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Therapeutic Targeting of Osteopontin in Breast Cancer Cells

Gopal C. Kundu et al.*
National Centre for Cell Science, NCCS Complex, Pune, India

1. Introduction

Osteopontin (OPN), a cytokine like ECM associated member of Small Integrin Binding LIgand N-linked Glycoprotein (SIBLING) family of protein plays an important role in determining the metastatic potential of many cancers. The function of OPN in various pathophysiological conditions, especially in cancer indicated that the variation in post-translational modification generate different functional forms that might alter its normal physiological functions. Recent data indicated that OPN regulates tumor growth through induction of pro-angiogenic and metastatic genes like COX-2, and VEGF expressions and activation of matrix metalloproteinase (MMP) in cancer cells. The exact role of stroma- and tumor-derived OPN in regulation of tumor growth and angiogenesis in various cancers is not well understood. Therefore, it is important to delineate the mechanism by which both tumor and stroma-derived OPN control the cell migration and tumor growth. p70S6 kinase, STAT3 and VEGF are directly involved in regulation of breast tumor growth and angiogenesis. But, the mechanism by which OPN regulates p70S6 kinase and STAT3 activation and VEGF expression leading to breast cancer cell migration, tumor growth and angiogenesis are not well defined. We have recently shown that OPN induces p70S6 kinase phosphorylation in a site specific manner. Interestingly, OPN has no effect on mTOR phosphorylation, but overexpression of mTOR does not regulate OPN-induced phosphorylation of p70S6 kinase. Overexpression of mTOR/p70S6 kinase suppresses OPN-induced ICAM-1 expression, while treatment with rapamycin enhances OPN-induced ICAM-1 expression. Our recent data also indicated that OPN upregulates JAK2 dependent STAT3 activation in breast cancer cells. Wild type STAT3 enhanced whereas mutant STAT3 suppressed OPN-induced breast tumor cell migration. Cells overexpressing STAT3 upregulate whereas mutant STAT3 downregulate OPN-induced tumor growth leading to

* Supriya Saraswati, Megha Sanyal, Anuradha Bulbule, Anuja Ramdasi, Dhiraj Kumar, Reeti Behera[1], Mansoor Ahmed[2], Goutam Chakraborty[3], Vinit Kumar[4], Shalini Jain[5], Gowrishankar S. and Pompom Ghosh
[1]*Present Address: H. Lee Moffitt Cancer Center and Research Institute, FL*
[2]*Present Address: University of Virginia, VA*
[3]*Present Address: Memorial Sloan-Kettering Cancer Center, NY*
[4]*Present Address: H. Lee Moffitt Cancer Center and Research Institute, FL*
[5]*PresentAddress: The University of Texas MD Anderson Cancer Center, TX USA*

Bcl2 and cyclin D1 expressions. Our data also revealed that OPN augments breast cancer cell migration, angiogenesis and tumor growth through induction of VEGF expression. Thus, targeting OPN and its regulated signalling cascade may develop an effective therapeutic approach for the management of breast cancer.

2. General features of breast cancer

The critical features that define cancer encompass the six core hallmarks of the disease as described recently (Hanahan and Weinberg, 2011). These hallmarks are sustained proliferative signalling, evading growth suppressors, activating invasion and metastasis, overcoming replicative senescence, inducing angiogenesis and resisting cell death (Hanahan and Weinberg, 2000). Breast cancer represents malignant transformation of the epithelial cells lining the ducts or lobules of the breast, occurring as a result of unrestricted cellular proliferation possibly owing to accumulation of a series of somatic or germ line mutations. Majority of the breast cancer is a result of somatic or acquired mutations and it is the most common form of cancer affecting women worldwide. Benign breast tumors are treatable and hence not a grave threat in contrast to malignant breast cancer where many complex processes are involved that are difficult to target. Invasion, angiogenesis and metastasis are the defining attributes of malignancy and occur as early events in cancer progression.

Mutations in certain genes lead to sporadic cases of breast cancer. Tumor suppressor genes like p53 control unrestricted proliferation of cells. It is noteworthy to mention about two related genes such as p63 and p73 which are yet to assume importance as candidates for alternative regimens for the treatment of cancer. These are reported to be involved in embryonic development and their roles in attenuating cancer progression are under study. Boominathan has provided mechanistic insights into how p53, p63, and p73 regulate the components of the miRNA processing and how p53, TA-p63, and TA-p73 regulated miRNAs inhibit tumorigenesis, EMT, metastasis, and cancer stem cell proliferation (Boominathan, 2010). The first clinical trials of attempting to use p73 to combat a hard-to-treat-type of breast cancer have been initiated (Leslie, 2011).

The other two breast cancer specific tumor suppressor genes, BRCA1 and BRCA2 protect the cells from dysregulation leading to unrestrained cellular proliferation (Stefansson et al, 2009). A member of EGF receptor superfamily called erbB2 or HER2/neu is a receptor for human epidermal growth factor that is present on the breast cancer cells and stimulates the cells to grow and divide. Overexpression of HER2/neu due to gene amplification is associated with transformation of human breast epithelium. Apart from mutations in tumor suppressor genes and oncogenes, breast cancer is also associated with the presence of ER and PR. Breast cancers are sub-divided into four groups based on IHC profile of ER/PR and Her2/neu expression: luminal A (ER and/or PR +ve, HER2 –ve), luminal B (ER and/or PR +ve, HER2 +ve), HER2 positive (ER and PR -ve, HER2 +ve) and triple negative (all –ve).

These classifications of breast cancers are based on which hormone fuels their growth and helps decide the course of hormone targeted therapy. Triple negative breast cancer is marked by the absence of hormone receptors and HER2/neu and forms belligerent tumors that are unresponsive to hormonal therapies (tamoxifene, aromatase inhibitors) or HER2 directed therapies (herceptin, lapatinib) (Chen and Russo, 2009). Staging of breast cancer is performed by employing the widely accepted TNM classification which describes the individual stages of the tumor, node and metastases (TNM) of the cancer. The tumor grade of invasive carcinomas is classified according to the Scarff–Bloom–Richardson (SBR) system.

