Role of osteopontin in adhesion, migration, cell survival and bone remodeling

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Osteopontin (OPN) is a secreted adhesive glycoprophosphoprotein expressed by several cell types. It is normally produced in bone, teeth, kidney and epithelial lining tissues and is found in plasma and breast milk. It is involved in a number of physiological and pathologic events including angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis. In this review focus will be on OPN in bone and its role in adhesion, migration and cell survival. These aspects of OPN biology are important in tumorigenesis.

OPN — general properties. OPN's versatile functions are illustrated by early articles describing this protein. Initially, OPN was described as a major noncollagenous protein in bone and named bone sialoprotein I [1].

The paper by Senger and colleagues [2] describes a protein whose secretion was elevated in cultures of transformed cells, indicating a function of it in tumor biology. In addition, OPN is important in immune activity and bacterial resistance and was named early T-lymphocyte activation 1 protein (Eta-1) by Patarca et al [3] in 1989. Today the protein is designated OPN and is known to be involved in bone resorption, wound repair, immune function, angiogenesis, cell survival and cancer biology.

OPN is an acidic protein consisting of about 300 amino acids. The protein is highly phosphorylated and glycosylated and has an arginine–glycine–aspartic acid (RGD)—binding domain as well as two heparin—binding sites, one thrombin cleavage site and a putative calcium binding site [4]. Matrix metalloproteases 3 and 7 also cleave OPN [5]. Cleavage of OPN by proteases (most notably thrombin) generates two functional fragments, an RGD—containing N—terminal part that binds to integrin receptors and a C—terminal fragment which interacts with CD44 variants. The C—terminal fragment also contains the two heparin—binding sites [4]. Both cleaved and native OPN can bind integrins, although some cells adhere more strongly to cleaved than native OPN [6—9]. Cleavage of OPN by thrombin unmasks epitopes that are hidden in the native protein, and also makes the RGD—motif more accessible in some cases.

OPN is encoded by a single gene, and its promoter is responsive to a number of different transcription factors [10, 11].

Cell survival. Both soluble OPN, which works as a cytokine, and immobilized OPN, which function as an extracellular matrix protein, protect against apoptosis and induce survival and proliferation in several cell types.

OPN has a pro—survival and/or proliferative function in adherent cell types such as smooth muscle cells [12] and epithelial cells [13]. Endothelial cells plated on OPN—coated surfaces are protected against apoptosis induced by serum deprivation or by TRAIL [14, 15]. OPN’s survival function on endothelial cells is mediated through αvβ3 integrin and nuclear factor kappa B (NF—kB) activation [14]. Furthermore, it was recently shown that OPN—induced NF—kB activation in endothelial cells leads to enhanced expression of osteoprotegerin (OPG). OPG forms a complex with TRAIL which subsequently blocks apoptosis [15, 16]. In another study, soluble OPN was found to inhibit apoptosis of adherent endothelial cells deprived of growth factors [17]. In contrast to the NF—kB pathway probably involved in immobilized OPN's anti—apoptotic effect, stimulation by soluble OPN was shown to cause an up—regulation and re—distribution of Bcl—XL. Although both immobilized and soluble OPN seem to protect adherent endothelial cells, soluble OPN has no anti—apoptotic effects on endothelial cells held in suspension [18].

However, other cells grown in suspension are protected from apoptosis by OPN. An example of this is the none—adherent IL—3—dependent pro—B cell line BA/F3. When these cells are stressed (deprived of IL—3), OPN protects against apoptosis via CD44 interaction and seems to involve PI 3K—Akt downstream signaling pathway [19, 20]. OPN thus seems to give pro—survival signals both through integrins and through CD44, and the downstream signaling pathways seem to differ and depend both on which receptors are engaged and on cell type.

