Role of the metastasis-promoting protein osteopontin in the tumour microenvironment

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Received: May 27, 2010; Accepted: June 23, 2010

Abstract

Osteopontin (OPN) is a secreted protein present in bodily fluids and tissues. It is subject to multiple post-translational modifications, including phosphorylation, glycosylation, proteolytic cleavage and crosslinking by transglutamination. Binding of OPN to integrin and CD44 receptors regulates signalling cascades that affect processes such as adhesion, migration, invasion, chemotaxis and cell survival. A variety of cells and tissues express OPN, including bone, vasculature, kidney, inflammatory cells and numerous secretory epithelia. Normal physiological roles include regulation of immune functions, vascular remodelling, wound repair and developmental processes. OPN also is expressed in many cancers, and elevated levels in patients' tumour tissue and blood are associated with poor prognosis. Tumour growth is regulated by interactions between tumour cells and their tissue microenvironment. Within a tumour mass, OPN can be expressed by both tumour cells and cellular components of the tumour microenvironment, and both tumour and normal cells may have receptors able to bind to OPN. OPN can also be found as a component of the extracellular matrix. The functional roles of OPN in a tumour are thus complex, with OPN secreted by both tumour cells and cells in the tumour microenvironment, both of which can in turn respond to OPN. Much remains to be learned about the cross-talk between normal and tumour cells within a tumour, and the role of multiple forms of OPN in these interactions. Understanding OPN-mediated interactions within a tumour will be important for the development of therapeutic strategies to target OPN.

Keywords: osteopontin • tumour microenvironment • bone microenvironment • metastasis • angiogenesis

Structure and functional properties of osteopontin (OPN)

OPN is a secreted, integrin-binding glycoprophosphoprotein that was discovered independently in several different settings [1, 2]. It was identified as bone sialoprotein I, a major non-collagenous protein in bone [3], as a secreted protein produced by many transformed cells in culture [4] and as a protein important for early T-cell-dependent resistance to bacterial infections [5]. Although OPN has long been associated with cancer [1, 6, 7] and is an attractive candidate target for cancer therapy [2], it is also up-regulated in a variety of acute and chronic inflammatory conditions, such as wound healing, fibrosis, autoimmune disease and atherosclerosis, and plays a role in certain developmental processes and tissue differentiation [7, 8]. OPN's expression in both cancer and inflammatory processes is consistent with the notion that tumours are 'wounds that do not heal' [9] or 'tissues that never cease to develop' [10]. The interactions between OPN, inflammatory/immune cells and the stromal microenvironment likely play an important role in tumour development and progression.

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doi:10.1111/j.1582-4934.2010.01115.x
Fig. 1 Schematic diagram of the structure of human OPN. The human OPN protein contains various highly conserved structural elements which are essential for its various biological functions. These include a secretion signal sequence (S), an aspartic acid rich sequence (DDD) important for binding hydroxylapatite in bone and contributing to the overall negative charge of OPN and glutamine residues (Q-Q) which serve as a substrate for transglutaminase crosslinking. It also has several highly conserved binding domains, such as RGD and SVVYGLR sequences involved in integrin receptor binding, and putative binding domains for heparin (Hp) and calcium (Ca). OPN contains a thrombin proteolytic cleavage site (black arrow) and proteolytic cleavage sites for MMPs-3, -7 and -9 (red arrows). OPN has also up to 36 potential serine/threonine phosphorylation sites, appearing in clusters (circled P’s) throughout the molecule, and contains a cluster of five glycosylation sites (black hexagon). Splice variants OPN-c and OPN-b are lacking exon 4 (4) and exon 5 (5), respectively. N: N-terminus; C: C-terminus.

Human OPN (Fig. 1) is a highly negatively charged protein of 314 amino acids (Mr 34-kD) and appears to lack complexity in secondary structure [11]. It has a number of highly conserved structural elements, including RGD (arginine-glycine-aspartate) and SVVYGLR (serine-valine-valine-tyrosine-glycine-leucine-arginine) domains for integrin binding, a calcium binding site and heparin binding domains that mediate CD44 receptor binding [7]. In addition to full length OPN (OPN-a), two other splice variants, OPN-b and OPN-c, are known to occur and likely display functional heterogeneity [12, 13]. All forms of OPN are subject to extensive post-translational modifications including serine/threonine phosphorylation, glycosylation and tyrosine sulphation, which allow for a monomeric molecular weight ranging from 41 to 75 kD [14]. These modifications can be cell type specific [15], may depend on other physiological and pathophysiological factors and likely impact both OPN structure and function [16, 17]. For example, Al-Shami et al. [18] have reported that dephosphorylation of highly phosphorylated OPN reduces its ability to stimulate the migration of human choriocarcinoma cells. Interestingly, however, bacterially produced OPN (which lacks eukaryotic post-translational modifications) has been shown to be functional in some experimental assays, such as adhesion and migration of MDA-MB-435 cells and 21T series mammary epithelial cells [19–23]. Additionally, transglutaminase crosslinking of OPN via two glutamine residues in the N-terminal half of the molecule can be important in its biological activity by altering adhesion and migration abilities [24]. Finally, proteolytic fragmentation of OPN by thrombin [25] or matrix metalloproteinases (MMPs) [26, 27] can generate OPN forms having potential physiological and pathological importance [11, 28]. However, much remains to be learned about the functional properties of these various forms of the OPN protein.

