The role of osteopontin in inflammatory processes

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Abstract Osteopontin (OPN) is a matricellular protein that mediates diverse biological functions. OPN is involved in normal physiological processes and is implicated in the pathogenesis of a variety of disease states, including atherosclerosis, glomerulonephritis, cancer, and several chronic inflammatory diseases. Through interactions with several integrins, OPN mediates cell migration, adhesion, and survival in many cell types. OPN also functions as a Th1 cytokine, promotes cell-mediated immune responses, and plays a role in chronic inflammatory and autoimmune diseases. Besides its function in inflammation, OPN is also a regulator of mineralization and a potent inhibitor of vascular calcification.

Keywords Inflammation · Matricellular protein · Osteopontin

Abbreviations
DC Dendritic cell
IFN Interferon
MMP Matrix metalloproteinase
OPN Osteopontin
PBMCs Peripheral blood mononuclear cells

Introduction

OPN is a secreted phosphorylated glycoprotein that mediates diverse biological functions. Originally isolated from bone, OPN was later shown to have a wider distribution (Brown et al. 1992). In adults, OPN expression is normally limited to the bone, kidney, and epithelial linings, and is secreted in bodily fluids including milk, blood and urine (Chen et al. 1993). In contrast to its restricted distribution in normal tissue, OPN is strikingly upregulated at sites of inflammation and tissue remodeling (Liaw et al. 1998; O’Brien et al. 1994). OPN exists both as a component of the extracellular matrix and as a soluble cytokine. Physiologically OPN is thought to regulate mineralization in bone tissue, and to reduce growth and aggregation of calcium crystals in epithelial tissues (Wesson et al. 2003). OPN has also been implicated in a variety of disease states, where it mediates diverse cellular functions such as adhesion, migration, and survival of several different cell types, including regulating and propagating inflammatory responses of macrophages, T-cells, and dendritic cells. The pleiotropic nature of OPN may reflect the various isoforms, post-translational modifications, and diversity of cell types which OPN can interact with. Clinically, OPN plasma levels are correlated with chronic inflammatory diseases such as Crohn’s disease (Agnholt et al. 2007), cancer (El-Tanani et al. 2006), atherosclerosis, aortic abdominal aneurysms (Golledge et al. 2007), and autoimmune diseases including lupus (Kariuki et al. 2009), multiple sclerosis (Comabella et al. 2005), and rheumatoid arthritis (Sennels et al. 2008). In this review we will focus on the role of OPN in inflammation biology.

OPN structure

OPN was originally isolated from bone and was later independently identified as secreted phosphoprotein I
(SppI) and early T-lymphocyte activation 1 (Eta-1) (Senger et al. 1989; Patarca et al. 1989). OPN is a member of the SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) family of proteins, which map to human chromosome 4 (Fisher et al. 2001). OPN is synthesized as an approximately 32 kDa protein, but due to extensive post-translational modifications its apparent molecular mass ranges from 45 to 75 kDa (Kazanecki et al. 2007). OPN possesses a negative charge due to a preponderance of acidic amino acids and serine phosphorylation. OPN also contains calcium binding sites and two putative heparin binding domains (Kon et al. 2008). OPN can interact directly with extracellular matrix proteins including fibronectin (Mukherjee et al. 1995) and collagen type I (Chen et al. 1992; Martin et al. 2004).

Adhesion motifs

OPN has multiple functional adhesive motifs, which allows interactions with many cell types including smooth muscle cells, endothelial cells, and inflammatory cells, thus mediating a broad range of biological functions. The OPN protein is poorly conserved among species (63% human to mouse, 30% human to chicken) however its functional domains are conserved. The highly conserved motifs include: abundance of acidic residues, the RGD integrin binding domain, similar phosphorylation and glycosylation motifs, and at least one site of controlled proteolysis (Bellahcene et al. 2008). The adhesive RGD domain of OPN mediates interactions via $\alpha_\beta_1$, $\alpha_\beta_3$, $\alpha_\beta_5$, $\alpha_\beta_6$, $\alpha_\beta_1$, and $\alpha_\beta_1$ integrins (Liaw et al. 1995; Yokosaki et al. 2005; Denda et al. 1998; Hu et al. 1995). Immediately C-terminal to the RGD motif is a cryptic SVVYGLR (SLAYGLR in mice) sequence that becomes exposed upon cleavage with thrombin and mediates interactions with fibronectin (Mukherjee et al. 1995) and collagen type I (Chen et al. 1992; Martin et al. 2004).

