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Osteopontin Overexpression in Breast Cancer: Knowledge Gained and Possible Implications for Clinical Management

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Abstract Osteopontin (OPN) is a secreted protein that is overexpressed in a number of human cancers, and has been associated with increased metastatic burden and poor prognosis in breast cancer patients. The OPN protein contains several conserved structural elements including heparin- and calcium-binding domains, a thrombin-cleavage site, a CD44 binding site, and two integrin-binding sites. Experimental studies have shown that the ability of OPN to interact with a diverse range of factors, including cell surface receptors (integrins, CD44), secreted proteases (matrix metalloproteinases, urokinase plasminogen activator), and growth factor/receptor pathways (TGFα/EGFR, HGF/Met) is central to its role in malignancy. These complex signaling interactions can result in changes in gene expression, which ultimately lead to alterations in cell properties involved in malignancy such as adhesion, migration, invasion, enhanced tumor cell survival, tumor angiogenesis, and metastasis. Therefore, OPN is not merely associated with cancer, but rather it plays a multi-faceted functional role via complex molecular cross-talk with other factors. This review will focus on the role of OPN in breast cancer, in particular on the malignancy-promoting aspects of OPN that may reveal opportunities for new approaches to the clinical management of breast cancer. J. Cell. Biochem. 102: 859–868, 2007. © 2007 Wiley-Liss, Inc.

Key words: osteopontin; breast cancer; clinical management; targeted therapy

Osteopontin (OPN) is a secreted glycophosphoprotein that has been implicated in a number of different normal physiologic and pathologic processes. Over the years, due to independent isolation from different sources, OPN has also been known as "bone sialoprotein I", "secreted phosphoprotein 1 (Spp1)", "2ar", "uropontin", and "early T-lymphocyte activation (ETA-1) factor" [Tuck and Chambers, 2001; Wai and Kuo, 2004]. In normal tissues, OPN is expressed in osteoclasts, osteoblasts, vascular smooth muscle cells, inflammatory cells (T-cells, macrophages, NK cells, Kupffer cells), and numerous types of epithelium (e.g., breast, kidney, skin, salivary gland) [Tuck and Chambers, 2001; Wai and Kuo, 2004]. It has been shown to have a role in some developmental processes and tissue differentiation, including that of mammary gland [Rittling and Novick, 1997; Nemir et al., 2000] and bone [Yamate et al., 1997; Rittling et al., 1998], as well as in wound repair [Liaw et al., 1998]. In addition, it has known involvement in inflammatory responses, vascular remodeling and mineralization /calcification [Tuck and Chambers, 2001; Wai and Kuo, 2004].

OPN has also been implicated in a variety of pathologic processes, such as sepsis and inflammatory response (including granulomatous inflammation) to a variety of different agents, fracture repair, regulation of stone formation in kidney and other sites, (including mammary calcifications), atherosclerosis and other cardiovascular diseases [Tuck and Chambers, 2001;

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Wai and Kuo, 2004]. In addition, OPN has been implicated in neoplastic processes of a number of different tissue origins [Tuck and Chambers, 2001; Wai and Kuo, 2004]. This review will focus on OPN in breast cancer, in particular on the malignancy-promoting aspects of OPN that may reveal opportunities for new approaches to the clinical management of breast cancer.

OPN IN BREAST CANCER—CLINICAL STUDIES

Early work of Brown et al. [1994] and Bellahcene and Castronovo [1995], established that in general, higher levels of OPN mRNA or protein (respectively) are commonly found in mammary and other tumor types than in matching benign tissues. In addition to being present in the cellular components of the tissue, OPN protein is also found to be present in association with calcifications [Bellahcene and Castronovo, 1995]. Although it was initially suggested that tumor OPN is supplied primarily by tumor infiltrating inflammatory cells (mainly macrophages and T-cells) [Bellahcene and Castronovo, 1995; Hirota et al., 1995] and taken up secondarily by carcinoma cells, several later studies have shown that breast carcinoma cells themselves also synthesize OPN [Tuck and Chambers, 2001].

