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

Pieter H. Anborgh [a], **Jennifer C. Mutrie** [a, b], **Alan B. Tuck** [a, b, c, d], **Ann F. Chambers** [a, b, d], *

[a] *London Regional Cancer Program, London, Ontario, Canada*
[b] *Department of Pathology, University of Western Ontario, London, Ontario, Canada*
[c] *Department of Pathology, London Health Sciences Centre, London, Ontario, Canada*
[d] *Department of Oncology, University of Western Ontario, London, Ontario, Canada*

- Structure and functional properties of osteopontin (OPN)
- OPN in the tumour microenvironment
- Effect of OPN on the tumour microenvironment
- Effect of OPN in the tumour microenvironment on tumour progression
- OPN in the bone microenvironment
- Conclusions

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 glycophosphoprotein 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.

*Correspondence to: Ann F. CHAMBERS,
London Regional Cancer Program, 790 Commissioners Road East,
London, Ontario N6A 4L6 Canada.

Tel.: 519–685-8652
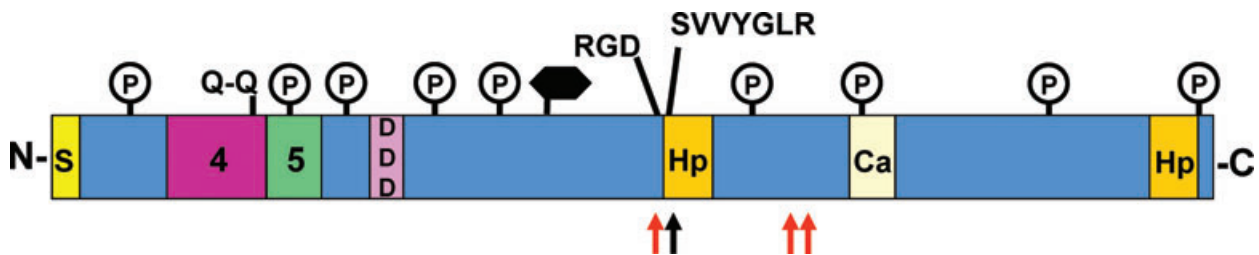Fax: 519–685-8646
E-mail: ann.chambers@Lhsc.on.ca

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

genetically manipulated animal models and the use of transformed cell lines [33].

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.
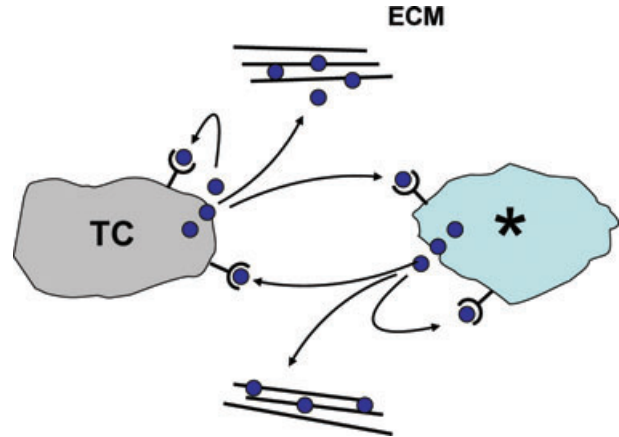

Fig. 2 OPN signalling in the tumour microenvironment. Both tumour cells (TC) and non-tumour cells (*), such as macrophages, NK cells, other immune cells, fibroblasts, myofibroblasts, endothelial cells, etc., may produce and secrete OPN (solid circles) in the tumour microenvironment. In either case, the secreted OPN may act on the secreting cell itself, *via* autocrine mechanisms, or may act on a different cell in the tumour microenvironment, *via* paracrine mechanisms, provided those cells have the appropriate integrin or CD44 family cell surface receptors. Secreted OPN may also be incorporated into the extracellular matrix.