In addition to interacting with integrins, OPN has also been reported to interact with CD44, the hyaluronic acid receptor (Weber et al. 1996). A number of different CD44 isoforms exist due to alternative splicing of the 10 variant exons and while OPN can bind some CD44 splice variants, notably v6 and v7, OPN does not bind to the standard isoform, CD44H (Smith et al. 1999; Katagiri et al. 1999). Further, interactions between OPN and CD44 appears to be mediated via $\beta_1$ integrins in an RGD independent manner (Katagiri et al. 1999). Other studies indicate interactions between the C-terminal fragment of thrombin cleaved OPN and a CD44 variant (Weber et al. 2002). However, the precise domain of OPN that interacts with CD44 has not been identified. In addition, growing evidence suggests that OPN is a major regulator of CD44 surface expression, especially in osteoclasts (Chellaiah et al. 2003; Marroquin et al. 2004).

Post-translational modifications

OPN is subject to extensive post-translational modifications including serine and threonine phosphorylation. Phosphorylation is cell specific with phosphorylation levels varying depending on the tissue type. OPN in milk is highly phosphorylated with human milk OPN containing 36 phosphate sites (Christensen et al. 2005). Phosphorylation tends to occur in clusters separated by stretches of unmodified residues. Normal rat kidney cells can secrete both phosphorylated and non-phosphorylated forms, indicating regulated control of phosphorylation (Singh et al. 1990). In some cases, OPN function is tightly controlled by phosphorylation state. Calcification of smooth muscle cells in vitro is inhibited by native OPN, but desphosphorylated or recombinant bacterially produced OPN has no effect on calcification (Jono et al. 2000). Similarly, in vivo in OPN-null mice, phosphorylated OPN, but not non-phosphorylated, prevents ectopic calcification in a subcutaneous model of bioprosthetic valve mineralization (Ohri et al. 2005). In contrast, the adhesive activity of OPN is not dependent on post-translational modifications, since bacterially derived recombinant OPN has been shown to support cell adhesion in a wide variety of cell types (Gao et al. 2004; Xuan et al. 1994). OPN is also subject to sulfation (Nagata et al. 1989), glycosylation (Sorensen et al. 1995) and transglutamination (Beninati et al. 1994). Interestingly, polymerization of OPN by transglutaminase 2 has been reported to increase the adhesive activity of OPN via the $\alpha_\beta_1$ integrin, independent of the SVVYGLR adhesion domain (Nishimichi et al. 2009). Precise regulation of OPN post-translational modifications may represent a mechanism to control OPN function.

Proteolytic processing

The bioactivity of OPN can be further regulated by proteolytic processing. OPN is a substrate for thrombin and the matrix metalloproteinases, MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-2 and MMP-9 (Agnihori et al. 2001; Dean and Overall 2007; Takafuji et al. 2007). Human OPN contains three cleavage sites for MMPs; Gly166-Leu167, Ala201-Tyr202, and Asp210-Leu211. The thrombin cleavage site generating a RGD and SVVYGLR (SLAYGLR in mice) is conserved in human and mice. Human OPN contains three cleavage sites for MMPs; Gly166-Leu167, Ala201-Tyr202, and Asp210-Leu211. The thrombin cleavage site generating a RGD and SVVYGLR (SLAYGLR in mice) is conserved in human and mice. Thrombin cleavage of OPN, Arg168-Ser169 in humans and Arg153-Ser154 in mice, reveals a cryptic SVVYGLR binding domain capable of fostering interactions with $\alpha_\beta_1$ (Smith...
and Giachelli 1998; Smith et al. 1996) and $\alpha_4\beta_1$ (Bayless and Davis 2001) integrins. Thus, the known adhesive functional domains of OPN are located in the thrombin cleaved N-terminal fragment. Little is known about the role of C-terminal fragments; however, as described above, it has been suggested to contain a CD44 binding domain (Weber et al. 2002; Takafuji et al. 2007).