When assessing primary tumor levels of OPN by immunohistochemistry, it has been shown that higher level of immunodetectable OPN within the tumor cells themselves is associated with poor prognosis of breast cancer [e.g., Tuck et al., 1998; Rudland et al., 2002; Wang et al., 2006]. Thus, whether the tumor cells are elaborating the protein themselves, or taking it up from the environment (as supplied by tumor infiltrating inflammatory cells), its presence in the tumor cells appears to impart increased aggressiveness. The potential significance of tumor cell versus host-derived OPN is discussed further below. This association of tumor cell OPN with worse prognosis appears to be independent of nodal status and other established clinical prognostic indicators [Rudland et al., 2002].

OPN can also be measured by ELISA in the blood, either in plasma [Singhal et al., 1997; Bramwell et al., 2006], or serum [Fedarko et al., 2001], although the serum assay requires heating of the sample at 100°C with DTT to disrupt binding of OPN to complement Factor H [Fedarko et al., 2001]. The serum assay has been used to show that increased OPN is associated with the presence of a number of different types of carcinoma, including those of breast origin [Fedarko et al., 2001]. The plasma ELISA assay for OPN has been used by Singhal et al. [1997] and Bramwell et al. [2006] to show that in patients with metastatic breast cancer, elevated baseline OPN levels are associated with worse prognosis and increased tumor burden (Fig. 1). Bramwell et al. [2006] further showed that *changes* in OPN plasma level over time after therapy are also associated with outcome (increasing levels, worse prognosis; Table I). This latter finding opens the door to the possibility of using OPN blood levels to monitor response to therapy and disease progression. Thus, there is potential clinical utility for the assessment of OPN both for prognostic (primary tumor OPN by IHC, blood OPN by ELISA) and predictive (blood OPN, in terms of response to therapy) purposes.

Finally, OPN has also been detected in high levels in calcifications of both benign and malignant breast tissues [Hirota et al., 1995; Oyama et al., 2002]. Although the significance of the presence of OPN in these calcifications is uncertain (in terms of whether it promotes or inhibits their formation), it may mean that some

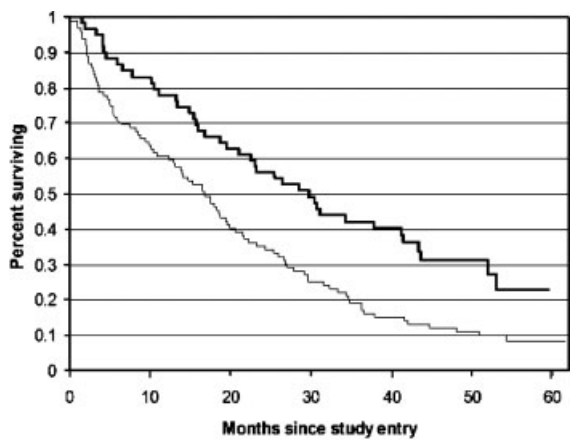

Fig. 1. Relationship between baseline plasma OPN concentration and survival in patients with metastatic breast cancer. Kaplan-Meier product limit survival curves for the entire study population (N = 157 patients) by plasma OPN level at study entry (baseline OPN). Patients were divided into those whose baseline OPN values were elevated relative to the upper level of normal values (gray line, baseline OPN >123 ng/mL), versus those whose baseline values were below this level (black line, baseline OPN <123 ng/mL). Patients with elevated baseline OPN values had significantly poorer survival than those whose initial OPN values were not elevated ($P = 0.0012$). From Bramwell et al. [2006]; reprinted with the permission of the American Association of Cancer Research.

TABLE I. Multivariate Analysis (109 Patients) Exploring Factors Prognostic for Duration of Survival in Women With Metastatic Breast Cancer, Including OPN Increase >250 ng/ml*

Variable	Relative risk of death (95%CI)	*P*-value
OPN increase >250 ng/ml	3.261 (1.716–6.198)	0.0003
Baseline OPN value	1.000 (0.999–1.001)	0.8171
ECOG status 2–4	1.993 (1.034–3.843)	0.0394
Visceral metastases	1.656 (0.822–3.337)	0.1585
Metastasis-free interval	1.001 (0.938–1.069)	0.9661
Metastatic burden	1.010 (0.480–2.127)	0.9791
PR positive	0.861 (0.434–1.712)	0.6703
ER positive	0.620 (0.321–1.197)	0.1546

95% CI, 95% confidence interval; PR, progesterone receptor; ER, estrogen receptor.
*From Bramwell et al. [2006]; reprinted with the permission of the American Association of Cancer Research.