Clinical studies have revealed that higher expression of OPN is found in tumor tissue and serum of breast cancers (Shevde et al, 2006). Enhanced expression of OPN can be correlated with increase in tumor growth and metastasis, suggesting that OPN can be used as a diagnostic and prognostic biomarker for breast cancer. Earlier micro array analysis data revealed that expression of OPN is upregulated in metastatic breast cancers (Cook, 2005). OPN is an extracellular matrix (ECM)-associated, SIBLING family of cytokine-like, noncollagenous, sialic acid rich phosphoglycoprotein (Rangaswami, et al 2006). OPN controls normal physiological and various pathophysiological processes such as myocardial necrosis, restenosis, atherosclerosis and autoimmune diseases (Panda et al, 1997). OPN acts as an important oncogenic molecule which is involved in all the stages of cancer progression including tumor invasion, angiogenesis and metastasis. Previous reports have indicated that OPN is also overexpressed in tumor-educated stromal cells suggesting its involvement in the crosstalk between tumor and stromal compartment that ultimately leads to cancer progression (Osterreicher, 2011). Earlier results indicate that OPN could regulate the expression of several oncogenic and angiogenic molecules through activation of various signalling mechanism (Chakraborty et al, 2006).

3. Structure, functions and mediators of osteopontin

Osteopontin was initially characterized in 1979 as a phosphoprotein secreted by transformed malignant epithelial cells and has since been under extensive study. The human OPN gene sprawls across 8 kilobases and is localized at chromosome 4q13 in human as a single copy gene with seven exons and six introns (Wai and Kuo, 2004). Alternative splicing yields three distinct splice variants- OPN-A, the full-length transcript, OPN-B, lacking exon 5 and OPN-C lacking exon 4 (He et al, 2006). Two isoforms of OPN, a full-length secreted OPN (Opn-s) and an intracellular OPN (Opn-i) are generated from alternative translation of a non-AUG site downstream of the canonical AUG sequence (Shinohara et al, 2008). These two isoforms occupy characteristic intracellular sites and mediate distinct functions in dendritic and T cells (Shinohara et al, 2008).

A full length human OPN consists of about 314 amino acid residues with a molecular weight in the range of 44-75 kDa, resulting from the varying degree of posttranslational modifications. Within the functional domains of OPN, there are specific motifs essential for the binding of OPN to its cell surface receptors, integrins and CD44 for mediating its biological activities (Figure 1). Whereas the N-terminal fragment contains the RGD motif, the SVVYGLR motif, a thrombin cleavage site and an aspartic acid rich site, the C-terminal fragment contains a calcium-binding site and CD44 binding site. The RGD motif necessary for the attachment of integrins such as $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 1$ and $\alpha 5\beta 1$ is embedded within exon 6. A central thrombin cleavage site distal to the RGD motif divides OPN into two similar-sized fragments. The SVVYGLR motif binds to integrins, $\alpha 9\beta 1$ and $\alpha 4\beta 1$ and the aspartic acid rich site binds hydroxyapatite in bones. The CD44 interacts through the C-terminal of OPN. OPN is involved in maintaining calcium homeostasis via its calcium binding site. OPN upon binding with integrins or CD44 regulates breast cancer cell proliferation, migration, invasion and chemotaxis. OPN plays an important role in regulation of tumor progression, angiogenesis and metastasis in breast cancer. OPN is detected in many biological fluids like plasma of metastatic breast cancer patients, urine, milk and seminal fluids. The ligation of OPN to its receptors stimulates a cascade of signalling pathways which cross talk and foster neoplastic growth in breast cancer (Rangaswami et al, 2006).

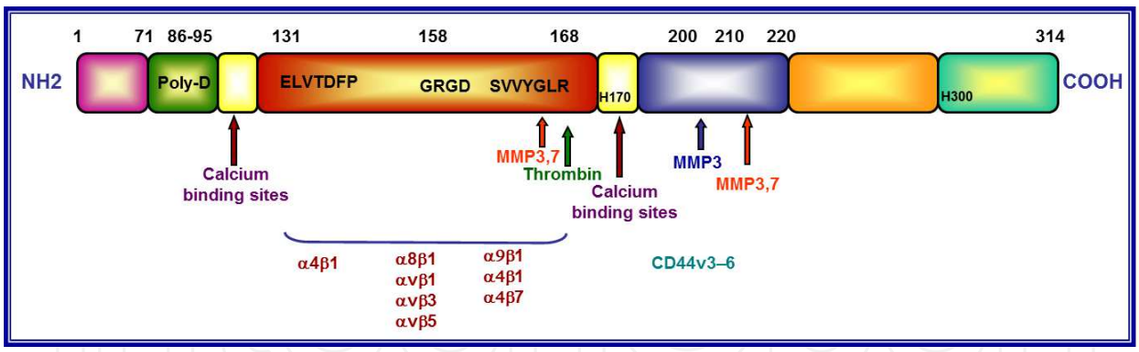

Fig. 1. Schematic representation of the domain structure of OPN. The N-terminal fragment contains a poly D rich region, calcium binding site, RGD motif and SVVYGLR. Various integrins interact with the N-terminal domain of OPN while C-terminal domain of it interacts with CD44, v3-6.

4. Pleiotropic function of OPN in breast cancer

Breast cancer progression depends on an accumulation of metastasis supporting cell signaling molecules that target various signal transduction pathways. These complex signaling mechanisms can result in changes in gene expression, which ultimately lead to alterations in cellular properties involved in malignancy such as adhesion, migration, invasion, enhanced tumor cell survival, angiogenesis and metastasis (Figure 2). Increased expressions of OPN and its receptors, integrins and CD44 correlate with enhanced breast tumor epithelial cell migration, tumor progression and metastasis. Among all splice variants of OPN, OPN-C is a highly specific marker for transformed breast cancer cells (He et al, 2005). Rittling et al have reported that OPN associated with tumors is primarily soluble, and that OPN can neither support endothelial cell proliferation nor prevent apoptosis of these cells in the absence of adhesion (Rittling et al, 2002).