Proteolytic processing may represent a way to locally regulate the function of OPN as the functional properties of cleaved OPN differ from those of the intact molecule. Of particular interest, rather than mediating degradation and inactivating OPN-mediated functions, proteolytic processing of OPN can increase the biological activity of the molecule (O'Regan et al. 1999). OPN and MMPs are co-localized during wound healing and tumorigenesis, indicating there may be an in vivo role for proteolyzed forms of OPN (Senger et al. 1994). Few studies suggest that OPN fragments may play a functional role in vivo. Enhanced production of the thrombin cleaved form of OPN is found in the synovial fluid of patients with rheumatoid arthritis (Ohshima et al. 2002) and the cryptic SLAYGLR motif was found to play an essential role in a murine and a primate model of rheumatoid arthritis (Yamamoto et al. 2003, 2007). An antibody against the SLAYGLR sequence inhibited inflammatory cell influx into arthritic joints and attenuated the severity of disease. The human OPN derived peptide SVVYGLR has also been found to induce angiogenesis in vitro and in vivo (Hamada et al. 2003, 2007). Several in vitro studies have demonstrated that the N-terminal fragments generated both by thrombin cleavage and MMP cleavage induced enhanced adhesion when compared to the full length molecule. This appears to be due mostly to increased activity of the RGD site, perhaps an indication of conformational change resulting in higher affinity binding (Senger and Perruzzi 1996; Smith and Giachelli 1998; Agnihotri et al. 2001). The SVVYGLR cryptic domain exposed following thrombin cleavage is also able to induce adhesion and migration through the $\alpha_4$ and $\alpha_6$ integrins (Smith and Giachelli 1998). However, the $\alpha_9$-dependent adhesion and migratory functions are completely lost in the N-terminal MMP generated fragment, as Arg168 seems to be required for $\alpha_9$-dependent binding (Yokosaki et al. 2005; Ito et al. 2009). In contrast, $\alpha_4$-dependent adhesion and migratory functions are only partially lost in the N-terminal MMP generated fragment (Ito et al. 2009). The C-terminal fragment of OPN generated by thrombin and MMP cleavage does not contain any integrin adhesive domains, it does not mediate adhesion when presented in immobilized form to cells and, in contrast, it appears to suppress OPN mediated adhesion and migration in monocyte-derived cells (Gao et al. 2004; Smith et al. 1996; Maeda et al. 2001; Takahashi et al. 1998)

Intracellular OPN

An intracellular form of OPN (iOPN) has been reported to be expressed in dendritic cells and macrophages (Shinohara et al. 2006, 2008a, b; Zohar et al. 2000). Studies by Shinohara et al. suggest that the intracellular form of OPN is generated due to translation initiation downstream of the usual start site in bone marrow-derived DCs and transfected 293T cells. Utilization of this downstream start site generates the truncated iOPN form that lacks the N-terminal signal sequence and consequently localizes to the cytoplasm, where it may associate with TLR9 and the MyD88 adaptor molecule (Shinohara et al. 2008a). The interaction of iOPN with MyD88 appears to activate the transcription factor IRF7 and to induce expression of IFN-$\alpha$ ultimately leading to Th1 cell-mediated immunity and pro-inflammatory responses (Shinohara et al. 2006). The same group has also suggested that iOPN expression in conventional DCs is permissive for Th17 T cell responses (Shinohara et al. 2008b). Th17 T cells are a subset of T helper cells producing IL-17. They are considered developmentally distinct from Th1 and Th2 cells and are thought to play a key role in autoimmune diseases including the tissue injury associated with these conditions (Steinman 2007). Thus iOPN expression may allow for autoimmune type disease progression. Indeed, OPN accelerates the progression of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (Chabas et al. 2001; Hur et al. 2007; Jansson et al. 2002; Shinohara et al. 2006, 2008b). Others have found that iOPN may associate with the intracellular domain of CD44 and with the ezrin/radixin/moesin (ERM) protein ezrin. iOPN in this context may modulate cytoskeletal rearrangements important for macrophage migration and osteoclast fusion (Zhu et al. 2004; Zohar et al. 2000).

A summary of the structural features of OPN is shown in Fig. 1.