OPN-expressing tumors are more readily detectable clinically at early stages, on the basis of associated abnormal/irregular mammographic calcifications.

OPN IN BREAST CANCER—EXPERIMENTAL STUDIES ON FUNCTION

Given the clinical associations between increased OPN expression and malignancy of breast and other cancers, there has been a concerted effort in recent years to establish potential mechanisms by which OPN can affect tumor aggressiveness. From this work, evidence has accumulated for involvement of OPN in a number of different processes associated with malignancy, such as increased cellular migratory and invasive behavior (including induction of proteases such as uPA, certain MMPs), increased metastasis (both generally and to bone, with increased adhesion to bone marrow endothelial cells), protection from apoptosis, promotion of colony formation and 3D growth ability, induction of tumor-associated inflammatory cells (which in turn may release tumor-promoting cytokines), and induction of expression of angiogenic factors, with resultant increased tumor angiogenesis [El-Tanani et al., 2006b; Tuck and Chambers, 2001; Weber, 2001].

Important to understanding the potential actions of a protein is an appreciation of its primary, secondary and tertiary structure. The human OPN gene maps to chromosome 4q13, consists of seven exons, and extends over 8 kb [reviewed in Wai and Kuo, 2004]. Alternative splicing may give rise to at least three different potential transcripts in some cell types [He et al., 2006]. The full-length human protein contains about 314 amino acid residues and the predicted secondary structure consists of eight alpha-helices and six beta-sheet segments [Denhardt and Guo, 1993]. Due to post-translational modifications such as glycosylation/sialation and phosphorylation, the molecular weight of OPN in monomeric form varies widely (41–75 kDa). These modifications appear to be both cell type and condition/environment specific, such that OPN produced by tumor-infiltrating inflammatory cells may be of different structure (and possibly different functional activity) than that of the cancer cells themselves. Indeed, levels of sialation and phosphorylation of OPN have independently been shown to affect cell binding and migration towards OPN in various different cell types [review in Christensen et al., 2005]. In addition, due to a high concentration of glutamate residues in the OPN backbone, transglutaminase activity can result in cross-linking of OPN (or OPN fragments) to itself, to form polymers, as well as to different extracellular matrix proteins such as fibronectin [Beninati et al., 1994; Higashikawa et al., 2007]. These multimeric complexes are then in turn more adhesive to collagen, and potentially other extracellular matrix components as well, thus allowing for interaction of OPN with the ECM scaffolding [Kaartinen et al., 1999]. Interestingly, the presence and activity of transglutaminase-2 (TG2) at the cell surface has been reported to be important in fibronectin binding and malignancy of metastatic breast carcinoma, and this TG2 has been shown to associate with β1 and β5 integrins, which also may bind OPN [Mangala et al., 2007].

OPN is now known to contain various different functional domains, which are involved in specific cellular functions (Fig. 2). These