OPN activates αvβ3 integrin-mediated PI 3'-kinase/IKK-dependent NF-κB activation and uPA secretion leading to breast cancer cell migration (Das et al, 2003). Previous reports have shown that OPN induces αvβ3 integrin-mediated AP-1 activation and uPA secretion through c-Src/EGFR/ERK signaling pathways and all of these ultimately control breast cancer cell migration (Das et al, 2004). Recent studies suggest that mutant OPN lacking thrombin cleavable domain decreases cell adhesion and primary tumor latency time, and increases uPA expression, primary tumor growth and lymph node metastatic burden in MDA-MB-468 breast cancer cells (Beausoleil et al, 2011). Cook et al have shown that hyaluronan synthase 2 (HAS2) is found to be upregulated by OPN in breast cancer cells (Cook et al, 2006). It is reported that OPN induces NF-κB activation and NF-κB dependent AP1-mediated ICAM-1 expression through mTOR/p70S6 kinase pathways in breast cancer cells. The study suggests that inhibition of mTOR by rapamycin induces whereas overexpression of mTOR/p70S6 kinase suppresses OPN-induced ICAM-1 expression. Thus OPN stimulates p70S6 kinase phosphorylation at Thr-421/Ser-424, but not at Thr-389 or Ser-371 and mTOR phosphorylation at Ser-2448. Overexpression of mTOR has no effect in regulation of OPN-induced phosphorylation of p70S6 kinase at Thr-421/Ser-424 (Ahmed and Kundu, 2010). Recent reports also suggested that OPN induces αvβ3 integrin-mediated JAK2 dependent STAT3 activation in breast cancer cells. OPN protects the cells from staurosporine (STS)-induced apoptosis through JAK2/STAT3 pathway. Wt STAT3 in

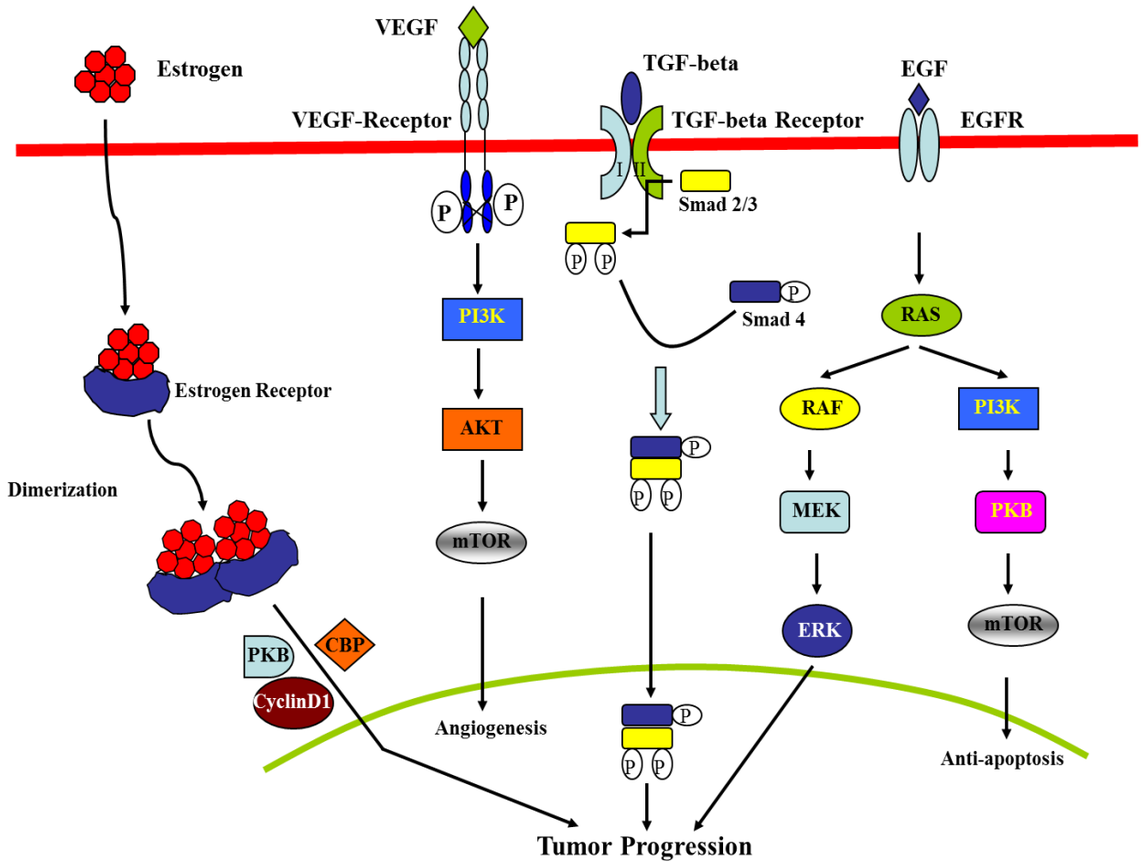

Fig. 2. Model depicting various signalling pathways involved in breast cancer cells. These pathways include estradiol, VEGF, TGF beta and EGF-induced signalling that promote cell growth, angiogenesis and prevention of cell death.

presence of OPN induces breast tumor progression through up regulation of Bcl2 and cyclin D1 expression in breast cancer cells (Behera et al, 2010). It has been also reported that both exogenous and tumor-derived OPN triggers vascular endothelial growth factor (VEGF)–dependent tumor progression and angiogenesis by activating breast tumor kinase (Brk)/NF-κB)/ATF-4 signaling cascades through autocrine and paracrine mechanisms in breast cancer models (Chakraborty et al, 2008). Curcumin inhibits OPN-induced VEGF expression leading to suppression of tumor angiogenesis in breast cancer (Chakraborty et al, 2008). Mi et al have demonstrated that OPN promotes CCL5-mesenchymal stromal cell (MSC) mediated breast cancer metastasis. They have shown that tumor derived OPN induces MSC expression of CCL5 through integrin mediated AP1 transactivation and further demonstrated that concomitant inoculation of MSC with MDA-MB-231 induces tumor growth and metastasis. These results suggested that tumor derived OPN promotes tumor progression through transformation of MSC into Cancer associated fibroblast (CAF) (Mi et al, 2011).

