ABSTRACT OF THE THESIS

Cell migration and degradation of the extracellular matrix are some of the crucial steps involved in tumor progression. Several proteinases including matrix metalloproteinases (MMPs) play important roles in degradation of extracellular matrix (ECM) components and hence contribute to cancer cell motility, tumor growth and metastasis. MMPs are highly regulated by several growth factors, cytokines and ECM components. Osteopontin (OPN) is a secreted, non-collagenous, sialic acid rich, chemokine like calcified ECM protein that plays a significant role in determining the metastatic potential of various cancers. Since its first identification in bone by biochemical means, OPN has been reported to play a multifaceted role in regulating a number of physiological and pathophysiological processes like atherosclerosis, bone remodeling, angiogenesis, wound healing and tissue injuries as well as certain diseases such as restenosis, arterial neointimal hyperplasia, myocardial necrosis, renal tubulointerstitial fibrosis, kidney stone formation and autoimmune diseases. It exerts its pro-metastatic effects by interacting with various integrins and CD44 receptors. Thus OPN and its receptors figure prominently in a wide spectrum of malignancies. Further studies in a variety of human cancers have correlated the high levels of OPN expression with the advanced stages of tumors. The pivotal role of OPN in tumor dissemination has been highlighted by gene transfer experiments. Overexpression of OPN results in an increase in malignant phenotype, whereas transfection with antisense oligonucleotides yields population with reduced malignant potential. Thus OPN has been recognized as a potential marker in the processes of tumorigenicity and metastasis. However, the molecular mechanism(s) by which OPN regulates pro-matrix metalloproteinase-9 (pro-MMP-9) activation, MMP-9 dependent cell motility and melanoma progression and involvement of upstream kinases in regulation of these processes in murine melanoma cells are not well defined.

Our data indicated that OPN induces αvβ3 integrin-mediated phosphorylation and activation of nuclear factor inducing kinase (NIK) and enhances the interaction between phosphorylated NIK and IκBα kinase α/β (IKKα/β) in B16F10 cells. Moreover, NIK is involved in OPN-induced phosphorylations of MEK-1 and ERK1/2 in these cells. OPN
induces NIK dependent NFκB activation through ERK/IKKα/β-mediated pathways. Furthermore, OPN enhances NIK regulated urokinase type plasminogen activator (uPA) secretion, uPA dependent pro-MMP-9 activation, cell motility and tumor growth. Wild type NIK, IKKα/β and ERK1/2 enhanced and kinase negative NIK (mut NIK), dominant negative IKKα/β (dn IKKα/β) and dn ERK1/2 suppressed the OPN-induced NFκB activation, uPA secretion, pro-MMP-9 activation, cell motility and chemoinvasion. Pretreatment of cells with anti-MMP-2 antibody along with anti-MMP-9 antibody drastically inhibited the OPN-induced cell migration and chemoinvasion whereas cells pretreated with anti-MMP-2 antibody had no effect on OPN-induced pro-MMP-9 activation suggesting that OPN induces pro-MMP-2 and pro-MMP-9 activations through two distinct pathways. The level of active MMP-9 in the OPN-induced tumor was higher compared with control. Thus our data indicated that NIK plays crucial role in OPN-induced NFκB activation, uPA secretion and pro-MMP-9 activation through MAPK/IKKα/β-mediated pathways and all of these ultimately control the cell motility, invasiveness and tumor growth.

NIK is a member of the MAP3K family that has been implicated in NFκB activation. Few reports indicate that NIK may also be involved in the regulation of transcription factor, AP-1 as its activation leads to the induction of c-Fos that associates with c-Jun to form an AP-1 heterodimeric complex that can promote targeted gene expression. However the molecular mechanism by which OPN regulates NIK and MEKK1-mediated AP-1 transactivation and whether JNK1 is involved in both these pathways is not clearly understood. Various MAPK cascades (eg. ERK1/2, JNK, p38) are often portrayed as linear cascades and indications of cross talk between the various cascades are limited. In this respect, we also examined whether any cross talk exists between OPN-induced NIK/ERK and MEKK1/JNK signaling pathways.

The data indicated that OPN induces αvβ3 integrin-mediated MEKK1 phosphorylation and MEKK1 dependent JNK1 phosphorylation and activation. Overexpression of NIK enhances OPN-induced c-Jun expression, whereas overexpressed NIK had no role in OPN-induced JNK1 phosphorylation and activation. Sustained activation of JNK1 by overexpression of wild type MEKK1 resulted in suppression of ERK1/2 activation. But this did not affect the OPN-induced NIK-dependent ERK1/2 activation.
activation. OPN stimulated both NIK and MEKK1-dependent c-Jun expression leading to AP-1 activation whereas NIK-dependent AP-1 activation is independent of JNK1. OPN also enhanced JNK1-dependent/independent AP-1-mediated uPA secretion, uPA dependent pro-MMP-9 activation, cell motility and invasion. OPN stimulates tumor growth and the levels of c-Jun, AP-1, uPA and MMP-9 were higher in OPN-induced tumor compared to control. Thus our data suggested that OPN induces NIK/MEKK1-mediated JNK1-dependent/independent AP-1-mediated pro-MMP-9 activation and regulates the negative cross talk between NIK/ERK1/2 and MEKK1/JNK1 pathways that ultimately controls the cell motility, invasiveness and tumor growth.

Melanoma is one of the most aggressive cancers with high mortality and hence the identification of new markers that correlate with melanoma progression may aid in efficient prognosis. This transformation-associated protein, osteopontin (OPN), induces MelCAM expression through FAK/NIK-mediated pathways. OPN also induces FAK phosphorylation and silencing of FAK completely abrogated the OPN-induced NIK phosphorylation. Treatment of cells with MelCAM specific blocking peptide reduced the OPN-induced FAK dependent NIK activation indicating the existence of a reciprocally regulated loop between FAK and MelCAM. OPN also induces FAK and NIK dependent AP-1 activation and AP-1 mediated Sp-1 activation leading to uPA secretion and MMP-9 activation in these cells. OPN also induces NIK-dependent spheroid formation, cell motility, tumor growth and lung metastasis. The levels of pNIK, MelCAM and MMP-9 are significantly higher in the primary tumor and metastasized lung compared to control. Clinical data revealed that enhanced OPN, pNIK and MelCAM expressions correlate with Clark’s level and Breslow thickness which in turn reflects the melanoma grades. These results highlight the potential role of OPN and the molecular mechanism underlying the genesis of metastasis signature in melanoma and hence may be useful in developing novel molecular diagnostics and targeted therapy for the treatment of malignant melanoma.