The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

Sir William Bragg
4. AIMS AND OBJECTIVE OF THIS STUDY

Regulation of metastatic signature and angiogenic switch have been considered as the most crucial steps during progression of tumor towards malignancies but currently used diagnostic techniques are unable to accurately reflect the degree of neovascularization and metastatic spread of cancers. Recent evidences suggested that development of cancer progression, metastatic spread and angiogenesis could be determined by the expression profiling of specific proteins, considered as the markers. OPN, a member of SIBLING family of ECM associated protein has been considered as one the most promising among all of the biomarkers.

OPN has known to promote tumor progression by interacting with cell surface receptors integrins and CD44, which in turn regulate the activation of several downstream kinases (i.e. c-Src, PI 3-kinase, ERK, AKT, MAPK, IKK etc.), transcription factors (i.e NF-κB, AP-1, SP-1 etc.), that ultimately augments the expression of various oncogenic molecules like MMPs, uPA, COX-2. Moreover, earlier studies demonstrated that OPN produced from either tumor or host, regulates tumor metastasis. Recently it has also been shown that targeting OPN by its specific siRNA or blocking antibody significantly attenuates tumor progression and metastasis in various cancer models.

Vascular endothelial growth factor (VEGF) has been considered as the most potent angiogenic factor that plays crucial role in tumor angiogenesis and leads to tumor malignancies. Till date, VEGF based anti-cancer approaches show great promises in anti-cancer therapy. Therefore, understanding the molecular mechanism underlying the expression of VEGF in tumor cells and the role of VEGF receptors in VEGF-regulated tumor angiogenesis will be helpful to develop therapeutic approach for next generation of cancer management. Therefore, this study is aimed to delineate the molecular mechanism(s) by which OPN via multiple signaling cascades regulates VEGF expression, VEGF dependent tumor growth and angiogenesis in \textit{in vivo} animal model and in human breast cancer clinical specimens. Moreover, we have demonstrated that down-regulation of tumor as well as stroma/host-derived OPN significantly attenuates breast tumor progression in various murine models. Briefly,

1. To investigate the role of OPN in regulation of VEGF expression in human breast carcinoma cells.
2. To understand the role of exogenous and tumor-derived OPN on induction of VEGF expression in tumor as well as stromal cells.
3. To delineate the molecular mechanism by which OPN regulates VEGF expression through c-Src-PI 3-kinase-NIK-IKKα/β-NF-κB mediated signaling pathway both at transcriptional and translation levels.
4. To study the role of OPN in the regulation of Brk kinase activity, phosphorylation, and subsequent interaction with phosphorylated NIK.

5. To examine the role of Brk on NF-κB activation and NF-κB dependent/independent ATF-4 nuclear localization and whether there would be any crosstalk between NF-κB and ATF-4 in response to OPN.

6. To ascertain whether OPN-induced VEGF regulates tumor cell motility by interacting with its cell surface receptor NRP-1 via autocrine loop.

7. To check whether OPN-induced tumor-derived VEGF interacts with KDR on endothelial cells and induce its phosphorylation and regulates KDR mediated endothelial cell migration, and in vivo Matrigel based angiogenesis via paracrine mechanism.

8. To demonstrate whether OPN-induced VEGF dependent tumor-endothelial interaction occurs through NRP-1-KDR mediated juxtacrine pathway.

9. To study the role of exogenous and tumor-derived OPN on VEGF-NRP-1 dependent orthotopic breast tumor growth in nude mice.

10. To check whether intratumoral injection of OPN or NRP-1 specific siRNA curbs breast tumor growth in nude mice.

11. To analyze the effect of stromal/host-derived OPN in regulation of breast tumor growth by using wild type and OPN-knockout mice.

12. To determine the expression profile of OPN and VEGF in various grades of human breast cancer clinical specimens and their correlation with activation and expression profiles of NF-κB, ATF-4, AP-1, Brk, NRP-1, MMP-2, MMP-9, MT-1MMP, uPA and vWF.

13. To investigate the role of natural carcinogen pristane on OPN-dependent breast tumor growth and angiogenesis in mice models.

Thus, this study is aimed to delineate the molecular mechanism by which the tumor or stroma-derived OPN regulates c-Src-PI 3-kinase-NIK-IKK-NF-κB or Brk-ATF-4 mediated VEGF expression, which in turn regulates breast tumor growth and angiogenesis. Moreover, our study also aimed to understand the role of both tumor- and stroma-derived OPN in regulation of breast cancer and melanoma progression and angiogenesis.