OPN is strongly associated with tumorigenesis. In patients with breast cancer [21—23], multiple myeloma [24] and prostate cancer [25] high plasma levels or tumor expression of OPN are associated with poor prognosis. Thus, not surprisingly, OPN is expressed by several types of cancer cells [26—29]. Some of them also respond to OPN with enhanced survival and proliferation. A recent study [30] indicates that epidermal growth factor (EGF) dependent proliferation of prostate cancer cells is amplified by OPN. This is probably due to an interaction of OPN with beta1 integrins on the surface of the cancer cells, leading to sustained activation of the epidermal growth factor receptor (EGFR). Epidermal growth factor (EGF) is an important growth factor for prostate cancer cells and this interaction thereby promotes proliferation of the cancer cells. Similarly, IL—6 is an important growth promoting/survival factor for myeloma cells, and OPN has been shown to promote my—

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Abbreviations used: OPN — osteopontin.
eloma cell growth in combination with IL–6. Receptors involved are αβ, and αβ3 [31] but the precise molecular mechanisms are currently not known. Breast carcinoma cells are protected against apoptosis when they adhere to OPN via αβ3 [32]. Although cancer cells often produce OPN, they also give rise to enhanced OPN production by host cells such as stromal cells/osteoblasts [29, 33] and osteoclasts [31]. OPN effects on tumor cells can thus be both autocrine and paracrine.

**Adhesion and migration.** OPN’s association with cancer can partly be explained by its anti–apoptotic and proliferative effect on tumor cells. However, OPN’s role as an adhesive and migratory factor might be even more important in tumorigenesis. Increased adhesion and migration may confer metastatic capacity and invasiveness to tumor cells.

Most cells adhere to OPN through integrins. Both α5 (β1, β2, β3) and (α9, α2, α3)β1 on several cell types bind to OPN [7, 34–36]. The binding of αβ, αββ3, and αββ3 integrins to OPN is RGD–dependent [6, 37–39], whereas the binding of αβ, and αββ3 involves other amino acid sequences [40, 41]. Cells can also bind to OPN via some splice forms of CD44. The binding of CD44–variants to OPN is RGD–independent [42] but seems to require β1 containing integrins [43].

The chemotaxtactic property of OPN has been demonstrated in the migration of monocytic cells/macrophages, T–cells, smooth muscle cells, endothelial cells, epithelial cells, and several malignant cells [8, 39, 42, 44, 45]. Malignant cells often show an increased responsiveness to OPN as compared to their normal counterparts. In cancer, integrin expression is often aberrant on malignant cells [46–48]. Tumorigenic and highly metastatic mammary epithelial cells migrate toward OPN in an αββ3–dependent way whereas non–malignant and less malignant epithelial cells do not express αββ3. Migration of the latter cells to OPN is dependent on αββ2 and β3 [49, 50]. Transfection of the less malignant cells lacking αββ3 with β3 leads to increased adherence, migration, and invasiveness in vitro and also leads to increased tumorigenesis in vivo [50]. Cells expressing αββ3 are more responsive to OPN than cells lacking this integrin and the increased responsiveness may enhance the malignancy–inducing effect of OPN. Migration induced by OPN via integrin is dependent on at least two different growth factor/receptor pathways (HGF/Met and EGF ligands/EGF) and multiple signaling pathways [49, 51, 52].

Increased motility in combination with increased protease expression may lead to increased invasiveness. Breast cancer cells invade through basement membrane in response to OPN [45] and the invasion seems to be dependent on secretion of uPA [53]. This important protease is secreted due to OPN signaling via αβ, and further activating c–Src/EGFR/ERK/AP–1 and/or PI–3 kinase/Akt/NF–κB [54, 55]. Also prostate cancer cells respond to OPN in an αββ3–dependent way with increased chemotaxis, invasion and up–regulation of plasminogen activators [56].

The findings discussed above indicate that OPN and in particular OPN interacting with αββ3 is important for the spread of breast and prostate cancer. OPN’s role as a metastasis–enhancing protein is further emphasized by the fact that OPN–deficient mice have reduced number of metastases to bone and soft tissue [57] and other studies linking OPN expression and responsiveness to increased cancer risk [45, 58, 59]. Although OPN–αβ interaction seem to be of particular importance in breast and prostate cancer metastases other receptors, such as CD44, may be of importance in the malignancy and spread of other cancer cells [27, 43].