domains, along with appropriate references to their function, are very nicely described in a review of Wai and Kuo [2004]. Within exon 6 of human OPN is an RGD (Arginine-Glycine-Aspartate) motif important for binding to integrins αvβ3, αvβ5, αvβ1, and α5β1. Six amino acids distal to the RGD motif is a thrombin cleavage site, which serves to separate OPN into two similar-sized fragments. The N-terminal fragment, in addition to containing the RGD motif, includes an SVVYGLR motif involved in binding to integrins α9β1 and α4β1, as well as an aspartate-rich domain, which in bone is involved in binding to hydroxyapatite. The C-terminal fragment in turn contains a calcium-binding domain and a CD44-binding domain, the latter potentially binding CD44 variant 6 directly, and/or CD44 variant 3 indirectly (via a heparin bridge). It has been suggested that thrombin-mediated cleavage of OPN may enhance integrin-binding, and hence pro-adhesive and migratory effects of the N-terminal fragment of OPN, and that N-terminal phosphorylation is also important in these activities [Weber et al., 2002]. Similarly, cleavage of OPN by certain matrix metalloproteinases (MMP-3, MMP-7) has been shown to increase cell adhesive and migratory effects [Agnihotri et al., 2001]. In addition, some have ascribed a more chemotactic function to the C-terminal half of cleaved OPN, mediated by CD44 binding, and a more haptotactic function to the N-terminal fragment, mediated by integrin binding [review in Weber, 2001]. In murine mammary epithelial tumor cells (4T1, 4T07), the chemotactic (and invasive) effects of the C-terminal fragment of OPN have been shown to be mediated through binding to cyclophilin C and the cell surface extracellular matrix metalloproteinase inducer CD147, to activate Akt 1/2 and MMP-2 [Mi et al., 2007].

Studies of cultured human breast cancer cell lines have shown that adhesion and migration responses to OPN are indeed dependent on interaction with several of the cell surface integrins mentioned above, including αvβ3, αvβ1, and αvβ5 [Tuck et al., 2000; Furger et al., 2003]. Although it is known that different mammary epithelial cells do have different expression profiles with respect to these integrins [e.g., Tuck et al., 2000; Allan et al., 2006], whether OPN interaction with different integrins triggers the same or different intracellular signals has yet to be determined. As alluded to above, cell migration response of breast cancer cell lines to OPN has also been shown to involve interaction of OPN with specific CD44 isoforms, either alone or in combination with integrin interaction [Katagiri et al., 1999; Khan et al., 2005]. Even more interesting is the recent finding that in some cell lines (e.g., gastrointestinal carcinoma), CD44 (variant 6) and specific (β1) integrins show synergistic effect in terms of cell binding to OPN, that OPN binding to CD44 promotes cell survival, and that this effect is via CD44-mediated activation of these integrins [Lee et al., 2007]. In myeloma cells, OPN binding to CD44 (variant 6) has been found to promote cell survival and cell migration, and the cell migration effect has been shown to also involve the αvβ3 integrin [Caers et al., 2006]. Some degree of cross-talk between these two cell surface receptor pathways is thus quite likely involved in different OPN-induced cellular

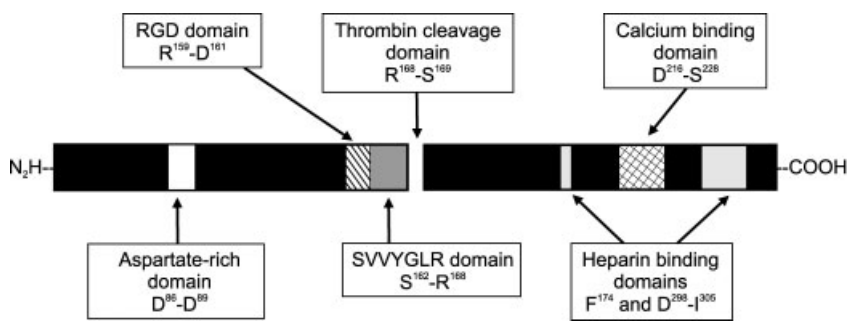

Fig. 2. The human OPN protein contains several highly conserved structural elements reflective of its varied biological functions. The aspartate domain serves to bind hydroxyapatite in bone. The RGD domain and the SVVYGLR domain are integrin-binding sites that mediate OPN binding to the integrins αvβ1, αvβ3, αvβ5, and α5β1 (RGD), as well as α4β1 and α9β1 (SVVYGLR). The thrombin cleavage domain allows for proteolytic cleavage of OPN into two similar-sized fragments. The C-terminal fragment contains a calcium-binding domain and two heparin-binding domains, the latter of which can also mediate binding of OPN to CD44 variant 3. D, aspartate; R, arginine; S, serine; V, valine; Y, Tyrosine; G, glycine; L, leucine; F, phenylalanine; I, isoleucine.

