: Amino acid numbers identified in this review are identified using the initial methionine (Met) residue as aa 1 and, therefore, differ from some publications in which the first residue after the 16 aa signal peptide is cleaved off, aa 17 Isoleucine, as aa 1.
equally well to full-length OPN or to the RGD sequence at its N-terminal end. Several members of the MMP family have also been reported to cleave OPN at various sites. These include MMP-2, MMP-3, MMP-7, MMP-9 and MMP-12. Interestingly, cleavage of OPN by MMP-3 and MMP-7 within the SVVGLR motif sequence interferes with $\alpha_4\beta_1$ and $\alpha_9\beta_1$ binding. Evidence points to OPN fragments generated by thrombin and MMP cleavage having important pathophysiological functions. For instance, the N-terminal OPN fragment is associated greater degrees of inflammation in carotid plaques in patients with hypertension (HTN). Additionally, the thrombin-cleaved N–terminal fragment with SVVYGLR motif promotes synthesis of collagen type III in cardiac fibrosis. And finally, several in vitro studies have shown that the N-terminal fragment of OPN, rather than full length OPN, promotes adhesion due to a conformational change that increases its binding activity.

Osteopontin Isoforms—Humans express five OPN isoforms due to alternative splicing of a single SPP1 mRNA transcript to generate: 1) full length OPN, known as OPNa (NP_001035147.1), 2) OPNb, which lacks exon 5 (NP_000573.1), 3) OPNc, which lacks exon 4 (NP_001035149.1), 4) OPN4, which lacks exons 4 and 5 (NP_001238758.1), and 5) OPN5, which contains an extra exon due to the retention of a portion of intron 3 (NP_001238759.1). Whether OPN4 and OPN5 are translated to protein remains to be determined; therefore, in this review we focus on OPNa, OPNb and OPNc. OPN splice variants were first described in glioma cells, are differentially expressed, and display isoform-specific biologic functions. The RGD and SVVYGLR integrin receptor binding domains and the CD44 binding domain are conserved across all three isoforms. Despite intact receptor binding domains, OPN isoforms clearly exhibit different intrinsic biological functions. Isoforms differ in that OPNb lacks three serine/threonine residues and one glutamine residue due to deletion of exon 5, whereas OPNc lacks two tyrosine, two serine/threonine, and three glutamine residues due to deletion of exon 4. Whether these missing exons lend to changes in protein folding, as suggested by predictive modeling, and/or receptor binding remains to be investigated and additional studies are required to define the molecular mechanisms underlying OPN isoform-specific biological effects. With a growing interest in the roles OPN isoforms may play in cardiovascular physiology and pathophysiology, we will discuss what is established thus far in this review.

3. Osteopontin in Vascular Physiology and Pathophysiology

Osteopontin in Acute and Chronic Ischemia

Inflammation is a central component of numerous diseases and can promote tissue damage and inhibit healing if unresolved; however, it is also well understood that immune suppression can limit successful tissue regeneration and recovery of homeostasis. Osteopontin is a secreted protein that is highly upregulated in settings of both acute and chronic ischemia and functions, in part, as an inflammatory cytokine that can promote recruitment of multiple inflammatory cell types and, thus, modulate the inflammatory response. In this section, we will discuss OPN and the “Goldilocks” principle using ischemia-mediated neovascularization as a model, since OPN expression is upregulated in
response to ischemia in stroke, myocardial infarction, and peripheral artery disease.

OPN expression is often upregulated 20–50 fold in response to ischemic insult, but typically resolves over time in murine models of stroke, myocardial infarction, and hindlimb ischemia. Studies have established that OPN is a clear driver of the immune response in ischemic conditions, demonstrating that OPN is necessary for macrophage infiltration in vivo. Indeed, OPN is required for post-ischemic neovascularization and ischemia-induced OPN expression is reactive oxygen species (ROS)-dependent. More recently, it was established that OPN isoforms have differential effects on macrophage migration and accumulation and arteriogenesis in vivo, where OPNc was the most potent mediator of these processes. With respect to macrophage function, phagocytosis is diminished in OPN−/− macrophages, but can be rescued by recombinant OPN. Pharmacological inhibition or genetic ablation of OPN has also been found to greatly impair macrophage infiltration in various models of acute inflammation. Furthermore, OPN induces macrophage migration via interaction of C-terminal fragment with CD44 surface receptors and more recently, via SLAYGLR domain (SVVYGRGL in human OPN) with α4 and α9-integrin receptors. Effects of OPN on macrophage polarization remain controversial. While one group reported OPN knockdown polarizes macrophages toward an M2c subtype, recently accepted as a pro-regenerative phenotype, another study using OPN−/− macrophages showed no effects on polarization. Similarly, we did not observe differences in OPN−/− macrophage polarization, nor did macrophage polarization shift in response to stimulation with purified recombinant human OPN isoforms, suggesting OPN isoforms do not differentially effect polarization of macrophages. One recent study reports that CD206+ macrophages strongly express OPN and suggests that specific macrophage subtypes involved in tissue repair may differentially express OPN, which requires further investigation. Another point that requires further investigation is whether macrophages at different points within the polarization spectrum respond differently to OPN.