Effect of OPN on the tumour microenvironment

Because it is a secreted protein, OPN produced by tumour cells can influence cells in the tumour microenvironment and vice versa (Fig. 2). Whether tumour-derived OPN differs, structurally or functionally, in its effects compared to stromal-derived OPN is largely unknown. Although there is evidence that different OPN isoforms and post-translational modifications (*e.g.* phosphorylation, sialation, proteolytic cleavage, transglutaminse crosslinking, proteolytic processing) may affect different OPN functions [11, 14, 15, 18, 59], there is very scant literature on how tumour-derived OPN may differ from stroma-derived OPN, either structurally or functionally.

That being said, there is evidence for a variety of different effects of OPN (regardless of its source) on the tumour microenvironment. For example, although OPN is able to attract macrophages by chemotaxis to a tumour site, its action on macrophages includes inhibition of function, at least in part through down-regulation of nitric oxide production (*via* down-regulation of inducible nitric oxide synthase [iNOS]), resulting in decreased apoptotic effect on the tumour cells themselves [60–64]. In addition, as there is evidence that the presence of macrophages in the tumour microenvironment can promote tumour invasion and metastasis *via* elaboration of epidermal growth factor and activation of tumour epidermal growth factor receptor responses [65] and secretion of various proteases (*e.g.* MMPs, urokinase plasminogen activator, cathepsin B, etc. [66]),

the recruitment of macrophages to the tumour microenvironment could have multiple indirect effects to promote malignancy. In contrast, as 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 pathophysiological 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 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.

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

Conclusions

As seen above, OPN is expressed in tumour cells, inflammatory cells and stromal cells in a variety of cancers. It influences

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. **Rangaswami H, Bulbule A, Kundu GC.** Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol.* 2006; 16: 79–87.

2. **Weber GF.** The metastasis gene osteopontin: a candidate target for cancer therapy. *Biochim Biophys Acta.* 2001; 1552: 61–85.

3. **Franzen A, Heinegard D.** Isolation and characterization of two sialoproteins present only in bone calcified matrix. *Biochem J.* 1985; 232: 715–24.

4. **Senger DR, Perruzzi CA, Papadopoulos A.** Elevated expression of secreted phosphoprotein I (osteopontin, 2ar) as a conse-

quence of neoplastic transformation. *Anticancer Res.* 1989; 9: 1291–9.

5. **Patarca R, Freeman GJ, Singh RP, *et al.*** Structural and functional studies of the early T lymphocyte activation 1 (Eta-1) gene. Definition of a novel T cell-dependent response associated with genetic

resistance to bacterial infection. *J Exp Med.* 1989; 170: 145–61.

6. **Rittling SR, Chambers AF.** Role of osteopontin in tumour progression. *Br J Cancer.* 2004; 90: 1877–81.

7. **Tuck AB, Chambers AF, Allan AL.** Osteopontin overexpression in breast cancer: knowledge gained and possible implications for clinical management. *J Cell Biochem.* 2007; 102: 859–68.

8. **Cho HJ, Cho HJ, Kim HS.** Osteopontin: a multifunctional protein at the crossroads of inflammation, atherosclerosis, and vascular calcification. *Curr Atheroscler Rep.* 2009; 11: 206–13.

9. **Balkwill F, Mantovani A.** Inflammation and cancer: back to Virchow? *Lancet.* 2001; 357: 539–45.

10. **Pollard JW.** Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer.* 2004; 4: 71–8.

11. **Kazanecki CC, Uzwiak DJ, Denhardt DT.** Control of osteopontin signaling and function by post-translational phosphorylation and protein folding. *J Cell Biochem.* 2007; 102: 912–24.

12. **He B, Mirza M, Weber GF.** An osteopontin splice variant induces anchorage independence in human breast cancer cells. *Oncogene.* 2006; 25: 2192–202.