activities (e.g., promotion of cell migration, inhibition of apoptosis). In terms of CD44 activation, in addition to the possibility of direct interaction between OPN and specific CD44 isoforms (particularly variant 6), indirect activation of CD44 by OPN may occur via upregulation of the synthesis of another CD44 ligand, hyaluronan (HA). Cook et al. [2006] have shown that constitutive upregulation of OPN in a breast cancer cell line results in increased expression of hyaluronan synthetase 2 (HAS2), which in turn is associated with increased HA production, increased matrix retention, increased cell adhesion to (bone marrow) endothelial cells and increased anchorage-independent growth.

Upon OPN interaction with these specific classes of cell surface receptors, a number of different signaling pathways appear to be activated. More specifically, cell surface integrin binding by OPN has been shown to induce activation of PKC, PLC, and PI3K pathways. Inhibition of any of these three pathways has been shown to inhibit OPN-induced migration of human breast cancer cells [Tuck et al., 2003]. Das et al. [2004] have further shown that OPN regulates $\alpha v\beta 3$ integrin-mediated PI3K/Akt/NFκB dependent uPA expression, which is associated with cellular invasiveness. In fact, it was later shown that this process, (activation of uPA expression upon integrin activation by OPN), also involves ILK activation and results in AP-1 mediated increase in MMP-2 as well as uPA expression [Mi et al., 2006]. Furthermore, Rangaswami et al. [2004] have shown that OPN-induced NFκB activation in melanoma cells involves nuclear factor-inducing kinase (NIK), and results in activation of not only uPA, but also of pro-MMP-9, and that this is mediated via activation of the MAPK pathway. In addition, ligation of integrins by OPN leads to activation of EGFR and the HGF receptor, Met [Tuck et al., 2000; Tuck et al., 2003]. In the case of EGFR, this has been said to occur via src-dependent transactivation, with resulting activation of downstream signaling pathways such as PI3K, Ras-MAPK, PLC, and PKC, in turn activating AP-1 dependent uPA expression [Das et al., 2004]. Although not yet shown for Met, a similar transactivation process is likely after OPN-integrin engagement, as (a) sensitivity to HGF/Met activation has also been shown to be influenced by src [Elliott et al., 2002], (b) OPN is known to increase sensitivity to the cell migration promoting effects of HGF/Met [Tuck et al., 2000], and (c) HGF/Met can also induce AP-1 mediated increase in uPA expression [Ried et al., 1999]. Although less is known about the consequences of OPN binding to CD44, it has been shown at least in some cell types that upon interaction of OPN with CD44, "inside-out" activation of integrins may also occur via src-dependent transactivation, and that this process is somehow associated with promoting cell survival [Lee et al., 2007]. It has been speculated that activation of CD44 by OPN may also induce integrin-independent activation of PLC/PKC/PI3K pathways more directly to promote cell survival [Chakraborty et al., 2006], although to our knowledge this has yet to be proven in breast cancer cells.

In addition to these various influences of OPN on the tumor cells themselves, the presence of OPN in the tumor microenvironment has been shown to have profound influences on various cells of the stromal compartment, including tumor-infiltrating T-cells, macrophages, and NK(T) cells, endothelial cells, and smooth muscle cells, and OPN is produced by many of these cells when they are activated. In T-cells and NK(T) cells, OPN has been shown to induce activation, and inhibit activation-induced apoptosis [O'Regan et al., 2000; Larkin et al., 2006]. Reported effects of OPN on macrophages include induction of cell migration/chemotaxis, cell activation, and promotion of cell survival [Weber et al., 2002; Wai et al., 2006], and the effects on cell migration and activation have been found to be phosphorylation-dependent [Weber et al., 2002]. Activated macrophages in turn are known to synthesize and secrete a number of mediators, including EGF and TNFα, various other cytokines including several interleukins, VEGF, various proteases, and additional macrophage-derived OPN, which may then feed back on the tumor cells and their environment in a paracrine fashion to influence malignant behavior [review in Shih et al., 2006]. In addition to induction of VEGF expression, there is evidence that OPN may promote the process of angiogenesis, through chemotactic effects on endothelial and vascular smooth muscle cells [Liaw et al., 1995; Senger et al., 1996].