OPN binds to a number of cell surface receptors, including members of the integrin and CD44 families, and indirectly influences the activity of growth factor receptors such as epidermal growth factor receptor and Met [7, 29]. This signalling results in the activation of various pathways, including phosphoinositide 3-kinase (PI3K)-Akt pathway, mitogen-activated protein kinase pathway, phospholipase C pathway, Src kinase pathway, urokinase plasminogen activator secretion and MMP-9 activation, leading to increased cell motility, cell survival, tumour growth and metastasis [1]. The nature of a given cell’s response to OPN will thus depend on both the numbers and types of receptors on the cell surface, as well as the structural modifications of the OPN present in the cell’s microenvironment.

OPN in the tumour microenvironment

Tumour cells and their microenvironment mutually influence each other during tumour formation and progression [30]. The tumour microenvironment consists of a variety of non-tumour cell types, including fibroblasts, immune, vascular and smooth muscle cells in conjunction with extracellular matrix (reviewed in [31, 32]). The presence of a tumour leads to stromal changes including the recruitment of immune and endothelial cells. These cells secrete growth and matrix remodelling factors and support angiogenesis and lymphangiogenesis to promote further tumour growth and metastasis. Stroma formation in neoplastic processes represents both the host’s reaction to neoplastic cells as well as the ability of tumour cells themselves to modify their environment to influence growth and progression [30, 32]. It has long been known that OPN can be produced by many cells that are present in the tumour microenvironment, including tumour, inflammatory and stromal cells; however, its various contributions to tumour growth, progression and the tumour microenvironment are still incompletely understood. Knowledge about the presence and role of OPN in the tumour microenvironment has accumulated through investigations of established human tumours, studies of
A number of studies have established that tumour cells themselves can synthesize OPN in vivo. OPN expression in tumour cells has been shown in a variety of cancers [34, 35] including carcinomas of breast [36], prostate [37], colon [38], stomach [39], liver [40] and lung [41, 42], mesotheliomas [43], squamous cell carcinomas [44], sarcomas [45, 46] and multiple myeloma [47]. However, OPN is also often produced by other cells in the tumour microenvironment, such as macrophages and stromal cells. For example, Tuck et al. [36] demonstrated that OPN mRNA and protein were detected in both tumour cells and tumour infiltrating inflammatory cells in a cohort of lymph node negative breast cancer patients. Similarly, another study looking at OPN expression in breast cancer observed cytoplasmic staining of breast cancer cells and OPN expression in tumour-infiltrating inflammatory cells, whereas stromal fibroblasts and endothelial cells were OPN− [48]. In pulmonary artery sarcomas, tumour cells as well as macrophages and extracellular matrix were OPN+, and tumour cell staining was most frequent at the tumour cell surface and at sites of invasion [49]. Chang et al. [50] investigated OPN expression in cutaneous squamous cell carcinoma and found that in most cases, OPN was expressed in cancer cells and found in extracellular matrix adjacent to the tumour, and not in other stromal regions. In contrast, in some tumour types, OPN expression has been found primarily in macrophages and stromal cells, and less frequently in the tumour cells themselves (e.g. oesophageal adenocarcinoma [51]).

Immunohistochemical staining often reveals heterogeneous expression of OPN in the tumour and its microenvironment (e.g. [34, 45, 52]). In addition, patterns of OPN cellular staining may change as tumours progress. This is seen in colon carcinomas, where well-differentiated tumours show OPN immunostaining at the apical cell surface, whereas in poorly differentiated tumours the staining is found on the basal cell surface, at the stromal interface [34]. This raises the interesting question as to whether the cellular localization or presentation of OPN affects whether or not the cell responds, or the nature of that response. Indeed, in many tumours, OPN+ cancer cells are often found scattered at the periphery of invasive tumours adjacent to stromal cells, suggesting its involvement in paracrine tumour and host cellular interactions (e.g. [53, 54]).