5. OPN as a chemoattractant cytokine and pro-angiogenic factor

OPN mediates RGD dependent chemotaxis, attachment and migration in many epithelial cell types (Celetti et al, 2005). It aids preferential metastasis of breast cancer cells to bone

(Kang et al, 2003). OPN functions in cell adhesion, chemotaxis, macrophage-directed interleukin-10 (IL-10) suppression, stress-dependent angiogenesis, prevention of apoptosis, and anchorage-independent growth of tumor cells by regulating cell-matrix interactions and cellular signaling through binding with integrin and CD44 receptors (Wai et al, 2004). Correlative evidence has shown that the αvβ3 integrin receptor appears to be preferentially used by more malignant breast epithelial cell lines in binding and migrating toward OPN (Tuck et al, 2000). Cancer metastasis involves invasion by the cancer cells, angiogenesis, circulation of cancer cells, colonization at a distant site and finally evasion of the host immune response. Motility of the cancer cells and degradation of extracellular matrix are essential for invasion. Cells cross the basement membrane and move to secondary organ sites. This phenomenon occurs due to the secretion of chemokines. Extracellular matrix degradation, by both tumor and host cells occurs by the secretion of proteases (Wong et al, 1998). On the molecular level, the metastatic phenotype is generated by the deregulation of cell surface receptors, their ligands, their downstream signaling molecules and extracellular matrix proteases. Unlike oncogenes, the genes involved in metastasis are not mutated but their expression is deregulated. OPN overexpression or exogenous addition in breast cancer cell lines increases the invasiveness of the cells and uPA expression through cell surface interactions between integrin and uPA/uPAR. Constitutive activation of NF-κB has been detected in lymphomas, melanomas and breast cancers and has been shown to correlate with oncogenesis.

A large number of proangiogenic factors and their cognate receptors have been identified including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), angiopoietin-1, transforming growth factor beta-1 (TGF-β1), transforming growth factor alpha (TGF-α), and epidermal growth factor (EGF) (Liotta et al, 2001). VEGF is one of the best characterized pro-angiogenic factors among other growth factors in terms of its specificity for the vascular endothelium (Mcmahon, 2000). OPN is involved in angiogenesis through αvβ3 integrin-mediated upregulation of VEGF expression. It can stimulate adhesion and migration of endothelial cells. Therefore, OPN and αvβ3 integrin play significant roles in vascular repair and regeneration. It has been reported that OPN protects the endothelial cells from apoptosis. This interaction is mediated by αvβ3 integrin and NF-κB dependent pathway.

6. Osteopontin regulates various signaling pathways in breast cancer

6.1 OPN controls tumor angiogenesis through VEGF/VEGFR signaling pathway

The molecular mechanism of OPN-induced VEGF expression and its potential role in regulating in vitro cell motility which ultimately controls in vivo tumor growth and angiogenesis in breast cancer model was described earlier (Chakraborty et al, 2008). The study highlighted the role of OPN in induction of neovascularization by enhancing VEGF expression through activation of breast tumor kinase (Brk)/NF-κB/ATF-4 pathways (Figure 3). OPN was shown to trigger VEGF-dependent tumor progression and angiogenesis by activating Brk/NF-κB/ATF-4 signaling cascades through autocrine and paracrine mechanisms in breast cancer cells. VEGF promoter activity and its expression in human breast carcinoma cell lines was found to be regulated by OPN. OPN induces Brk/NIK-dependent NF-κB-mediated ATF-4 activation that leads to VEGF expression. The study revealed that OPN-induced VEGF binds with neuropilin-1 (NRP-1) and enhances VEGF–NRP-1-dependent tumor cell migration through autocrine pathway. Moreover, OPN

induces VEGF dependent KDR phosphorylation leading to increased endothelial cell migration and angiogenesis in a paracrine manner. Tumor-endothelial cell interaction through binding with NRP-1 and KDR in endothelial cells was observed to be regulated by tumor derived VEGF in response to OPN in a juxtacrine manner. Blocking tumor-derived VEGF or silencing tumor-derived OPN and NRP-1 significantly suppressed breast tumor progression and angiogenesis in nude mice model. Clinical specimen analysis of solid human breast tumors exhibited strong correlation between the OPN and VEGF expression with different pathologic grades of tumors. Previous reports have also shown that VEGF induces mRNA encoding OPN in endothelial cells (Sengar et al, 1996). OPN plays a crucial role in determining spontaneous metastatic performance of orthotopic human breast cancer xenografts. Changes in levels of OPN induced by silencing with its shRNA or upregulation by cDNA altered the ability of breast cancer cells to colonize to distant organs. It has been shown that silencing of OPN resulted in reduction of *in vivo* tumorigenicity through down regulation of molecules like uPA, MMP-2 and -9. OPN knocked out mice showed slower progression of tumor growth in breast cancer model as compared to wild type mice (Chakraborty et al, 2008).