The role of OPN in inflammation

OPN regulates the immune system at many different levels. It serves as a chemotactic molecule to promote the migration of inflammatory cells to the wound site and acts as an adhesive protein to retain cells at the site. OPN also functions as a pro-inflammatory cytokine and can modulate the immune response by enhancing expression of Th1 cytokines and matrix degrading enzymes (Weber et al. 2002; Bruemmer et al. 2003). OPN plays a pivotal role in T cell and macrophage responses during cell mediated immune responses against bacterial and viral pathogens (Ashkar et al. 2000). More recently, OPN has also been shown to modulate dendritic cell responses and neutrophil chemotaxis.
Macrophages

OPN is not expressed in circulating monocytes, but is dramatically upregulated during macrophage differentiation and constitutes one of the major macrophage products (Krause et al. 1996). OPN is known to be induced in macrophages by several inflammatory cytokines, including TNF-α, IL-1β, IFN-γ, and IL-6, and other factors including angiotensin-II, oxidizedLDL, and phorbol-ester are known inducers of OPN in macrophages (Nakamachi et al. 2007; Ogawa et al. 2005; Bruemmer et al. 2003). More recently, Liver X Receptor and Peroxisome proliferator-activated receptor α antagonists have been shown to suppress OPN expression (Nakamachi et al. 2007; Ogawa et al. 2005). Functionally OPN plays a key role in macrophage biology by regulating migration, survival, phagocytosis, and pro-inflammatory cytokine production (Bruemmer et al. 2003; Nyström et al. 2007).

We and others have shown that OPN serves as a potent chemoattractant for macrophages (Bruemmer et al. 2003; Persy et al. 2003; Giachelli et al. 1998; Panzer et al. 2001). Functional inhibition of OPN and genetic ablation of OPN in mice greatly impair macrophage recruitment in several models of acute inflammation. In OPN-null mice acute macrophage infiltration was greatly diminished compared to wild-type mice in an obstructed kidneys model (Ophascharoensuk et al. 1999), and in a thiglycollate induced peritonitis model (Bruemmer et al. 2003). Further, purified OPN induced macrophage accumulation when injected in rat dermis and following intradermal injection of N-formyl-met-leu-phe (FMLP), a potent macrophage chemotaectic peptide. The OPN effect was neutralized by an anti-OPN blocking antibody (Giachelli et al. 1998). In a rat model of heart injury OPN was highly expressed in the granulation tissue associated macrophages, and it was downregulated with healing progression and formation of the fibrotic scar (Murry et al. 1994). Wound healing studies in mice also indicate that OPN is expressed during the acute inflammatory phase at very high levels in infiltrating leukocytes and other cell types where it appears to regulate leukocyte infiltration and activation as well as proper matrix organization (Liaw et al. 1998). Interestingly, downregulation of OPN at the wound site with antisense mRNA diminished macrophage infiltration and accelerated wound healing (Mori et al. 2008).

OPN also modifies chronic inflammatory responses. Chronic inflammation is characterized by the persistence of macrophages at sites of injury and disease. Deficits in macrophage accumulation have been noted in OPN-null mice when challenged with chronic inflammatory conditions, including atherosclerosis, delayed-type hypersensitivity (Yu et al. 1998; Ashkar et al. 2000), granulomatous disease (Weber et al. 2002; Nau et al. 1999), and biomaterial implantation (Steitz et al. 2002; Tsai et al. 2005). These data suggest that OPN may be particularly important in promoting migration and retention of macrophages at sites of acute and chronic inflammation. We have also shown that OPN regulates foreign body giant cell (FBGC) formation in vitro and in vivo. In a recent paper we described that despite the defect in macrophage recruitment, OPN-null mice formed more FBGCs on the surface of the implant. In vitro, OPN inhibited macrophage fusion to form FBGCs in a dose dependent manner (Tsai et al. 2005).

In vitro, OPN-null macrophages exhibit reduced basal migration and impaired migration towards MCP-1, despite the fact that wild type and OPN-null macrophages express comparable levels of CCR-2, the MCP-1 receptor. This may be a consequence of a lack of a permissive pro-migratory substrate, and the reduced expression of CD44 observed in OPN-null macrophages. CD44 is well known to be essential for macrophage migration (Marcondes et al. 2008), and its expression is upregulated by OPN in macrophages (Chellaiah et al. 2003). Macrophages from OPN-null mice are also more susceptible to programmed cell death (Bruemmer et al. 2003). Together with impaired migration, macrophage apoptosis may further contribute the
impaired macrophage accumulation observed in OPN-null mice in response to acute and chronic inflammatory stimuli.