There is conflicting evidence on whether tumour-derived OPN is incorporated into the extracellular matrix. In some tissues, such as bone, OPN is an established component of the extracellular matrix and is important in cell attachment [55]. OPN has been found in the extracellular matrix of various cancers by immunohistochemistry [49–51, 56, 57]. However, it has been argued that at least in some instances, tumour-derived OPN may be more soluble and not incorporated into the extracellular matrix [58]. Thus, it is unclear whether it is tumour- or stromal-derived OPN (or both) that can be incorporated into the extracellular matrix and affect tumour growth and progression.
the recruitment of macrophages to the tumour microenvironment could have multiple indirect effects to promote malignancy. In contrast, OPN has been shown to promote the development and activity of type I natural killer T (NK T) cells [67] and to inhibit their apoptosis via binding and activation of the CD44 receptor [68], OPN may induce anti-tumour activity via these NK cells [69]. Thus it may be the balance between the macrophage and type I NK T cell response elicited by OPN that determines whether the overall effect of the inflammatory response will be tumour promoting or tumour inhibiting.

Studies have also implicated OPN in angiogenesis. Tumour angiogenesis involves several processes, such as proliferation, migration and tissue infiltration of pericytes, vascular smooth muscle and endothelial cells from pre-existing blood vessels, and activation of these cells, in which OPN can be overexpressed [70–73]. The role of OPN in tumour angiogenesis is tightly associated with vascular endothelial growth factor, and both are frequently simultaneously up-regulated during angiogenesis [74–77]. In a mouse xenograft model, OPN stimulated angiogenesis by inducing vascular endothelial growth factor expression, through activation of PI3K/AKT and ERK-mediated pathways in endothelial cells [78]. Also, OPN itself can be up-regulated by fibroblast growth factor-2 in endothelial cells in vitro and in vivo, leading to the recruitment of pro-angiogenic monocytes to the tumour microenvironment [79]. Therefore, OPN most likely influences angiogenesis in multiple ways, by activating pro-angiogenic signalling pathways and by helping to recruit cells to angiogenic sites.

Recently, a role for tumour-derived OPN has been revealed in the activation and mobilization of bone marrow derived cells to the microenvironment of disseminated tumour cells (i.e. establishment of a so-called ‘pre-metastatic niche’) [80]. McAllister et al. [80] found that the secretion of soluble OPN by a tumour supports distant tumour/metastatic outgrowth by releasing and activating bone marrow derived cells, which then incorporate into the distant tumour microenvironment, conditioning that environment and promoting tumour growth. This may represent a distinct pathophysiologic role for circulating OPN in the blood of cancer patients [80].

OPN may also play a role in the formation of calcifications in breast cancers [81]. High levels of OPN have been detected in calcifications of both benign and malignant breast tissues [82]. Calcifications are more frequent in tumours overexpressing OPN than in OPN− tumours [83]. Although the significance of the presence of OPN in these calcifications is uncertain, in terms of whether OPN promotes or inhibits their formation, it may mean that some OPN-expressing tumours are more readily detectable at early stages on the basis of associated abnormal or irregular mammographic calcifications. OPN has also been found in psammoma bodies and other forms of dystrophic calcification associated with other types of cancers [84–88], although the significance of this finding is again uncertain.

Effect of OPN in the tumour microenvironment on tumour progression

In addition to OPN produced by tumour cells, OPN produced by stromal cells and infiltrating inflammatory cells can affect the tumour microenvironment and alter cell function. For example, OPN produced by macrophages is able to restore metastatic potential, as shown in a human hepatoma cell line in which OPN expression was silenced [89]. However, in some contexts, host-derived OPN may inhibit tumour growth, as OPN-deficient macrophages have been shown to display impaired anti-tumour activity against ras-transformed 3T3 fibroblast cells [90]. As mentioned above, whether the overall inflammatory response elicited by (in this case, macrophage) OPN promotes or inhibits tumour progression is likely context-dependent, and may vary with tumour type.

Cancer-associated stromal fibroblasts and pericytes are also influenced by OPN, and as these cells perform critical functions in angiogenesis, extracellular matrix remodelling and metastasis, their presence can be beneficial to tumour growth. Stromal fibroblasts and myofibroblasts play important roles in the tumour microenvironment. Although tumour-derived myofibroblasts are believed to favour tumour progression and have been linked with OPN expression in neoplasia [31, 91], normal fibroblasts are believed to inhibit tumorigenesis [92]. OPN has been shown to be up-regulated during and required for the differentiation of fibroblasts into myofibroblasts [93, 94] and this may be one mechanism by which OPN facilitates tumour progression. Increased OPN expression in fibroblasts has also been seen in cell senescence [95], and senescent fibroblasts have also been shown to promote pre-neoplastic growth in vitro and in vivo [96]. Conversely, reduction of OPN expression in senescent fibroblasts by RNA interference has been shown to reduce their growth-promoting influence [95]. Consistent with these findings, cancer-associated fibroblasts recruited into the stroma by platelet-derived growth factor-CC elaborated by tumour cells have also been shown to overexpress OPN and promote tumour growth [97].