6.2 OPN inhibits staurosporine (STS)-induced apoptosis through JAK2/STAT3 signaling pathway

Earlier reports have indicated that enhanced expression of STAT3 correlates with increased tumor growth and poor survival in breast cancer (Garcia et al, 1997). Behera et al have recently demonstrated that OPN induces αvβ3 integrin-mediated JAK2 dependent STAT3 activation in breast cancer cells (Behera et al, 2010). The mechanism by which OPN controls JAK2/STAT3 signaling pathway and regulates apoptosis and breast tumor growth was studied. OPN was found to activate STAT3 by inducing its phosphorylation through αvβ3 integrin mediated pathway. OPN has been observed to regulate STAT3 nuclear translocation through αvβ3 integrin mediated and JAK2 dependent pathway. It was further established that OPN, through promoting STAT3-DNA binding ultimately regulates the expression of downstream molecules such as cyclin D1 and Bcl2 and thus influences survival and cell migration in breast cancer (Figure 3). Cells transfected with wt STAT3 showed enhanced cell migration as well as anti-apoptotic function in response to OPN, as opposed to cells transfected with the mutant forms of STAT3. The study revealed that OPN protects the cells from staurosporine (STS)-induced apoptosis through JAK2/STAT3 pathway. Cells stably transfected with wt STAT3 and not with mutant STAT3 were observed to enhance tumor growth in response to OPN in mice models. Enhanced expressions of Bcl2 and cyclin D1 in STAT3- overexpressed tumors in response to OPN were indicative of the significance of STAT3 in OPN-induced Bcl2 and cyclin D1 expression and tumor progression. Clinical specimen analysis revealed an enhanced expression of OPN and phosphorylated STAT3 and their correlation with higher grades of breast cancer as compared to the peripheral normal and lower grades.

6.3 OPN regulates breast cancer cell motility through mTOR/p70S6 kinase pathway

mTOR, a serine threonine kinase regulates both cell growth and cell cycle progression (Ahmed and Kundu, 2010). mTOR initiates translation by activating the p70S6 kinase. Inhibition of mTOR by rapamycin attenuates its ability to control cell cycle progression, cell growth and proliferation in normal and malignant cells. They have recently reported

that OPN regulates p70S6 kinase and mTOR phosphorylation in breast cancer cells (Ahmed and Kundu, 2010). The results revealed that OPN controls NF-κB mediated ICAM-1 expression in these cells. The data also showed that OPN induced NF-κB controls AP-1 transactivation indicating a cross talk between NF-κB and AP-1 which in turn regulates ICAM-1 expression in these cells (Figure 3). The study suggested that inhibition of mTOR by rapamycin enhanced whereas overexpression of mTOR/p70S6 kinase inhibited OPN-induced ICAM-1 expression. OPN-induced NF-κB and AP-1-DNA binding and transcriptional activity was inhibited by mTOR overexpression whereas rapamycin was noted to enhance these OPN-induced effects. In the same study, OPN was shown to selectively phosphorylate p70S6 kinase at Thr-421/Ser-424 through MEK/ERK pathway but it did not phosphorylate p70S6 kinase at Thr-389 and Ser-371 sites which further suggested that mTOR inhibitor, rapamycin suppresses p70S6 kinase phosphorylation at Ser-371 and does not affect p70S6 kinase phosphorylation at Thr-421/Ser-424 and Thr-389 sites indicating that Ser-371 phosphorylation is primarily responsible for p70S6 kinase activation in these cells (Figure 3).

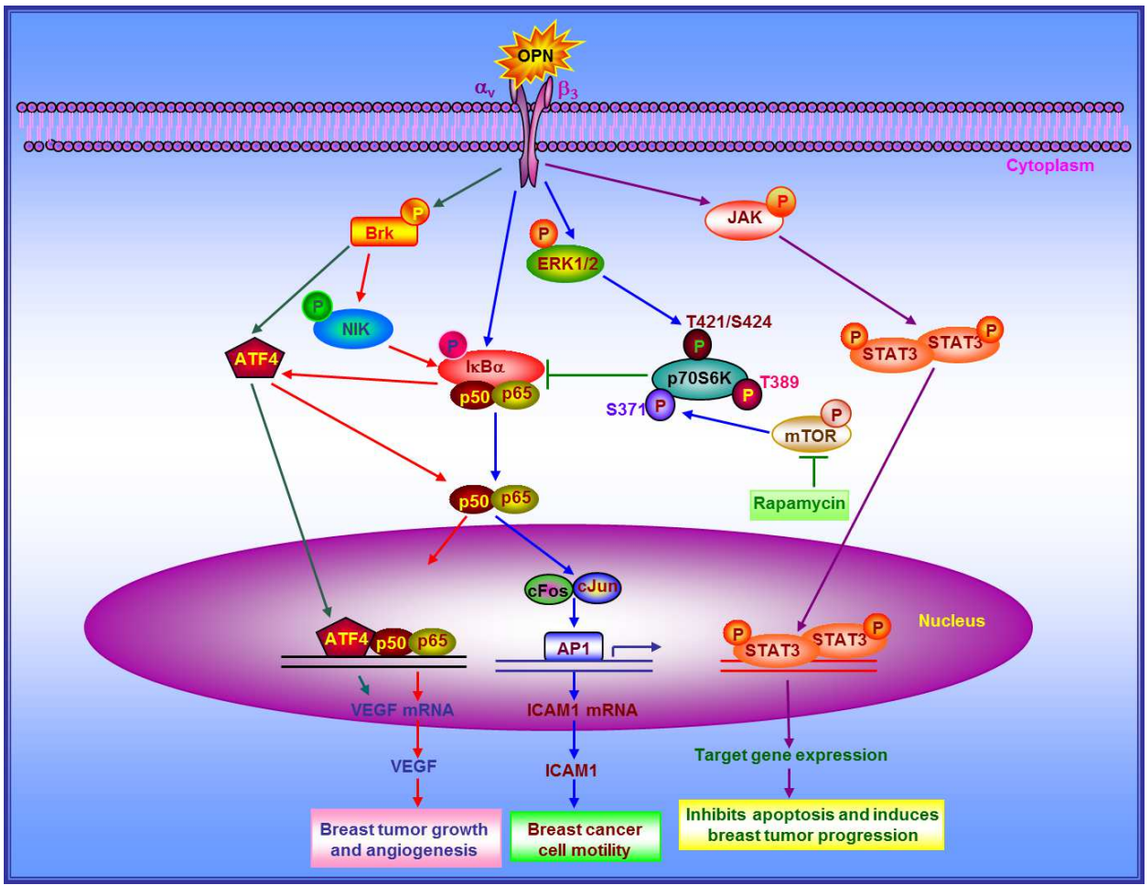

Fig. 3. Diagramatic representation of OPN-induced signaling cascades mediated by its cell surface receptor, integrin. These signaling pathways lead to upregulation of various oncogenic and angiogenic molecules that augment breast cancer cell migration, tumor growth, angiogenesis and inhibition of apoptosis, (Adapted from Chakraborty et al., 2008; Ahmed and Kundu, 2010; Behera et al., 2010 with modification).