In addition to regulating macrophage migration, OPN can also modulate the cytokine production by macrophages. OPN stimulates production of IL-12 while inhibiting the production of IL-10, thereby promoting Th1 cell mediated responses (Weber et al. 2002; Ashkar et al. 2000). Interestingly, these results were mediated by different receptors. IL-12 production was mediated via an N-terminal fragment interaction with αvβ3 integrin, while IL-10 was inhibited via a C-terminal fragment, possibly via the CD44 receptor. OPN regulation of IL-12 and IL-10 were also demonstrated in vivo in an angiotensin II (AngII)-accelerated model of atherosclerosis. In this model, Bruemmer et al. showed reduced expression of IL-12 and an increased expression of IL-10 in ApoE-/- OPN-/- AngII treated mice compared to ApoE-/-OPN+/+ AngII treated control mice by RT-PCR of whole mouse aortas. These results correlated with less macrophage rich lesions and lower expression of the macrophage marker CD68 (Bruemmer et al. 2003).

While OPN is generally classified as a pro-inflammatory cytokine, it also appears to have anti-inflammatory effects. OPN is a potent trans-repressor of inducible nitric oxide synthase (iNOS) expression in macrophages (Rollo et al. 1996). OPN represses inducible nitric oxide synthase (iNOS) by increasing Stat1 ubiquitination and proteasome mediated degradation of Stat1, consequently inhibiting Stat1 mediated iNOS transcription and protein expression (Gao et al. 2007). NO feedback inhibits its own synthesis by increasing transcription of OPN (Guo et al. 2001). OPN inhibition of NO may be particularly relevant for tumor cell evasion of inflammation (Wai et al. 2006; Crawford et al. 1998).

Finally, a recent study suggests that OPN may play a role in macrophage differentiation. Using siRNA to stably silence the expression of OPN in RAW 264.7 cells, Nystrom et al. showed that OPN silenced cells displayed an altered phenotype with monocyte-like characteristics (Nystrom et al. 2007). Further, OPN silenced cells had decreased expression of macrophage scavenger receptor A type 1 (Msr-1), a macrophage differentiation marker. While these studies are intriguing, the phenotype could not be rescued by the addition of exogenous OPN suggesting non-receptor mediated effects or iOPN may be involved.

Together, these data suggest that OPN may be particularly important in promoting migration and retention of macrophages at sites of acute and chronic inflammation by regulating multiple macrophage functions. These studies also emphasize the importance of macrophage-derived OPN in the regulation of OPN’s functions, suggesting that macrophages are both a source and target of OPN.

### Neutrophils

While much is known about the role of OPN in macrophage biology, relatively few studies have explored the function of OPN in neutrophils. Neutrophils express low levels of OPN (Koh et al. 2007). However, OPN is important for the recruitment and migration of neutrophils, as neutrophils from OPN-null mice display reduced chemotaxis toward fMLP and in vivo the recruitment of neutrophils to the peritoneal cavity in response to sodium periodate is impaired in OPN-null mice (Koh et al. 2007). OPN-null mice also have impaired neutrophil infiltration into liver when challenged with concanavalin A induced hepatitis (Diao et al. 2004). Despite these defects in migration and chemotaxis, OPN-null neutrophils do not display reduced destructive capacity in terms of phagocytosis, the generation of reactive oxygen species, or cytokine production (Koh et al. 2007). Recent reports indicate that polymeric OPN interacts with αvβ3 on neutrophils and serves as a potent neutrophil chemoattractant (Nishimichi et al. 2009).