The presence of OPN in the microenvironment of a metastasis may also lead to increased tumour take at a secondary site. Allan et al. [98] identified OPN as a key molecular player involved in lymphatic metastasis of breast cancer, and showed increased OPN expression in the intranodal metastatic tumour deposits, relative to levels in the primary tumours. In addition, in an experimental murine metastasis assay, injection of low OPN-producing B16 melanoma cells led to significantly reduced numbers of metastatic tumours in lung and bone in OPN-deficient mice compared with wild-type mice, pointing to a role of host OPN in melanoma metastasis to these sites [99]. In gastric cancer, a high degree of OPN expression was seen in gastric cancer cells that had metastasized to the lymph node, as well as in surrounding macrophages [39]. These data would thus suggest that OPN may promote survival and establishment of tumour cells at secondary sites such as lymph node, lung and bone.
OPN in the bone microenvironment

OPN is produced by osteoblasts, osteoclasts, osteocytes and macrophages in the bone microenvironment [100]. In bone metastases, expression of OPN has been detected in both osteoclasts and metastatic tumour cells [101]. Kang et al. [102] identified OPN in a bone metastasis gene signature, such that breast cancer cells that spread to bone had increased OPN expression. Consistent with this finding, it has been shown that melanoma cells with decreased OPN expression have lower incidence of bone metastases [99]. In addition, OPN expression may differ between bone metastases with a more osteolytic versus osteoblastic pattern of involvement [103]. For instance, cancer cells in bone metastases from breast carcinomas (mixed osteolytic and osteoblastic/sclerotic pattern) have strong OPN expression, compared to lower OPN expression in bone metastases from prostate carcinomas (more osteoblastic/sclerotic pattern) [103].

A number of studies have indicated that OPN may play a role in early tumour cell colonization of the bone [33, 99, 102, 104]. Interaction between cancer cells and bone marrow endothelial cells is thought to be an early step in bone metastasis formation [105]. Breast cancer cells without OPN have decreased ability to bind bone marrow endothelial cells [33, 106]. OPN expression by (prostatic) carcinoma cells is also induced because of physical contact with bone marrow stromal cells [107]. As contact among cancer cells, bone marrow stromal cells and bone marrow endothelial cells is important for both survival and proliferation, OPN’s presence may be important for metastatic cancer growth in the bone microenvironment. OPN also helps regulate osteoclast activation and adhesion to the bone surface, activating the mechanism of resorption which aids in bone metastasis formation [88, 101, 104]. Thus, OPN is present in tumour cells which have spread to the bone, as well as in a number of cell types in the bone itself, and may play a functional role in helping cancer cells survive and grow within the bone microenvironment.

Conclusions

As seen above, OPN is expressed in tumour cells, inflammatory cells and stromal cells in a variety of cancers. It influences recruitment and survival of cells, may be incorporated into the extracellular matrix and is associated with poor prognosis in cancer patients. OPN may affect the tumour microenvironment by attracting inflammatory cells and fibroblasts/myofibroblasts, aiding in angiogenesis, protecting from apoptosis and increasing migratory and invasive abilities of cells to aid in metastasis. OPN is highly post-translationally modified, and effects of the various modifications on its function are as of yet poorly understood. Although there may be structural differences in tumour-derived versus stromal-derived OPN, there may also be functional differences in how the target cell responds, depending on the cell type and tissue context. It is now known that the tumour microenvironment plays an important role in tumour development and progression. As more therapies are developed to target aspects of the tumour environment, such as anti-angiogenesis drugs, it will become increasingly important to understand how the tumour environment develops and influences tumour growth. In many contexts, OPN may be an attractive target for cancer therapy, as it would allow for a simultaneous attack on both the tumour cells themselves and on interactions with the microenvironment that promote tumour progression. There is still much to learn about this multifaceted protein, and future work should continue to investigate its mechanisms of action both within tumour cells and the surrounding microenvironment.

Acknowledgements

This work is supported by an award from the Lloyd Carr-Harris Foundation. J.C.M. is supported by a Canadian Graduate Award from Canadian Institutes of Health Research and a Studentship from the Translational Breast Cancer Research Unit at the London Regional Cancer Program. A.F.C. is Canada Research Chair in Oncology, supported by the Canada Research Chairs Program.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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