

C-Jun N-terminal Kinase is Activated in Non-Small Cell Lung Cancer and Confers Neoplastic Properties to Human Bronchial Epithelial Cells

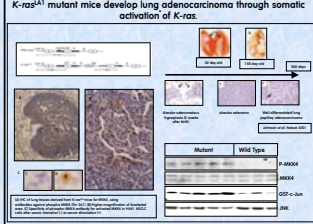
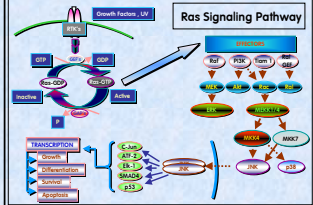
Abstract # 2646

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Abstract
c-Jun N-terminal kinase (JNK) has been reported to either potentiate or inhibit oncogenesis, depending upon the cellular context, but its role in lung neoplasia has not been fully defined. Here we investigated the role of JNK in lung neoplasia by examining evidence of JNK phosphorylation in NSCLC biopsy samples and by using genetic and pharmacologic approaches to modulate JNK expression and activity in cells. First, immunohistochemical analysis was performed to examine Thr183/Tyr185-phosphorylated JNK in NSCLC biopsy samples. Staining was detected in 45% (114/252) of NSCLC biopsy samples and was predominantly nuclear, providing evidence of JNK activation in a subset of NSCLC. On the basis of these findings, we hypothesized that JNK activation is oncogenic in the bronchial epithelium and investigated whether the introduction of an upstream activator of JNK, mitogen-activated protein kinase kinase-4 (MKK4), is sufficient to confer neoplastic properties to human bronchial epithelial (HBE) cells. Introduction of a doxycycline-inducible, constitutively active MKK4 (MKK4-ED mutant) into the HBE cell lines BEAS-2B and H560B increased cell proliferation, migration, invasion, and clonogenicity. Depletion of JNK in MKK4-ED-transformed BEAS-2B cells by introduction of JNK1/2 shRNA reversed the transformed phenotype, indicating that JNK was oncogenic in these cells. The NSCLC cell lines HCC97 and H2009 exhibited constitutive phosphorylation of JNK and its substrate, c-Jun, and these cell lines were sensitive to treatment with SP600125, a JNK kinase inhibitor. We conclude that JNK is activated in a subset of NSCLC biopsy samples and promotes oncogenesis in the bronchial epithelium.



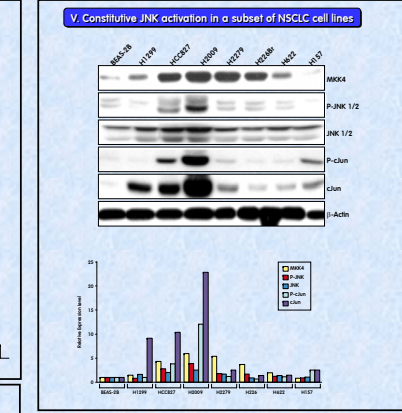
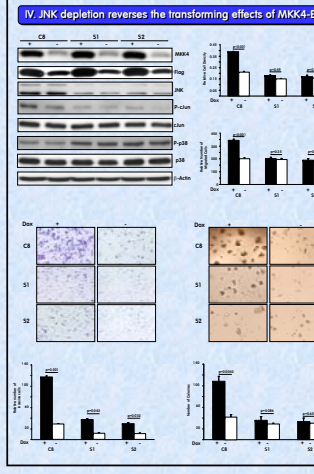
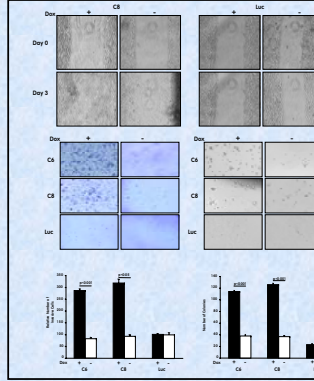
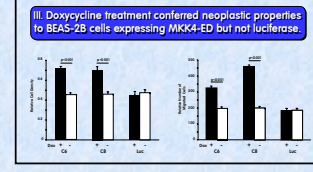
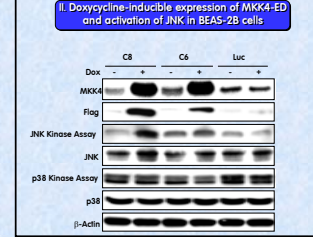
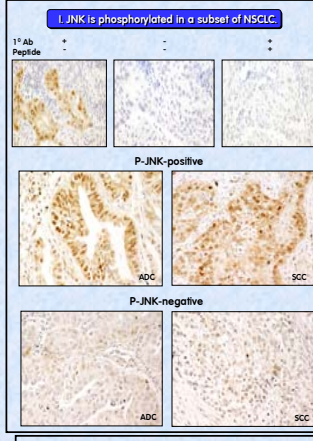
Mitogen Activated Protein Kinase Kinase-4 (MKK4)
MKK4 is a member of the stress activated protein kinase (SAPK) signaling pathway. It directly phosphorylates and activates JNK at Thr183/Tyr185 and p38, and ultimately controls the transcription factors such as c-Jun. It plays a role in processes like cellular proliferation, differentiation and apoptosis. In cancer, activation of MKK4-dependent signaling has demonstrated widely disparate effects (pro-survival or pro-apoptotic).