### T-cells

OPN is also known as Eta-1 (early T lymphocyte activation gene 1) for its high expression in activated T cells and it plays an important role in the induction of cell mediated immune responses through the regulation of T cells. Following activation, naïve CD4 T cells can differentiate towards Th1, Th2, or Th17 cells which differ in effector function. The development of Th1 cells leads to cell-mediated immunity while development of Th2 cells provides humoral immunity. Th17 cells are associated with autoimmunity. OPN is not expressed in naïve T-cells but it is strongly upregulated in response to T cell receptor ligation (Shinohara et al. 2005). OPN functions in T cells by mediating migration, adhesion, and co-stimulating T cell proliferation (O’Regan et al. 1999; Patarca et al. 1993). Shinohara et al. have shown that OPN gene expression in T cells is controlled by T-bet, a transcription factor that promotes CD4+ T helper cell lineage commitment to Th1 (Shinohara et al. 2005). Further, T-bet-dependent expression of OPN in T cells is essential for efficient skewing of CD4 T and CD8 T cells toward the Th1 and type 1 CD8 T cell (Th1) pathway, respectively (Shinohara et al. 2005). In vivo, OPN-null mice display impaired Th1 responses to the intracellular bacterium Listeria monocytogenes and the viral pathogen HSV1 (herpes simplex virus type 1) both of which depend on the induction of IL-12 for protection (Ashkar et al. 2000). Indeed, mice deficient in OPN have decreased IL-12 and IFN-γ production, while IL-10 levels are enhanced (Bruemmer et al. 2003). Further studies have shown that OPN regulates CD3-mediated T cell expression.
of IFN-γ and CD40L (O’Regan et al. 2000), which in turn stimulates IL-12 production from leukocytes. Together these findings suggest that OPN may play a role in polarizing early Th1 responses. More recently, OPN has also been shown to regulate IFN-γ and IL-17 production by T cells in an αvβ3-dependent manner and to dampen IL-10 in a CD44-dependent manner (Murugaiyan et al. 2008). This appears to be important for the progression of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis. The same authors found that during EAE, OPN expression was elevated in murine model of multiple sclerosis. The same authors experimental autoimmune encephalomyelitis (EAE), a This appears to be important for the progression of EAE. Moreover, OPN expression increases as monocytes adhere and differentiate into macrophages (Krause et al. 1996). These findings suggest differential regulation of OPN in DCs and macrophages. During macrophage differentiation, OPN expression increases as monocytes adhere and differentiate into macrophages (Krause et al. 1996).

In vitro OPN stimulates DC migration in a dose dependent manner in both the presence and absence of divalent cations, and studies with blocking antibodies have indicated a role for the OPN receptors αvβ3 and CD44 in OPN induced DC migration (Weiss et al. 2001). In vivo, DCs in OPN-null mice display a defect in DC trafficking to the lymph nodes resulting in reduced contact hypersensitivity responses (Weiss et al. 2001). Despite the influence of OPN on DC migratory capacity, treatment with recombinant OPN does not affect the expression of CCR5 and CCR7, chemokine receptors which are involved in DC migration (Schulz et al. 2008). Recently, it has also been suggested that OPN modulates different subset of DCs in the airway hypersensitivity reaction (a model of asthma). It appears that OPN increased the reaction during primary sensitization but decreased the reaction in the challenge phase, perhaps by mediating differential recruitment of different DCs (plasmacytoid and conventional) subsets to the lymph nodes (Xanthou et al. 2007).

OPN also influences DC cytokine production. In coculture systems, OPN induces DC secretion of TNF-α and IL-12p70 which stimulates secretion of IFN-γ by T-cells (Renkl et al. 2005). By augmenting DC production of IL-12, OPN can enhance Th1 polarization.

As mentioned earlier, recent studies illustrate the critical role of intracellular OPN in IFN-α production by plasmacytoid DCs, a specialized subset of DCs which produce high levels of type I interferons upon stimulation (Shinohara et al. 2006). IFN-α produced by pDCs activates NK cells, and consequently OPN deficient mice display impaired IFN-α dependent natural killer cell responses. Further, iOPN appears to decrease IL-27 in conventional DCs, leading to increases in Th17 responses (Shinohara et al. 2008b).