Finally, gene expression profiling of breast carcinoma cells overexpressing OPN (vs. appropriate control cells) has shown changes in the expression pattern of a number of genes, covering all functional categories of Hanahan and

Weinberg's "Hallmarks of Cancer" (e.g., self-sufficiency in growth signals/insensitivity to antigrowth signals, evading apoptosis, tissue invasion and metastasis, sustained angiogenesis, limitless replicative potential, and genetic instability) [Cook et al., 2005]. Evaluation of the relative functional contribution of these different OPN-regulated genes may provide even further clarification of the mechanisms of OPN effects on breast cancer cells, as well as other potential candidates for targeted therapy.

REGULATION OF OPN EXPRESSION

Data regarding regulation of OPN expression at the transcriptional level has been accumulating at a rapid pace in recent years, and is very nicely summarized in reviews of Weber [2001], Wai and Kuo [2004] and El-Tanani et al. [2006b]. It has become clear that OPN expression is regulated by a wide variety of stimuli, involving complex regulatory pathways. Study of the OPN promoter has revealed numerous potential regulatory elements for transcription factors, including TATA-like and CCAAT-like sequences, vitamin D-responsive motifs, GATA-1, AP-1 (FOS, JUN, ATF, MAF family factors), AP-2, AP-4, PEA1, PEA3, Ets-1, Ets-2, Runx2, BRCA1, β-catenin binding sequences and multiple Tcf-1 and TcF-4 recognition sequences [reviewed in Weber, 2001; Wai and Kuo, 2004; El-Tanani et al., 2006b]. In addition, there are suggested sites for binding of E4tf1, E2A, SP1, Oct-1, Oct-4, Sox-2 repressor, CBF-like factor (src response element), a response element (RE-1a) for Myc, Sp1, glucocorticoid receptor and E-box binding factor, estrogen response elements (SFREs), and response elements for Smad3 and Smad4 of the TGF-β signaling pathway [reviewed in Weber, 2001; El-Tanani et al., 2006b]. Ras-mediated activation of OPN transcription has also been reported, a phenomenon that is mediated in part by the Ets-related transcription factor MATF [Guo et al., 1995]. Inhibition of the OPN promoter has been observed by wild-type BRCA1, possibly through binding and inactivation of ERα, PEA3, and/or AP-1 [El-Tanani et al., 2006a]. The metastasis suppressor BRMS1 has also been reported to inhibit OPN transcription, through abrogation of NF-κB activation [Samant et al., 2007]. Most recently, agonists of the ligand-activated transcription factor PPARα have been found to suppress OPN expression (in macrophages) and to reduce plasma levels of OPN [Nakamachi et al., 2007]. In all, it has become apparent that the transcriptional regulation of OPN is very complex. However, one must keep in mind that, as most of these potential regulation systems have only been worked out for specific cell types, not all may be necessarily active in a mammary epithelial cell/carcinoma background.

OPPORTUNITIES FOR DIRECTED ANTI-OPN THERAPIES

The wealth of information gathered on expression and post-transcriptional processing of OPN, as well as cell surface receptor, signal transduction pathway and downstream effector mechanisms potentially influenced by OPN, provides numerous levels/targets at which potential therapeutic strategies can be directed (Fig. 3). In some instances this can mean new applications of existing therapies, whereas in others potential new targets are offered. Examples of experimental evidences for the validity of various of these approaches are provided in reviews of Weber [2001] and Jain et al. [2007].