Many of the stimuli known to promote OPN expression including ROS, angiotensin II (Ang II), high glucose and low oxygen tension also contribute to chronic vascular inflammation that, when unresolved, promote long-term, chronic expression of OPN. Recently, oxidized low-density lipoprotein was also shown to promote proliferation and migration of human coronary artery SMCs via upregulation of OPN and MMP-9. Additionally, hypoxia and hyperglycemia synergistically increase OPN expression in VSMCs. This is in line with clinical findings that show that OPN is highly and chronically upregulated in patients with peripheral artery disease and in patients with type 1 and type 2 diabetes mellitus and several studies establish OPN as a clear predictor of cardiovascular outcomes in these patient populations. Interestingly, a recent study demonstrated that high glucose-mediated OPN expression is mediated, in part, through changes in histone acetylation and methylation regulated by histone deacetylase and histone methyltransferase, respectively, which was reversed by histone methyltransferase inhibition. This suggests that high glucose-mediated OPN expression is regulated in some cells through epigenetic mechanisms; however, further studies are required to determine if this is true in vascular cells. Collectively, these findings point towards OPN as an integral part of the first line immune response to tissue injury and the tissue remodeling processes required for healing. However, in disease states in which the
inflammatory process fails to resolve and becomes chronic, OPN may be detrimental, as discussed further below.

**Osteopontin in Atherosclerosis and Neo-intimal Hyperplasia**

Atherosclerosis is characterized by a persistent inflammatory response in the vascular wall in response to noxious stimuli such as hypoxia, endothelial injury and hyperglycemia. The role of OPN in atherosclerotic plaque progression has been shown in human and murine vascular diseases and was reviewed in detail by Wolak. High concentrations of OPN are observed in human atherosclerotic plaques in the aorta, carotid and coronary arteries, and are primarily expressed in endothelial cells, macrophages and VSMCs. In a high fat diet-induced mouse model of atherosclerosis, OPN overexpression significantly increased fatty-streak and mononuclear cell rich lesion formation, as well as decreased levels of interleukin-10, an anti-inflammatory atheroprotective cytokine. Likewise, Matsui et al. reported that OPN−/− mice had significantly smaller atherosclerotic lesions, but this was only true in female OPN−/− mice and the authors predominantly attributed this to higher triglyceride and total cholesterol levels in male OPN−/− mice. Worth noting, estrogen has been shown to induce OPN expression while testosterone was shown to suppress it. However, there are conflicting reports in the literature of sex differences in OPN expression, which may be disease and/or context specific and this requires further investigation. In an Ang II-accelerated model of atherosclerosis and abdominal aortic aneurysm (AAA) formation, Brummer et al. showed that a partial or complete lack of OPN protects against atherosclerosis, partially due to reduced inflammatory macrophage accumulation and viability in atherosclerotic lesions. When Zheng et al. used a microarray to compare gene expression profiles in normal aortas and tissues from abdominal aortic aneurysm patients, they found that OPN mRNA is upregulated as much as 125 fold. In this same study, the authors demonstrate that stimulation with high doses of OPN (200 – 500 ng/mL) promotes increased autophagy, whereas lower doses of OPN have pro-survival effects; however, the source of OPN used in these studies was not disclosed, suggesting that further studies are required to determine if cellular source, dose, PTMs, and/or isoforms are important for the survival vs. autophagy effects of OPN. In clinical studies, increased plasma OPN expression levels are associated with the presence and severity of coronary artery disease. Interestingly, coronary revascularization and Ang II receptor blockers reduce plasma OPN levels, which is in line with in vitro studies that demonstrate that Ang II induces OPN expression. Several clinical studies have also independently demonstrated that the use of plasma OPN expression levels alone can predict CVD events and all-cause mortality commensurate with other atherosclerosis prognostic markers including lipid profile and hsCRP in patients with HTN, type 1 diabetes, and type 2 diabetes.