Other biological functions of OPN

Wound healing

OPN is a key cytokine regulating tissue repair. OPN is present at sites of wound healing where it serves as a chemotactic molecule to recruit inflammatory cells to the site of injury. Wound healing studies in OPN-null mice have elucidated the role of OPN in tissue repair. Compared to wild type mice, incisional wounds made in OPN-null mice displayed alterations in the matrix architecture especially collagen fiber diameter, and had more residual debris (Liaw et al. 1998). Furthermore, macrophages at the wound site in OPN-null mice expressed higher levels of mannose receptor, suggesting OPN may contribute to macrophage polarization and thus regulate healing responses. Mannose receptor expression in macrophages is associated with reduced pro-inflammatory (IL-1, IL-6, IL-12, and TNF-α) cytokine secretion, upregulation of pro-healing molecules (IL-10 and TGF-β), and certain phagocytic receptors (Sica et al. 2008).
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A more recent study by Mori et al. explored the use of local OPN knockdown at the site of wound healing by delivering antisense oligodeoxynucleotides from a drug delivery polymer gel. Consistent with the finding in OPN-null mice, in OPN knockdown wounds the diameter of collagen fibrils was also smaller than in control wounds. Further, OPN knockdown hindered the migration of inflammatory cells to the wound site and resulted in accelerated healing and a reduction in granulation tissue formation and scarring (Mori et al. 2008). Whether the infiltrated macrophages in OPN knockdown wounds also had a less pro-inflammatory and more pro-healing phenotype was not established, however, this may be behind the observed accelerated healing. The reduced size of the collagen fibers that was observed in the OPN-null and OPN knockdown wounds may be related to the finding that OPN has been shown to bind directly to collagen type I (Chen et al. 1992), and to interact with collagen types II, III, IV, and V (Bulter et al. 1995). Further, it has recently been shown that OPN is necessary for TGF-β1-induced myofibroblast differentiation and OPN-null fibroblasts exhibited less spreading, less resistance to detachment, and a reduction in collagen gel contraction (Lenga et al. 2008). These studies indicate a role for OPN in promoting proper collagen organization and regulating ECM and myofibroblast interactions.

Vascular disease

To date, several studies have investigated the role of OPN in the progression of atherosclerosis. In hyperlipidemic apoE-deficient mice, Matsui et al. showed that osteopontin deficiency significantly reduces atherosclerotic lesion size in female ApoE-/-OPN-/- mice compared to ApoE-/-OPN+/- mice after 36 weeks on a normal chow diet (Matsui et al. 2003). Similarly, studies in ApoE/LDLReceptor/OPN triple knockout mice showed that OPN deficiency resulted in decreased atherosclerotic lesion size and an increase in the number of apoptotic cells in lesions (Strom et al. 2004). Bone marrow transplantation studies in an angiotensin II-accelerated model of atherosclerosis indicated that leukocyte derived OPN contributes to OPN-mediated development of atherosclerosis (Bruemmer et al. 2003). These studies suggest that OPN promotes macrophage accumulation and retention in the atherosclerotic lesions, thus contributing to the chronicity of the disease.

OPN also modulates other vascular cells associated with vascular disease. In human atherosclerotic lesions, OPN is expressed in smooth muscle cells (SMC), angiogenic endothelial cells, and macrophages and it is re-expressed in SMCs associated with human restenotic lesions (Giachelli et al. 1993; Panda et al. 1997). Consistently, animal models have confirmed the role of OPN in promoting SMC migration and proliferation (Liaw et al. 1994; Isoda et al. 2002). All these data indicate that during injury, OPN enhances the proliferation, migration, and accumulation of smooth muscle and endothelial cells involved in repair and remodeling processes of the vasculature.

Cancer

OPN is highly expressed in transformed cells and is found in a variety of cancers (Wai and Kuo 2008). OPN overexpression can confer metastatic phenotype to non-metastatic, benign transformed cells, and increased OPN expression correlates with tumor progression, poor prognosis and increased invasiveness. In metastatic models, OPN has been shown to induce matrix proteases MMP2 and uPA in an integrin-dependent manner (Mi et al. 2006). Further, OPN has been shown to bind and activate MMP3. Thus, the ability of OPN to stimulate migration and matrix breakdown could contribute to invasiveness and to the metastatic potential of tumors cells. OPN may also promote tumorigenesis and metastasis by inhibiting apoptosis of tumor cells (Zhao et al. 2008), and by stimulating neovascularization (Wai and Kuo 2004). Finally, OPN is widely expressed by macrophages, which infiltrate tumor tissue (Brown et al. 1994; Chambers et al. 1996). Macrophage-derived OPN functions as a chemoattractant and was associated with reduced tumor burden while tumor-derived OPN appeared to inhibit macrophage function and enhance tumor growth (Crawford et al. 1998). It is possible that tumor cell-derived OPN may enhance cancer cell survival by downregulating iNOS expression and NO production in macrophages. OPN is currently being studied as a potential biomarker for cancer and there is interest in targeting OPN as a therapeutic treatment for cancer.