7. Clinicopathological significance of osteopontin in breast cancer

Effective management of breast cancer is possible by surgical removal of the tumor. Metastasis of tumor cells to secondary sites like bone, lung, liver and brain leads to poor survival. Although the detection system is not well established owing to the multifactorial nature and heterogeneity of cancer, early diagnosis can be made possible by identifying cancer biomarkers. Many earlier publications suggested that OPN may be considered as one of the potential candidate biomarkers in breast cancer. OPN is overexpressed in human breast cancer cells and tissues as well as in stromal compartment including CAFs. OPN plays a critical role in generation of calcification which is allied with breast cancer. Enhanced expression of OPN has been found in plasma and tumors of metastatic breast cancer suggesting that OPN may be considered as a prognostic marker (Bramwell et al, 2006). The plasma OPN level in women with known metastatic breast carcinoma is significantly higher than that of normal healthy individuals. The plasma OPN level in patients with metastatic breast cancer is higher than 138 ng/ml versus control groups which have 123 ng/ml. Gene profiles compared between lobular versus ductal breast carcinomas using microarray analysis reveal 11 genes including OPN, and a specific change in gene expression (Korkola et al, 2003). An mRNA transcript analysis of OPN in normal, non-invasive, invasive and metastatic human breast cancer specimens shows that it's level increases with enhanced malignancy. Moreover, a splice variant of OPN, namely OPN-C has been shown to be an important marker of breast cancer. It has been shown that OPN-C is selectively expressed in invasive, but not in non-invasive breast tumor cell lines. When the significance of OPN-C was studied in various tumor grades of breast cancer, the level of OPN-C increased from grade 1 to 3. Conclusively, these reports suggested that OPN-C is a selective marker of breast cancer (He et al, 2005).

8. Therapeutic potential of OPN and its receptors

Many OPN specific monoclonal and polyclonal antibodies have been generated. It has been observed that humanized anti-OPN antibody inhibits cell migration, adhesion, invasion, colony formation, tumor growth and lung metastasis in breast cancer (Dai et al, 2010). Thus for effective cancer management, targeting OPN by its specific blocking antibody may provide a novel therapeutic approach (Figure 4). The binding of OPN and its receptor controls the expression of various oncogenic molecules leading to tumor progression through various signalling pathways. Therefore, disruption of OPN and its receptor ligation may attenuate tumor growth and metastasis. αvβ3 integrin blocking antibody inhibits OPN-induced tumor growth and angiogenesis through attenuating various signaling cascades (Rangaswami et al, 2006). Decreased expression of OPN, integrin linked kinase (ILK), uPA and MMP-2 in murine mammary epithelial cancer cells was observed by blocking αvβ3 integrin (Mi et al, 2006). OPN can interact with various integrins and the specific blocking antibodies against these receptors can significantly suppress tumor-stromal interaction and reduce OPN-induced tumor progression (Figure 4). It has been recently documented that αvβ3 integrin blocking antibody inhibits AP1 activation in response to OPN in breast cancer cells (Ahmed and Kundu, 2010). Inhibition of OPN and αvβ3 integrin binding by LM609 and RGD peptide attenuates STAT3 DNA-binding and suppresses cell migration and breast tumor growth by down regulating the expression of cyclinD1 and Bcl2 (Behera et al, 2010). Previous results have demonstrated that non-RGD-based integrin binding peptide (ATN-161) suppresses breast tumor growth and metastasis (Khalili et al, 2006).