In addition to atherogenesis, OPN also contributes to the development of neo-intimal hyperplasia following vessel revascularization. Clinical studies have shown that higher baseline OPN levels are associated with rapid coronary plaque progress and in stent restenosis. OPN mRNA and protein were highly expressed during neo-intimal formation in mouse and human carotid vessels. In a rat carotid artery injury model, blockade of OPN before endothelial denudation by balloon catheter decreases neo-intimal thickening of the artery. Similarly, a study by Isoda et al. demonstrated increased neo-intimal...
thickening after femoral artery cuffing in OPN transgenic mice. More recently, OPN was found to be significantly upregulated over the first postoperative week in the porcine venous wall after saphenous-vein artery interposition graft. OPN expression also correlated well with the number of PCNA-positive cells and MMP expression, suggesting OPN is a key regulator of VSMC proliferation and migration. It is well-established that mature, differentiated VSMCs can dedifferentiate into a more proliferative, synthetic phenotype during vascular remodeling and OPN appears to downregulate two differentiation markers, α-SM actin and calponin in VSMCs. However, neither SM22-α nor tropomyosin marker expression was altered with overexpression of OPN in vitro hence, further studies will be required to understand the mechanism by which these genes are regulated by OPN.

Osteopontin in Vascular Calcification

Vascular calcification was once considered an end-stage, degenerative process of aging. However, calcification is now recognized as an active, tightly-regulated biomineralization process that may be treatable. One proposed major mechanism that drives vascular calcification is the loss of mineral inhibiting factors, such as matrix G1a (MGP) and osteoprotegerin. OPN is also an important inhibitor of mineral deposition in the vascular wall and in cardiac valves. In vitro, OPN−/− VSMCs calcify significantly more than wild-type VSMCs in the presence of elevated phosphate. Furthermore, the aortas of MGP−/−OPN−/− mice exhibit 2 and 3 fold more calcification at 2 and 4 weeks, respectively, compared to mice only lacking MGP. Several in vivo subcutaneous implantation models using OPN−/− mice have demonstrated an inhibitory role for OPN in vascular calcification. A study by Steitz et al. clearly demonstrated that aortic valve leaflets subcutaneously implanted in OPN−/− mice showed accelerated calcification (4 – 5 fold greater) compared to wild-type mice and, conversely, that increased OPN and carbonic anhydrase II accumulation correlates with calcification regression. These data were corroborated by another study that showed increased calcification of subcutaneously implanted bovine pericardium tissue in OPN−/− mice, which was mitigated by the administration of histidine-fused OPN at the implant site or adsorption of the OPN onto the implant materials. Interestingly, calcification was only reversed by OPN containing a functional RGD-motif and that was adequately phosphorylated. A separate, independent study showed that only phosphorylated OPN can inhibit calcification in cultured human VSMCs. More recently, OPN levels were shown to be elevated in asymptomatic calcific aortic valve disease patients; however, a separate study from the same group showed this circulating OPN is dephosphorylated. Importantly, OPN isoforms were differentially expressed during calcific aortic valve disease progression and functioned to inhibit bio-mineralization, but only when phosphorylated. Altogether, these data support that OPN inhibits calcification and promotes dissolution, however, OPN phosphorylation is critical for these effects.

Osteopontin in Hypertension

The vessel wall undergoes remodeling in response to elevated pressure and pulsatile flow. OPN expression is elevated in HTN, which is mediated, in part, by increased aortic strain and ROS production. Indeed, overexpression of catalase in VSMC in vivo reduced mechanical strain mediated OPN expression in hypertensive animals. One of the mechanism by which mechanical strain increases OPN expression in VSMCs is through
activation of the phosphatidylinositol-3 kinase/Akt1 signaling pathway. Ang II, a peptide hormone that causes vasoconstriction and increases blood pressure, promotes HTN and has been shown to upregulate OPN expression, in part, via Ang II-mediated increases in ROS production. Vessel remodeling in HTN involves inflammatory cell infiltration, as well as MMP-mediated degradation and reorganization of the extracellular matrix. OPN has been linked to HTN-related vascular remodeling and inflammatory cell recruitment. Indeed, early macrophage infiltration into the vascular wall in response to HTN is blunted in OPN−/− mice compared to wild-type animals. Finally, OPN expression levels are significantly higher in plasma and aortic tissues in hypertensive rodents and expression positively correlates with systolic blood pressure, suggesting that OPN could be used as a clinical marker for HTN-induced vascular remodeling. One such study showed that treatment with Ang II blocker and statins significantly reduces plasma OPN level.