Recent findings indicate that an intracellular form of OPN (iOPN) exists [60, 61]. In migrating fibroblasts, macrophages, osteoclasts and metastatic breast cancer cell lines iOPN co–localizes with CD44 in cell processes and at the cell membrane. Furthermore, iOPN and CD44 associate with ezrin–radixin–moesin proteins inducing cytoskeleton rearrangement and signal–involved in cell migration, indicating that iOPN plays an important role in cell movement [61–63].

**OPN in bone.** Bone remodeling is a regulated process in which removal of old bone by osteoclasts is followed by bone–formation by osteoblasts. OPN influences bone homeostasis both by inhibiting mineral deposition, by promoting differentiation of osteoclasts and by enhancing osteoclast activity.

**Inhibition of mineral deposition.** Bone matrix consists of an inorganic component in the form of hydroxyapatite (HA = [Ca10 (PO4)6 (OH)2]) and an organic component consisting of proteins and proteoglycans. OPN is one of the major non–collagenous proteins in bone. Its electronegative glutamic and aspartic acid residues as well as the putative Ca2+ binding motif make it bind tightly to HA. OPN is a potent inhibitor of the mineralization process, since binding of OPN to HA inhibits growth of HA crystals [64–66].

**Differentiation of osteoclasts.** Osteoclasts develop from precursor cells in the monocyte/macrophage family to become giant multinucleated cells capable of resorbing bone. The maturation and differentiation of macrophages to osteoclasts are dependent on two factors secreted or expressed by stromal cells/osteoblasts: macrophage colony stimulating factor (M–CSF) and receptor for activation of NF–κB ligand (RANKL) [67]. Engagement of these cytokines with their receptors, c–fms and RANK, on the osteoclast precursor cells induces maturation of the osteoclast osteoprotegerin (OPG) is a decay receptor for RANKL. It is secreted by osteoblasts/stromal cells and inhibits interaction of RANKL with RANK and thereby inhibits osteoclastogenesis.

OPN does not seem to be an essential factor in the development of osteoclasts during normal bone development since osteoclast number and distribution are normal in OPN knock–out mice [68, 69]. However, several studies show that in pathological situations, OPN is of importance in osteoclastogenesis. PTH–induced RANKL signaling normally results in either an increase in osteoclast number and/or activation, but this increase is disrupted in the absence of OPN [70]. Similarly, ovariectomy of normal mice (as a model of postmenopausal osteoporosis) results in a 3–fold increase of TRAP+ cells whereas ovariectomized
OPN$^{-/-}$ mice lack the capability to increase osteoclast number [71]. Furthermore, loss of mechanical stress normally leads to increased bone resorption and an increase in osteoclast number. This effect in not seen in OPN$^{-/-}$ mice, and OPN thus seems to be required for the effects of mechanical stress on bone [72].

*In vitro*, neutralization of OPN suppresses osteoclastogenesis, whereas addition of OPN enhances osteoclastogenesis in OPN$^{-/-}$ cells [73, 74]. OPN has also been shown to influence osteoclastogenesis by enhancing RANKL and decreasing OPG expression on stromal cells [74].

Although both Rittling et al [68] and Liaw et al [69] found that osteoclast number was normal in OPN$^{-/-}$ mouse, the number of osteoclasts in OPN$^{-/-}$ mice was about 3-fold higher than in normal mice in the study by Yoshitake et al [71]. This is also reported by Chelliah et al [75]. The increase in number of osteoclasts may be compensatory for the decreased activity of OPN$^{-/-}$ osteoclasts [75].

**Osteoclast activity.** In mineralized tissues, OPN is secreted by both osteoblasts and osteoclasts [73, 76, 77]. OPN is highly concentrated in sites where preexisting and newly formed bone meet (cement lines), and in bone surfaces interacting with cells (laminae limitantes) [78–80].