13. **Blasberg JD, Goparaju CM, Pass HI, et al.** Lung cancer osteopontin isoforms exhibit angiogenic functional heterogeneity. *J Thorac Cardiovasc Surg.* 2010; 139: 1587–93.

14. **Christensen B, Petersen TE, Sorensen ES.** Post-translational modification and proteolytic processing of urinary osteopontin. *Biochem J.* 2008; 411: 53–61.

15. **Christensen B, Kazanecki CC, Petersen TE, et al.** Cell type-specific post-translational modifications of mouse osteopontin are associated with different adhesive properties. *J Biol Chem.* 2007; 282: 19463–72.

16. **Zhang H, Hunter GK, Goldberg HA, et al.** An integrated procedure of selective injection, sample stacking and fractionation of phosphopeptides for MALDI MS analysis. *Anal Chim Acta.* 2007; 581: 268–80.

17. **Anborgh PH, Wilson SM, Tuck AB, et al.** New dual monoclonal ELISA for measuring plasma osteopontin as a biomarker associated with survival in prostate cancer: clinical validation and comparison of multiple ELISAs. *Clin Chem.* 2009; 55: 895–903.

18. **Al-Shami R, Sorensen ES, Ek-Rylander B, et al.** Phosphorylated osteopontin promotes migration of human choriocarcinoma cells *via* a p70 S6 kinase-dependent

pathway. *J Cell Biochem.* 2005; 94: 1218–33.

19. **Xuan JW, Hota C, Chambers AF.** Recombinant GST-human osteopontin fusion protein is functional in RGD-dependent cell adhesion. *J Cell Biochem.* 1994; 54: 247–55.

20. **Furger KA, Allan AL, Wilson SM, et al.** Beta(3) integrin expression increases breast carcinoma cell responsiveness to the malignancy-enhancing effects of osteopontin. *Mol Cancer Res.* 2003; 1: 810–9.

21. **Tuck AB, Hota C, Wilson SM, et al.** Osteopontin-induced migration of human mammary epithelial cells involves activation of EGF receptor and multiple signal transduction pathways. *Oncogene.* 2003; 22: 1198–205.

22. **Tuck AB, Arsenault DM, O'Malley FP, et al.** Osteopontin induces increased invasiveness and plasminogen activator expression of human mammary epithelial cells. *Oncogene.* 1999; 18: 4237–46.

23. **Tuck AB, Elliott BE, Hota C, et al.** Osteopontin-induced, integrin-dependent migration of human mammary epithelial cells involves activation of the hepatocyte growth factor receptor (Met). *J Cell Biochem.* 2000; 78: 465–75.

24. **Higashikawa F, Eboshida A, Yokosaki Y.** Enhanced biological activity of polymeric osteopontin. *FEBS Lett.* 2007; 581: 2697–701.

25. **Senger DR, Perruzzi CA, Papadopoulos-Sergiou A, et al.** Adhesive properties of osteopontin: regulation by a naturally occurring thrombin-cleavage in close proximity to the GRGDS cell-binding domain. *Mol Biol Cell.* 1994; 5: 565–74.

26. **Agnihotri R, Crawford HC, Haro H, et al.** Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). *J Biol Chem.* 2001; 276: 28261–7.

27. **Takafuji V, Forgues M, Unsworth E, et al.** An osteopontin fragment is essential for tumor cell invasion in hepatocellular carcinoma. *Oncogene.* 2007; 26: 6361–71.

28. **Schulze EB, Hedley BD, Goodale D, et al.** The thrombin inhibitor Argatroban reduces breast cancer malignancy and metastasis *via* osteopontin-dependent and osteopontin-independent mechanisms. *Breast Cancer Res Treat.* 2008; 112: 243–54.

29. **Bellahcene A, Castronovo V, Ogbureke KU, et al.** Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat Rev Cancer.* 2008; 8: 212–26.

30. **Polyak K, Haviv I, Campbell IG.** Co-evolution of tumor cells and their microenvironment. *Trends Genet.* 2009; 25: 30–8.