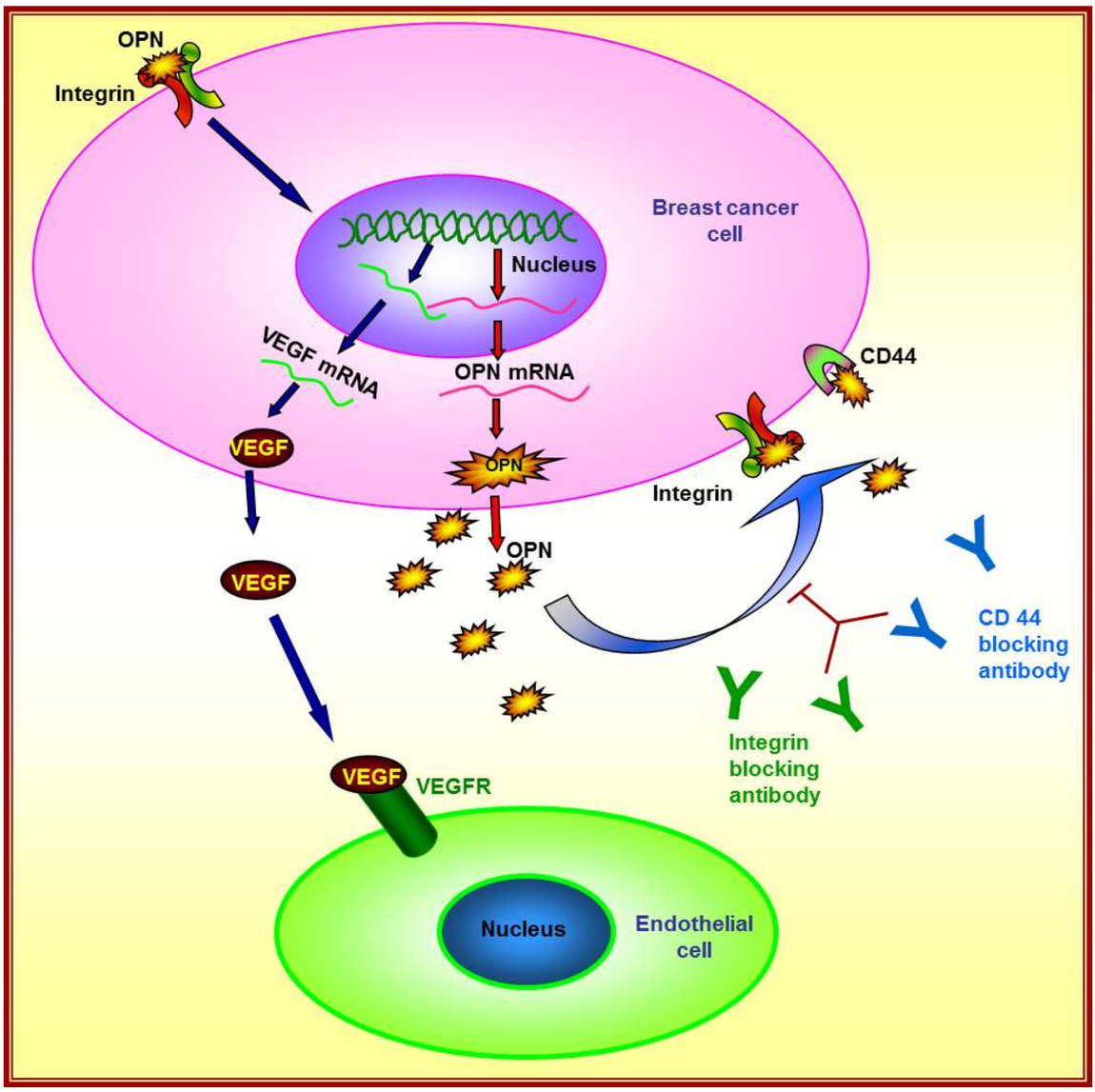

Fig. 4. Therapeutic targeting of OPN in breast cancer. The blocking antibody against OPN or its receptors such as integrin and CD44 impedes OPN regulated cancer signalling pathways leading to inhibition of breast tumor growth and angiogenesis through disruption of tumor-endothelial cell interaction.

9. Conclusion

Breast cancer accounts for major cancer related death in women around the world. Tremendous efforts are being made everyday in reducing the occurrence of breast cancer. Because of the complexity of the diseases, precise detection system is not available till date to diagnose the cancer at the early stages. Therefore, identification of novel biomarkers is the need of the hour. According to numerous publications, OPN may be considered as a potential biomarker in breast cancer because of its involvement in all the stages of tumor progression. Hence targeting OPN would be a rational approach for the treatment of cancer. In addition to tumor derived OPN, stromal OPN also plays a crucial role in regulation of tumor progression and angiogenesis. In conclusion, we have demonstrated that OPN regulates breast cancer cell migration through mTOR/p70S6 kinase dependent ICAM-1 expression. Moreover, OPN also induces breast tumor growth and inhibits apoptosis through induction of JAK2/STAT3 dependent expression of Bcl2 and cyclin D1. Furthermore, OPN controls VEGF dependent breast cancer growth and angiogenesis through tumor-endothelial cell interaction via Brk/NIK dependent NF-κB activation pathway. Thus in depth knowledge of OPN regulated signalling mechanism may be useful in developing novel molecular diagnostics and targeted therapy for the management of breast cancer.

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11. References

Ahmed, M. & Kundu, GC. (2010). Osteopontin selectively regulates p70S6K/mTOR phosphorylation leading to NF-κB dependent AP-1-mediated ICAM-1 expression in breast cancer cells. *Molecular Cancer*, Vol. 9, No. 101, (May 2010), pp 101-13.

Beausoleil, MS., et al. (2011). Deletion of the thrombin cleavage domain of osteopontin mediates breast cancer cell adhesion, proteolytic activity, tumorgenicity, and metastasis. *BMC Cancer*, Vol. 11, No. 25, (January 2011), pp 1-12.

Behera,R., et al. (2010). Activation of JAK2/STAT3 signaling by osteopontin promotes tumor growth in human breast cancer cells. *Carcinogenesis*, Vol. 31, No. 2, (February 2010), pp 192–200.

Boominathan, L. (2010). The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex. *PLoS One*, Vol. 5, No. 5, (May 2010), pp. 1-13.

Bramwell, V.H.C., et al (2006). Serial plasma osteopontin levels have prognostic value in metastatic breast cancer. *Clinical Cancer Research.* Vol.12, (June 2006), pp. 3337-43.

Celetti et al. (2005). Overexpression of the cytokine osteopontin identifies aggressive laryngeal squamous cell carcinomas and enhances carcinoma cell proliferation and invasiveness, *Clinical Cancer Research,* Vol.11, (November 2005), pp. 8019-27.

Chakraborty, G., et al. (2006). The multifaceted roles of osteopontin in cell signaling, tumor progression and angiogenesis. *Current Molecular Medicine*. Vol. 6, No. 8, (December 2006), pp. 819-30.

Chakraborty, G., et al. (2008). Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. *Cancer Research*, Vol. 68, No. 1, (January 2008), pp 152-61.

Chakraborty, G., et al (2008). Curcumin suppresses breast tumor angiogenesis by abrogating osteopontin-induced VEGF expression. *Molecular Medicine Reports*, Vol. 1, No. 5, (June 2008) pp. 641-46.

Chen, JQ. & Russo, J. (2009). ER alpha negative and triple negative breast cancer: molecular features and potential therapeutic approaches. *Biochimica et Biophysica Acta*, Vol. 1796, No. 2, (December 2009), pp. 162-75.

Cook, A.C., et al (2005). Osteopontin induces multiple changes in gene expression that reflect the six ''Hallmarks of Cancer'' in a model of breast cancer progression. *Molecular Carcinogenesis*, Vol. 43, No. 4, (August 2005), pp- 225-36.

Cook, A.C., et al (2006). Osteopontin induction of hyaluronan synthase 2 expression promotes breast cancer malignancy. *Journal of Biological Chemistry*, Vol. 281, No. 34, (August 2006), pp. 24381-89.