Jun-N-terminal Kinase (JNK)
The Jun kinase/stress-activated protein kinase (JNK/SAPK) pathway has been implicated in major cellular functions, such as cell proliferation and transformation, DNA repair and cellular stress response including apoptosis. JNK phosphorylates the transcription factor c-Jun at serine residues 63 and 73 and augments the AP-1 transcriptional activity. JNK activation and c-Jun phosphorylation are required for transformation induced by Ras, an oncogene that is mutually activated in almost 30% of non-small cell lung cancer NSCLC. Transforming potential of several oncogenes like EGFR is reduced upon introduction of anti-sense JNK oligonucleotides or dominant-negative versions of proteins of JNK pathway. However a study in jnk-null fibroblasts showed that JNK was not required for transformation induced by Ras, suggesting that JNK acts as a tumor suppressor in fibroblasts. Together these considerations indicate that JNK may play more than one role in tumor development.

Aims
Based on the fact that MKK4/JNK is involved in many cellular processes, and our previous findings that I. MKK4 and JNK are activated in K-rasA1 mutant mice, and II. the survival of NSCLC cells is dependent upon activation of PI3K and MKK4-dependent pathways, our main aim was to determine whether the activation of MKK4 and its downstream mediators like JNK and c-Jun are sufficient to induce malignant transformation in the immortalized Human Bronchial Epithelial cell lines (HBE).

Materials and Methods
Cell lines: BEAS-2B-Derived from SV40-transformed Human-Bronchial-Epithelial cell lines. H560-B-Spontaneously immortalized cell line derived from the bronchus of a healthy woman. Normal karyotype. Non-tumorigenic in nude mice. Partially intact differentiation potential.

1. Retrovirus and target cell production:
 - Tet Inducible retroviral gene expression system.
 - Infection of target cells to produce the stable cells.
2. Protein Expression Studies:
 - Western using Ab's to total and Phospho-specific proteins.
3. Protein Kinase assays:
 - Effect of over-expressed MKK4 on downstream targets.
4. Cell Proliferation:
 - MTT Assay.
5. Cell Migration and Invasion Assay:
 - Matrigel Assay.
6. Anchorage independent growth:
 - Clone formation assay on soft agar.



Summary and Conclusion

1. JNK was phosphorylated in a subset of NSCLC biopsy samples.
2. The introduction of an upstream activator of JNK, MKK4, conferred neoplastic properties in HBE cells.
3. JNK depletion reversed the oncogenic effects of MKK4 activation in HBE cells.
4. JNK was constitutively active in two NSCLC cell lines, and these cells were sensitive to pharmacologic inhibition of JNK.
5. We conclude that JNK is activated in a subset of NSCLC biopsy samples and promotes oncogenesis in the bronchial epithelium.

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