31. **De Wever O, Demetter P, Mareel M, et al.** Stromal myofibroblasts are drivers of invasive cancer growth. *Int J Cancer.* 2008; 123: 2229–38.

32. **Li H, Fan X, Houghton J.** Tumor microenvironment: the role of the tumor stroma in cancer. *J Cell Biochem.* 2007; 101: 805–15.

33. **Shevde LA, Das S, Clark DW, et al.** Osteopontin: an effector and an effect of tumor metastasis. *Curr Mol Med.* 2010; 10: 71–81.

34. **Brown LF, Papadopoulos-Sergiou A, Berse B, et al.** Osteopontin expression and distribution in human carcinomas. *Am J Pathol.* 1994; 145: 610–23.

35. **Coppola D, Szabo M, Boulware D, et al.** Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies. *Clin Cancer Res.* 2004; 10: 184–90.

36. **Tuck AB, O'Malley FP, Singhal H, et al.** Osteopontin expression in a group of lymph node negative breast cancer patients. *Int J Cancer.* 1998; 79: 502–8.

37. **Forootan SS, Foster CS, Aachi VR, et al.** Prognostic significance of osteopontin expression in human prostate cancer. *Int J Cancer.* 2006; 118: 2255–61.

38. **Agrawal D, Chen T, Irby R, et al.** Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. *J Natl Cancer Inst.* 2002; 94: 513–21.

39. **Imano M, Satou T, Itoh T, et al.** Immunohistochemical expression of osteopontin in gastric cancer. *J Gastrointest Surg.* 2009; 13: 1577–82.

40. **Ye QH, Qin LX, Forgues M, et al.** Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med.* 2003; 9: 416–23.

41. **Zhang J, Takahashi K, Takahashi F, et al.** Differential osteopontin expression in lung cancer. *Cancer Lett.* 2001; 171: 215–22.

42. **Chambers AF, Wilson SM, Kerkvliet N, et al.** Osteopontin expression in lung cancer. *Lung Cancer.* 1996; 15: 311–23.

43. **Pass HI, Lott D, Lonardo F, et al.** Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med.* 2005; 353: 1564–73.

44. **Chien CY, Su CY, Chuang HC, et al.** Comprehensive study on the prognostic role of osteopontin expression in oral

squamous cell carcinoma. *Oral Oncol.* 2009; 45: 798–802.

45. **Sulzbacher I, Birner P, Trieb K, *et al.*** Expression of osteopontin and vascular endothelial growth factor in benign and malignant bone tumors. *Virchows Arch.* 2002; 441: 345–9.

46. **Bramwell VH, Tuck AB, Wilson SM, *et al.*** Expression of osteopontin and HGF/met in adult soft tissue tumors. *Cancer Biol Ther.* 2005; 4: 1336–41.

47. **Saeki Y, Mima T, Ishii T, *et al.*** Enhanced production of osteopontin in multiple myeloma: clinical and pathogenic implications. *Br J Haematol.* 2003; 123: 263–70.

48. **Wang-Rodriguez J, Urquidi V, Rivard A, *et al.*** Elevated osteopontin and thrombospondin expression identifies malignant human breast carcinoma but is not indicative of metastatic status. *Breast Cancer Res.* 2003; 5: R136–43.

49. **Gaumann A, Petrow P, Mentzel T, *et al.*** Osteopontin expression in primary sarcomas of the pulmonary artery. *Virchows Arch.* 2001; 439: 668–74.

50. **Chang PL, Harkins L, Hsieh YH, *et al.*** Osteopontin expression in normal skin and non-melanoma skin tumors. *J Histochem Cytochem.* 2008; 56: 57–66.

51. **Casson AG, Wilson SM, McCart JA, *et al.*** ras mutation and expression of the ras-regulated genes osteopontin and cathepsin L in human esophageal cancer. *Int J Cancer.* 1997; 72: 739–45.