At the transcriptional level, in addition to inhibiting OPN expression by various RNA suppressive approaches (asRNA, siRNA, shRNA, hammerhead ribozymes) directed against the OPN transcript, numerous potential targets are offered by the abundance of upstream control elements and associated transcription factors. For example, the presence of estrogen and glucocorticoid response elements suggests that hormonal therapy may affect transcription of OPN. Binding and activity of specific transcription factors, such as JNK1, and NFκB might be blocked with inhibitors such as SP600125 and SN50 (or curcumin) respectively. Interference with either the TGF-β and/or Wnt [e.g., FJ9, Fujii et al., 2007] pathways would also have potential influence on OPN transcription. If transcriptional control of breast cancer cell OPN is influenced by PPARα, as it is in macrophages, agonists such as benzafibrate or WY14643 could also be useful in suppressing OPN expression [Nakamachi et al., 2007].

At the post-transcriptional level, there is abundant experimental evidence suggesting that cleaved OPN may have higher biological activity [Agnihotri et al., 2001; Weber et al., 2002]. Therefore, protease inhibitors,

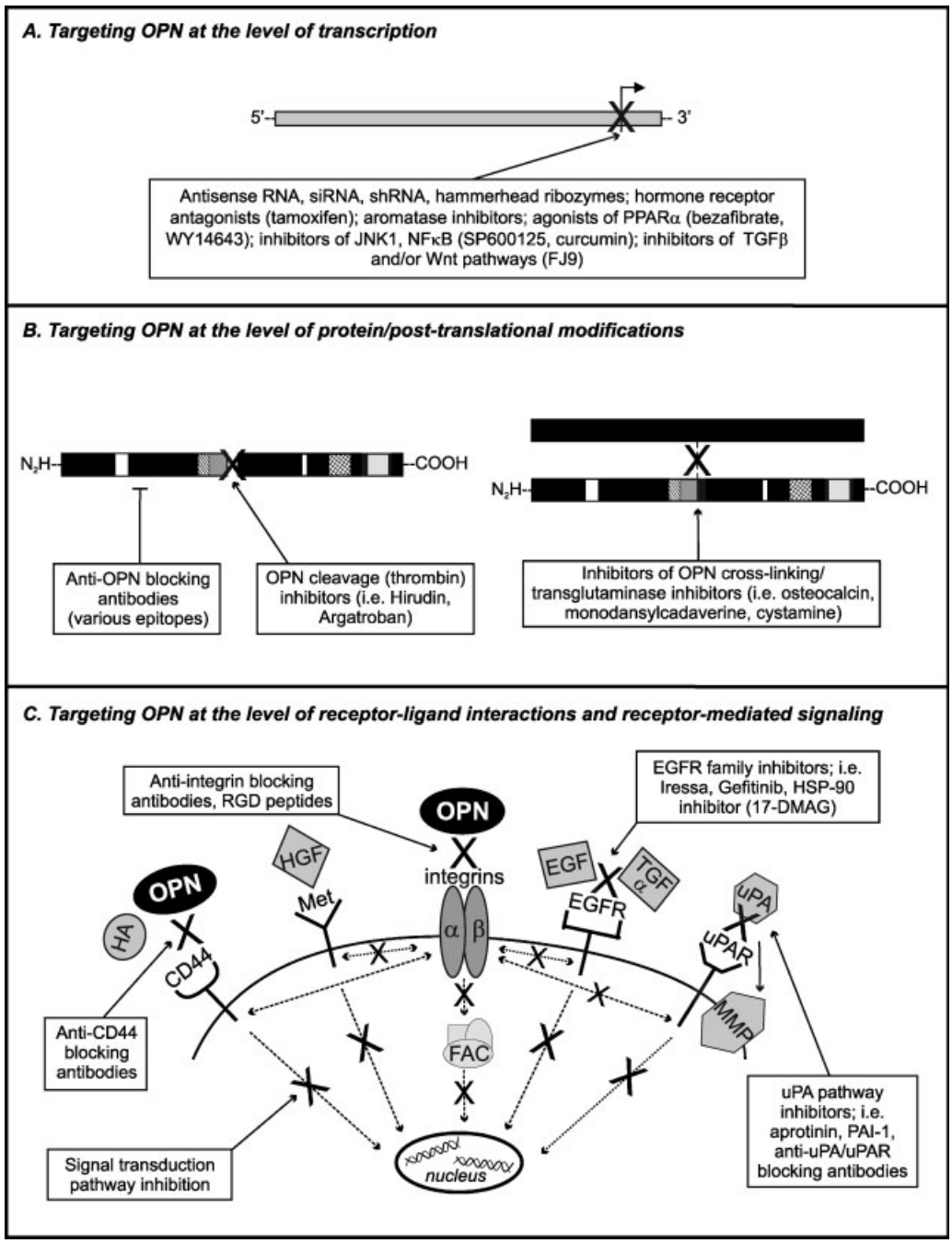

Fig. 3. Potential therapeutic strategies to target OPN and inhibit its malignancy-promoting activities. There are numerous levels/targets at which potential therapeutic strategies can be directed against OPN, including (**A**) at the transcriptional level, (**B**) at the level of the protein and post-translational modifications, or (**C**) at the level of receptor-ligand interactions and receptor-mediated signaling. In some instances this can mean new applications of existing therapies, whereas in others potential new targets are offered.

particularly thrombin inhibitors (e.g., Hirudin, Argatroban) may be of use to inhibit such processing of the OPN protein. Similarly, the evidence that transglutaminase cross-linking of OPN can be important in its biological activity [Beninati et al., 1994; Higashikawa et al., 2007] suggests that strategies using transglutaminase inhibitors (e.g., osteocalcin, monodansylcadaverine, cystamine) may also be of use.

From experimental evidence detailed above, numerous potential targets are available at the

cell surface. Interaction of OPN with its various receptors could potentially be blocked by antibodies specific to OPN, to specific OPN-binding integrins, CD44 isoforms, or sites of interaction with growth factor receptors (Met, EGFR family), uPAR and/or CD147. Alternatively, small molecule inhibitors of binding to cell surface receptors, such as RGD peptides, RGD peptidomimetic agents [e.g., S137, S247, Harms