Several studies suggest that OPN in laminae limitantes mediate the attachment of osteoclasts to bone. $\alpha_1\beta_1$ integrin is located in the sealing zone of osteoclasts and interacts with OPN in the bone matrix (96, 79, 81–83). OPN stimulate osteoclast migration through $\alpha_1\beta_1$ and CD44 [84–86] and also increases CD44 expression on osteoclasts. The expression of CD44 is necessary for osteoclast motility [75]. Osteoclasts deficient in OPN do not migrate and are unable to resorb bone [86, 87]. The bone-resorbing activity can only partially be restored by exogenous OPN, indicating that autocrine OPN is important to osteoclast activity [75]. OPN secreted by the osteoclast is present in the resorption lacunae in which exogenously added OPN does not have access. The need for autocrine OPN to get fully functional osteoclasts could also be due to the need of the expression of intracellular OPN [82].

**Osteolysis in myeloma and breast cancer.** Breast cancer cells frequently metastasize to bone and commonly give rise to osteolysis. Breast cancer patients with metastases to the skeleton have higher levels of OPN than patients without such metastases [21].

Breast cancer and prostate cancer cells that induce osteolytic lesions are able to enhance expression of OPN in osteoblasts, whereas cancer cells that induce osteosclerotic effects have no effect on OPN expression [33]. This finding indicates that osteolysis induced by tumor cells metastasizing to the skeleton could be due to enhanced OPN expression by osteoblasts. On the other hand, breast cancer cells themselves express OPN [23, 26, 88], so enhanced osteoclast activity could be due to both tumor cell and host cell OPN.

Osteolysis is also a common complication of multiple myeloma, and, similarly, myeloma cells express OPN [24, 29, 89] and increases OPN expression in osteoblasts/stromal cells [29] and osteoclasts [31]. Levels of circulating OPN in myeloma patients have recently been shown to be increased as compared to levels of OPN in healthy individuals [24, 29] and to correlate to bone disease and prognosis [24]. However, the correlation to bone disease and prognosis is not confirmed, since Standal et al [29] found no such correlations in their patient material (although a significant correlation to serum calcium could indicate a connection of OPN to bone disease). Both studies found that MM patients had higher plasma concentration of OPN than MGUS patients. A major difference between MM and MGUS patients is the lack of bone affection in MGUS patients. Taken together, this could indicate that OPN is implicated in the bone destruction in MM although more studies are needed to confirm this hypothesis. During the last few years, several reports show that bone homeostasis in patients with multiple myeloma is disrupted due to an imbalance in the RANKL/RANK/OPG system [90–94]. OPN appears to be an important downstream factor in RANKL-mediated bone resorption [70] and deregulation of OPN could thus contribute to the imbalance in bone homeostasis in this disease.

**Conclusion.** OPN is a player in several processes and functions in different ways in different cells in different systems. In this review, OPN’s role in tumorogenesis has been exemplified by giving a brief overview over its role in cell survival, adhesion, migration and bone remodeling. Other aspects of OPN biology that are important in malignancy are OPN’s role in angiogenesis and immune function. A recent review covering these topics was published [95]. The transcriptional control of OPN has not been mentioned here but are excellently reviewed in [11].

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and spreading and is chemotactic for smooth muscle cells in vitro. 


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РОЛЬ ОСТЕОПОТИНА В АДГЕЗИИ, МИГРАЦИИ И ВЫЖИВАНИИ КЛЕТОК ВОССТАНОВЛЕНИЯ КОСТОЙ ТКАНИ

Остеопринин, секретируемый адгезивными гликофосфопротеинами, экспрессируется некоторыми типами клеток. В норме он продуцируется в ткани костей, зубов, почек и эпителия и обнаруживается в плазме крови и грудном молоке. Остеопонин принимает участие в некоторых физиологических и патологических процессах, в том числе ангиогенезе, апоптозе, воспалении, заживлении ран и метастазировании опухолей. В обзоре рассматривается роль остеопонина в костной ткани, адгезии, миграции и выживании клеток, так как эти аспекты имеют важное значение в биологии опухолей.

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