52. **Weismann D, Briese J, Niemann J, *et al.*** Osteopontin stimulates invasion of NCI-h295 cells but is not associated with survival in adrenocortical carcinoma. *J Pathol.* 2009; 218: 232–40.

53. **Gotoh M, Sakamoto M, Kanetaka K, *et al.*** Overexpression of osteopontin in hepatocellular carcinoma. *Pathol Int.* 2002; 52: 19–24.

54. **Kim J, Ki SS, Lee SD, *et al.*** Elevated plasma osteopontin levels in patients with hepatocellular carcinoma. *Am J Gastroenterol.* 2006; 101: 2051–9.

55. **Reinholt FP, Hultenby K, Oldberg A, *et al.*** Osteopontin–a possible anchor of osteoclasts to bone. *Proc Natl Acad Sci USA.* 1990; 87: 4473–5.

56. **Sullivan J, Blair L, Alnajar A, *et al.*** Expression of a prometastatic splice variant of osteopontin, OPNC, in human pancreatic ductal adenocarcinoma. *Surgery.* 2009; 146: 232–40.

57. **Finak G, Bertos N, Pepin F, *et al.*** Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med.* 2008; 14: 518–27.

58. **Rittling SR, Chen Y, Feng F, *et al.*** Tumor-derived osteopontin is soluble, not matrix associated. *J Biol Chem.* 2002; 277: 9175–82.

59. **Kubota T, Zhang Q, Wrana JL, *et al.*** Multiple forms of Sppl (secreted phosphoprotein, osteopontin) synthesized by normal and transformed rat bone cell populations: regulation by TGF-beta. *Biochem Biophys Res Commun.* 1989; 162: 1453–9.

60. **Schwertfeger KL, Xian W, Kaplan AM, *et al.*** A critical role for the inflammatory response in a mouse model of preneoplastic progression. *Cancer Res.* 2006; 66: 5676–85.

61. **Wai PY, Guo L, Gao C, *et al.*** Osteopontin inhibits macrophage nitric oxide synthesis to enhance tumor proliferation. *Surgery.* 2006; 140: 132–40.

62. **Feng B, Rollo EE, Denhardt DT.** Osteopontin (OPN) may facilitate metastasis by protecting cells from macrophage NO-mediated cytotoxicity: evidence from cell lines down-regulated for OPN expression by a targeted ribozyme. *Clin Exp Metastasis.* 1995; 13: 453–62.

63. **Denhardt DT, Chambers AF.** Overcoming obstacles to metastasis–defenses against host defenses: osteopontin (OPN) as a shield against attack by cytotoxic host cells. *J Cell Biochem.* 1994; 56: 48–51.

64. **Crawford HC, Matrisian LM, Liaw L.** Distinct roles of osteopontin in host defense activity and tumor survival during squamous cell carcinoma progression *in vivo. Cancer Res.* 1998; 58: 5206–15.

65. **Patsialou A, Wyckoff J, Wang Y, *et al.*** Invasion of human breast cancer cells *in vivo* requires both paracrine and autocrine loops involving the colony-stimulating factor-1 receptor. *Cancer Res.* 2009; 69: 9498–506.

66. **Shih J, Yuan A, Chen JJ-, Yang P.** Tumor-associated macrophage: its role in cancer invasion and metastasis. *J Cancer Mol.* 2006; 2: 101–6.

67. **Diao H, Iwabuchi K, Li L, *et al.*** Osteopontin regulates development and function of invariant natural killer T cells. *Proc Natl Acad Sci USA.* 2008; 105: 15884–9.

68. **Larkin J, Renukaradhya GJ, Sriram V, *et al.*** CD44 differentially activates mouse NK T cells and conventional T cells. *J Immunol.* 2006; 177: 268–79.