Clinical Implications and Conclusions—Inflammation is a central component of numerous diseases and the discovery of inflammatory biomarkers highly predictive of CVD has the potential to improve targeted treatment strategies. Biomarkers are also of great interest because they can be utilized for diagnostic, prognostic and/or therapeutic purposes in the clinical setting. As a secreted protein, OPN is of particular interest as a biomarker because of its detectability in body fluids that include plasma, urine, breast milk, and cerebrospinal fluid; thus, OPN is measurable by minimally invasive means and this allows for rapid repeated measures over time. Recent clinical studies have demonstrated that OPN expression levels are a strong predictor of CVD events and mortality in several patient populations and may prove to be a useful prognostic for disease activity and severity. The recent finding that humans express multiple isoforms that have different functional effects has added a new layer of complexity to this protein and much remains to be discovered regarding the function of the individual OPN isoforms in vascular physiology and pathophysiology, what mediates OPN splicing, and if specific isoforms are possible therapeutic targets. Indeed, it was shown recently that OPN isoforms are differentially upregulated in patients with end-stage heart failure, where OPNa was significantly upregulated in patients with dilated cardiomyopathy, while expression of OPNb and OPNc were only detected in patients with ischemic cardiomyopathy. Therefore, additional studies are required to determine if individual OPN isoforms are better diagnostic and/or prognostic biomarkers of CVD severity than total OPN levels.

Substantial progress has been made recently toward our understanding of the biological functions of OPN in several vascular disease pathologies. The cellular sources of OPN have been identified, which has led to the discovery of many of its important cell- and tissue-type specific functions. Evidence presented in this review suggest that the role of OPN in vascular disease may follow the Goldilocks principle, with too little OPN impeding the tissue injury and wound healing responses, while too much OPN leads to deleterious vascular remodeling. We also require a better understanding of how specific vascular cell types differentially post-translationally modify OPN and if and how these modifications vary with disease state. Additional work is also necessary to determine the underlying molecular mechanisms of OPN isoform-specific biologic functions. The future development of “humanized” transgenic animals and isoform specific tools should greatly facilitate this
work and will further our understanding of the physiologic and pathophysiologic roles of these newly defined OPN splice variants.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

**Sources of Funding**

Supported by National Institutes of Health K99/R00 HL119567 (ANL), a Georgia Clinical and Translational Science Alliance (Georgia CTSA) pilot award through the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR002378 (ANL), and funds from Emory University’s Department of Medicine and Division of Cardiology (to ANL).

**Non-Standard Abbreviations and Acronyms**

- **Ang II**: Angiotensin II
- **CVD**: Cardiovascular Disease
- **HTN**: Hypertension
- **MMP**: Matrix Metalloproteinases
- **OPN**: Osteopontin
- **PTM**: Post-translational Modification
- **RGD**: Arginine-Glycine-Aspartate
- **ROS**: Reactive Oxygen Species
- **TG2**: Transglutaminase 2
- **VSMC**: Vascular Smooth Muscle Cells

**REFERENCES**


77. Gordin D, Forsblom C, Panduru NM, Thomas MC, Bjerre M, Soro-Paavonen A, Tolonen N, Sandholm N, Flyvbjerg A, Harjutsalo V, Groop PH and FinnDiane Study G. Osteopontin is a


Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2020 April 01.


Highlights

- Osteopontin (OPN) is a strong predictor of outcomes in patients with calcific aortic valve disease and ischemic vascular pathologies including stroke, myocardial infarction, and peripheral artery disease.
- Evidence clearly establishes that OPN is *driver* of cellular functions that impact physiologic and pathophysiologic processes in the vascular setting including cell survival, adhesion, migration and proliferation.
- Humans express multiple OPN isoforms, which have distinct biologic functions, and further investigation is required to determine what OPN isoform(s) are expressed in the setting of vascular disease and what role each isoform plays in vascular disease progression.
Figure 1. OPN isoform primary domain structure.
Each block corresponds to an exon (numbered). OPNa is full length (top; 314 aa), OPNb lacks exon 5 (middle; 300 aa), and OPNc lacks exon 4 (bottom; 287 aa). Expanded amino acid sequences of exons 4 and 5, absent in OPNc and OPNb, respectively, are included and glutamine residues (Q) that are potential sites for transglutamination are indicated in blue. Also indicated are OPN structural features and cleavage sites present within each exon. Corresponding amino acid numbers listed are for the OPNa isoform (with Met as aa 1).