et al., 2004], or peptide fragments of CD44 [Weber, 2001], may be used to interfere with OPN interactions. Given the involvement of FAK and src in transducing signals through integrin and CD44 receptors, inhibitors such as PF-573,228 (FAK inhibitor) or AZD0530 (src inhibitor) would be potentially effective as well. An arsenal of antibodies and tyrosine kinase inhibitors to growth factor receptors are either in development or already in clinical use at present, and would also be expected to be effective against tumors overexpressing OPN (e.g., antibodies to EGFR, HER2/neu, or Met, inhibitors such as Iressa, Gefitinib, DMAG). Similarly, as MAPK, PKC, PLC, and PI3K pathways have all been shown to be involved in transduction of OPN-mediated signals, potential targets are presented by the various members of these pathways [reviews in Weber, 2001; Jain et al., 2007].

At the level of the various effectors of OPN signaling, potential targets include the uPA/uPAR axis, various metalloproteinases (particularly MMP-2 and MMP-9), regulators of apoptosis, regulators of angiogenesis and hyaluronan synthetases (particularly HAS-2). This could again involve specific targeted antibody approaches or strategies utilizing any of a number of small molecule inhibitors presently in development. Finally, continued analysis and validation of expression array profile differences between cells expressing high versus low levels of OPN in various cell types will likely yield even more potential targets for therapy directed at these more aggressive, OPN overexpressing tumors.

CONCLUSIONS

Knowledge of the mechanisms of OPN involvement in malignancy of breast cancer has expanded rapidly in the last few years. It has become clear that OPN regulates a variety of cell properties involved in malignancy, from cell migration/invasion, to inhibition of apoptosis, clonogenicity, angiogenesis and influence on host inflammatory response, to cell properties involved in general and site-specific (particularly bone) metastatic ability. Details of the regulation of synthesis and biological processing of OPN, as well as information regarding cell surface receptors involved in OPN binding, signal transduction, collaborative relationships with other pathways (e.g., growth factor receptor), and effector mechanisms of OPN function continue to be elucidated. In all, these are providing a number of opportunities for directed therapy to block OPN-mediated effects on cancer. As is generally the case with systemic therapy, the challenge now will be to identify which of these approaches will have the optimum effect, in what combination, with the minimum of toxicity.

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