69. **Terabe M, Berzofsky JA.** NKT cells in immunoregulation of tumor immunity: a new immunoregulatory axis. *Trends Immunol.* 2007; 28: 491–6.

70. **Bostrom K.** Osteopontin, a missing link in PDGF-induced smooth muscle cell migration. *Cardiovasc Res.* 2007; 75: 634–5.

71. **Lu C, Bonome T, Li Y, *et al.*** Gene alterations identified by expression profiling in tumor-associated endothelial cells from invasive ovarian carcinoma. *Cancer Res.* 2007; 67: 1757–68.

72. **Du XL, Jiang T, Zhao WB, *et al.*** Gene alterations in tumor-associated endothelial cells from endometrial cancer. *Int J Mol Med.* 2008; 22: 619–32.

73. **Hanahan D, Folkman J.** Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell.* 1996; 86: 353–64.

74. **Chakraborty G, Jain S, Kundu GC.** Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis *via* autocrine and paracrine mechanisms. *Cancer Res.* 2008; 68: 152–61.

75. **Shijubo N, Uede T, Kon S, *et al.*** Vascular endothelial growth factor and osteopontin in stage I lung adenocarcinoma. *Am J Respir Crit Care Med.* 1999; 160: 1269–73.

76. **Shijubo N, Uede T, Kon S, *et al.*** Vascular endothelial growth factor and osteopontin in tumor biology. *Crit Rev Oncog.* 2000; 11: 135–46.

77. **Takahashi F, Akutagawa S, Fukumoto H, *et al.*** Osteopontin induces angiogenesis of murine neuroblastoma cells in mice. *Int J Cancer.* 2002; 98: 707–12.

78. **Dai J, Peng L, Fan K, *et al.*** Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells. *Oncogene.* 2009; 28: 3412–22.

79. **Leali D, Dell'Era P, Stabile H, *et al.*** Osteopontin (Eta-1) and fibroblast growth factor-2 cross-talk in angiogenesis. *J Immunol.* 2003; 171: 1085–93.

80. **McAllister SS, Gifford AM, Greiner AL, *et al.*** Systemic endocrine instigation of indolent tumor growth requires osteopontin. *Cell.* 2008; 133: 994–1005.

81. **Bellahcene A, Castronovo V.** Increased expression of osteonectin and osteopontin, two bone matrix proteins, in human breast cancer. *Am J Pathol.* 1995; 146: 95–100.

82. **Oyama T, Sano T, Hikino T, *et al.*** Microcalcifications of breast cancer and atypical cystic lobules associated with infiltration of foam cells expressing osteopontin. *Virchows Arch.* 2002; 440: 267–73.

83. **Wang X, Chao L, Ma G, *et al.*** Primary breast carcinoma: association of mammographic calcifications with osteopontin expression. *Radiology.* 2010; 254: 69–78.

84. **Hirota S, Nakajima Y, Yoshimine T, *et al.*** Expression of bone-related protein messenger RNA in human meningiomas: possible involvement of osteopontin in development of psammoma bodies. *J Neuropathol Exp Neurol.* 1995; 54: 698–703.

85. **Maki M, Hirota S, Kaneko Y, *et al.*** Expression of osteopontin messenger RNA by macrophages in ovarian serous papillary cystadenocarcinoma: a possible association with calcification of psammoma bodies. *Pathol Int.* 2000; 50: 531–5.

86. **Bai Y, Zhou G, Nakamura M, *et al.*** Survival impact of psammoma body, stromal calcification, and bone formation in papillary thyroid carcinoma. *Mod Pathol.* 2009; 22: 887–94.

87. **Kuroda N, Hamaguchi N, Ohara M, *et al.*** Large cell neuroendocrine carcinoma of the lung with calcification and expression of osteopontin. *Med Mol Morphol.* 2007; 40: 218–20.

88. **Modolo F, Biz MT, Martins MT, *et al.*** Expression of extracellular matrix proteins in adenomatoid odontogenic tumor. *J Oral Pathol Med.* 2010; 39: 230–5.