Biomineralization

OPN is one of the most abundant non-collagenous proteins in bone. Because of its abundance in bone, OPN has been studied as a regulator of biomineralization. OPN is a potent inhibitor of mineralization, prevents ectopic calcification, and is an inducible inhibitor of vascular calcification (Steitz et al. 2002). OPN binds hydroxyapatite and calcium ions thereby physically inhibiting crystal formation and growth in vivo. Studies in OPN-null mice have shown that OPN-/- bones are hypermineralized, with increased mineral content and crystal size (Boskey et al. 2002). OPN also plays a role in osteoclast differentiation and osteoblast recruitment and function (Rittling et al. 1998). OPN functions in osteoclast migration to sites of resorption and is crucial for normal resorption and bone turnover (Chellaiah et al. 2003).

OPN appears also to be an important regulator of vascular calcification and is associated with mineralized deposits in
humans (Giachelli et al. 1993). In mice, OPN levels are greatly elevated in the spontaneously mineralizing arteries of MGP" mice and we have recently shown that OPN is major inducible inhibitor of arterial medial calcification in this system (Steitz et al. 2002). Vascular calcification is now recognized as a marker of atherosclerotic plaque burden as well as a major contributor to loss of arterial compliance and increased pulse pressure seen with age, diabetes, and renal insufficiency. These findings suggest that OPN may be an important inhibitor of arterial mineral deposition under conditions of injury and disease, and that strategies to replenish OPN might be useful to prevent or treat ectopic calcification, including vascular calcification.

**Conclusions**

OPN is emerging as a key regulator of immune cell biology. Most of the evidence indicates that OPN is transiently expressed in leukocytes during acute inflammation. However, persistence of OPN expression by immune cells exacerbates chronic inflammatory diseases. Clinically, this is manifested by increased OPN plasma levels in Crohn’s disease (Agnholt et al. 2007), cancer (El-Tanani et al. 2006), atherosclerosis, and autoimmune diseases including lupus (Kariuki et al. 2009), multiple sclerosis (Comabella et al. 2005), and rheumatoid arthritis (Sennels et al. 2008).

Mechanistically, OPN appears to regulate innate immune cells (macrophages and DCs) and adaptive immune cells (T cells) at multiple levels (see Fig. 2). Recent data point to a role for OPN in the regulation of cross-talk between DCs and T cells and their subsequent polarization in Th1 and more recently in Th17 cells.

*In vitro* and *in vivo* studies show that both the thrombin and MMP proteolytically cleaved OPN fragments possess higher activity than the full-length form. In addition, at least the thrombin cleaved fragment also gains a new cell interacting domain (SVVYGLR). Antibodies specifically reacting toward the SVVYGLR (human and primate) or the SLAYGLR (murine) sequences have been shown effective in ameliorating rheumatoid arthritis symptoms in non-human primates and mice (Yamamoto et al. 2003, 2007).

![Fig. 2 OPN regulation of immune and inflammatory cells. OPN is secreted and modulates the function of macrophages, DCs and T Cells. OPN may induce macrophage accumulation by promoting migration and survival. Further, OPN induces IL-12 and inhibits IL-10 in macrophages, thus, propagating a Th1 response. In DCs OPN appears to modulate their function as an extracellular soluble cytokine and also as an intracellular molecule (iOPN). Both OPN forms appear to induce Th1 polarization. Extracellular OPN appears to induce expression of IL-12 and TNF-α, and iOPN appears to regulate the production of INF-α. In T cells OPN induces migration, proliferation, survival, and IL-17 secretion. These two latter functions have been correlated with Th17 responses and autoimmunity. Further, OPN appears to induce IFN-γ secretion by T cells thus propagating a Th1 response. Finally, the N-terminal OPN fragment (containing the activated adhesive domain SVVYGLR) may be important in the propagation of rheumatoid arthritis.](image-url)
Therefore, during inflammation, it is likely that the secreted, less potent, full-length OPN is rapidly cleaved and thus activated. Understanding differences in the mechanisms and structure/function relationships governing the proinflammatory properties of OPN could help create specific therapeutics aimed at targeting chronic inflammatory diseases selectively.

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