Dai, J., et al (2010). A humanized anti-osteopontin antibody inhibits breast cancer growth and metastasis in vivo. *Cancer Immunology and Immunotherapeutics*, Vol.59, No.3, (March 2010), pp. 355-66.

Das, R., et al. (2003). Osteopontin stimulates cell motility and nuclear factor kappaB-mediated secretion of urokinase type plasminogen activator through phosphatidylinositol 3-kinase/Akt signaling pathways in breast cancer cells. *Journal of Biological Chemistry*, Vol. 278, No. 31, (August 2003), pp 28593-606.

Das, R., et al. (2004). Osteopontin induces AP-1-mediated secretion of urokinase-type plasminogen activator through c-Src-dependent epidermal growth factor receptor transactivation in breast cancer cells. *Journal of Biological Chemistry*, Vol. 279, No.12, (March 2004), pp 11051-64.

Garcia, R., et al (1997). Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells. *Cell Growth and Differentiation*. Vol.8, No.12 (December 1997), pp. 1267-76.

Hanahan, D. & Weinberg, RA. (2000). The hallmarks of cancer. *Cell*, Vol. 100, No.1, (January 2000), pp. 57-70.

Hanahan, D. & Weinberg, RA. (2011). Hallmarks of cancer: The next generation. *Cell,* Vol. 144, No. 5, (March 2011), pp. 646-74.

He, B., et al. (2005). An osteopontin splice variant induces anchorage independence in human breast cancer cells. *Oncogene,* Vol. 25, No. 15, (April 2006), pp. 2192-202.

Kang, Y. et al., (2003). A multigenic program mediating breast cancer metastasis to bone, *Cancer Cell,* Volume 3, No. 6 (June 2003) pp. 537-49.

Khalili, P., et al (2006). A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo. *Molecular Cancer Therapeutics*, Vol.5, No.9, (September 2006), pp. 2271-80.

Korkola, J.E., et al (2003). Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. *Cancer Research,* Vol.63, No.21, (November 2003), pp. 7167-75.

Leslie, M. (2011). Brothers in arms against cancer. *Science*, Vol. 331, No. 6024, (March 2011), pp. 1551 - 52.

Liotta, L. A., & Kohn, EC. (2001). The microenvironment of the tumor-host interface, *Nature*, Vol. 411, No. 6835 (May 2001), pp. 375 –79.

Mcmahon (2000). VEGF receptor signaling in tumor angiogenesis, *The Oncologist,* Vol.5, (April 2000), pp. 3-10.

Mi, Z., et al (2006). Integrin-linked kinase regulates osteopontin-dependent MMP-2 and uPA expression to convey metastatic function in murine mammary epithelial cancer cells. *Carcinogenesis,* Vol.27, No.6, (June 2006), pp. 1134-45.

Mi, Z., et al (2011). Osteopontin promotes CCL5-mesenchymal stromal cell-mediated breast cancer metastasis, *Carcinogenesis*, Vol.32, No. 4, (April 2011), pp. 477-87.

Osterreicher, C.H., et al. (2011). Fibroblast-specific protein 1 identifies an inflammatory subpopulation of macrophages in the liver *Proceedings of the National Academy of Sciences,* Vol. 108 No. 1, (January 2011) pp 308-13.

Panda, D., et al. (1997). Potential roles of osteopontin and alphavbeta3 integrin in the development of coronary artery restenosis after angioplasty, *Proceedings of the National Academy of Sciences,* Vol. 94, No. 17, (August 1997), pp. 9308–13.

Rangaswami, H., et al. (2006). Osteopontin: role in cell signaling and cancer progression. *Trends in Cell Biology*, Vol. 16, No 2, (February 2006), pp. 79-87.

Rittling, S.R., et al. (2002). Tumor-derived osteopontin is soluble, not matrix associated. *Journal of Biological Chemistry*, Vol. 277, No. 11, (March 2002), pp. 9175-82.

Senger, D.R., et al. (1996). Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. *American Journal of Pathology,* Vol.149, No.1 (July 1996), pp. 293-305.

Shevde, L.A., et al (2006). Osteopontin knockdown suppresses tumorigenicity of human metastatic breast carcinoma, MDA-MB-435. *Clinical Experimental Metastasis*. Vol. 23, No. 2, (July 2006), pp. 123-33.

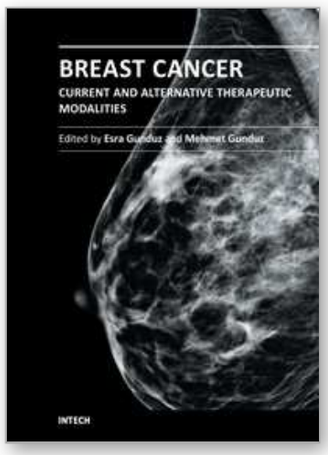
Shinohara, M.L., et al. (2008). Alternative translation of osteopontin generates intracellular and secreted isoforms that mediate distinct biological activities in dendritic cells. *Proceedings of the National Academy of Sciences*, Vol. 105, No 20, (May 2008), pp. 7235-39.

Stefansson, O.A., et al. (2009). Genomic profiling of breast tumours in relation to BRCA abnormalities and phenotypes. *Breast Cancer Research*, Vol. 11, No. 4, (July 2009), pp. 1-14.

Tuck et al. (2000). Osteopontin-induced, integrin-dependent migration of human mammary epithelial cells involves activation of the hepatocyte growth factor receptor (Met). *Journal of Cellular Biochemistry*, Vol.78, No. 3, (June 2000), pp. 465–75.

Wai, PY. & Kuo, PC. (2004). The role of Osteopontin in tumor metastasis. *Journal of Surgical Research*. Vol. 121, No. 2, (October 2004), pp 228-41.

Wong et al. (1998). Alphav integrins mediate adhesion and migration of breast carcinoma cell lines. *Clinical and Experimental Metastasis*, Vol.16, No. 1, (January 1998) pp. 50 – 61.



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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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