89. **Cheng J, Huo DH, Kuang DM, *et al.*** Human macrophages promote the motility and invasiveness of osteopontin-knockdown tumor cells. *Cancer Res.* 2007; 67: 5141–7.

90. **Bourassa B, Monaghan S, Rittling SR.** Impaired anti-tumor cytotoxicity of macrophages from osteopontin-deficient mice. *Cell Immunol.* 2004; 227: 1–11.

91. **Kaarteenaho R, Sormunen R, Paakko P.** Variable expression of tenascin-C, osteopontin and fibronectin in inflammatory myofibroblastic tumour of the lung. *APMIS.* 2010; 118: 91–100.

92. **Kalluri R, Zeisberg M.** Fibroblasts in cancer. *Nat Rev Cancer.* 2006; 6: 392–401.

93. **Pereira RO, Carvalho SN, Stumbo AC, *et al.*** Osteopontin expression in coculture of differentiating rat fetal skeletal fibroblasts and myoblasts. *In Vitro Cell Dev Biol Anim.* 2006; 42: 4–7.

94. **Lenga Y, Koh A, Perera AS, *et al.*** Osteopontin expression is required for myofibroblast differentiation. *Circ Res.* 2008; 102: 319–27.

95. **Pazolli E, Luo X, Brehm S, *et al.*** Senescent stromal-derived osteopontin promotes preneoplastic cell growth. *Cancer Res.* 2009; 69: 1230–9.

96. **Bhowmick NA, Neilson EG, Moses HL.** Stromal fibroblasts in cancer initiation and progression. *Nature.* 2004; 432: 332–7.

97. **Anderberg C, Li H, Fredriksson L, *et al.*** Paracrine signaling by platelet-derived growth factor-CC promotes tumor growth by recruitment of cancer-associated fibroblasts. *Cancer Res.* 2009; 69: 369–78.

98. **Allan AL, George R, Vantyghem SA, *et al.*** Role of the integrin-binding protein osteopontin in lymphatic metastasis of breast cancer. *Am J Pathol.* 2006; 169: 233–46.

99. **Nemoto H, Rittling SR, Yoshitake H, *et al.*** Osteopontin deficiency reduces experimental tumor cell metastasis to bone and soft tissues. *J Bone Miner Res.* 2001; 16: 652–9.

100. **Heinegard D, Andersson G, Reinholt FP.** Roles of osteopontin in bone remodeling. *Ann NY Acad Sci.* 1995; 760: 213–22.

101. **Ibrahim T, Leong I, Sanchez-Sweatman O, *et al.*** Expression of bone sialoprotein and osteopontin in breast cancer bone metastases. *Clin Exp Metastasis.* 2000; 18: 253–60.

102. **Kang Y, Siegel PM, Shu W, *et al.*** A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell.* 2003; 3: 537–49.

103. **Carlinfante G, Vassiliou D, Svensson O, *et al.*** Differential expression of osteopontin and bone sialoprotein in bone metastasis of breast and prostate carcinoma. *Clin Exp Metastasis.* 2003; 20: 437–44.

104. **Chellaiah MA, Kizer N, Biswas R, *et al.*** Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. *Mol Biol Cell.* 2003; 14: 173–89.

105. **Yoneda T.** Arterial microvascularization and breast cancer colonization in bone. *Histol Histopathol.* 1997; 12: 1145–9.

106. **Cook AC, Chambers AF, Turley EA, *et al.*** Osteopontin induction of hyaluronan synthase 2 expression promotes breast cancer malignancy. *J Biol Chem.* 2006; 281: 24381–9.

107. **Wang J, Levenson AS, Satcher RL Jr.** Identification of a unique set of genes altered during cell-cell contact in an *in vitro* model of prostate cancer bone metastasis. *Int J Mol Med.* 2006; 17: 849–56.