Double mutant P96S/S120G of Nm23-H1 abrogates its NDPK activity and motility-suppressive ability*

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Background: The Nm23-H1 gene is a metastasis suppressor gene. However, its biochemical mechanism of suppressing the metastatic potential of cancer cells is still unknown. The previous hypothesis that a histidine protein kinase activity may contribute to the motility-suppressive effect of Nm23-H1 could not explain why the H118F mutant, a kinase-deficient mutant, still had motility-suppressive ability.

Methods: We conducted a study on the double mutant P96S/S120G of Nm23-H1 and succeeded in introducing the RP-HPLC method in NDPK assay.

Results: The results showed that double mutant P96S/S120G, when expressed in the bacteria, was completely aggregated in inclusion bodies; prompted that the deficiency of motility-suppressive function of S120G, P96S, and P96S/S120G mutants was due to their altered structure, which might deprive Nm23-H1 of most activities including kinase activity or interactions with other proteins.

Conclusions: Double mutant P96S/S120G of Nm23-H1 abrogates its NDPK activity and motility-suppressive ability in the human high-metastatic large cell lung cancer cell line L9981.

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Novel mechanism of collagen-tumor cell interaction by integrin alpha-11 expression by cancer associated fibroblasts in non-small cell lung cancer cells

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Background: The integrin α11β1 subunit is commonly overexpressed in non-small cell lung carcinoma (NSCLC). α11β1 integrin is one of the four receptors for interstitial collagen. Immunofluorescence study localized the protein mainly to the tumor stroma. α11 is also commonly overexpressed in cancer-associated fibroblasts. We hypothesized that stromal expression of α11 may play important role in regulating the tumor formation of NSCLC cells.

Methods: SV40 immortalized mouse embryonic fibroblasts established from the wild type (WT) and α11 deficient (KO) mice were tested for their tumorigenicity in immune deficient mice when co-implanted with the A549 human lung adenocarcinoma cells. Fibroblasts and A549 alone served as controls. Total cellular RNA was isolated from tumors formed and profiled using the Affymetrix U133A microarray, and differentially expressed genes were identified. Stable gene expression downregulation was accomplished by retroviral mediated transduction of short hairpin (sh) RNA.

Results: A549 co-implanted with the fibroblasts showed markedly increased tumor growth rate as compared with all control cell lines alone, which formed only small tumors. Importantly, the growth was significantly greater for A549+WT compared to A549+KO tumors. The reduced tumorigenicity was also rescued by re-expression of human α11 in KO fibroblasts. The tumor-promoting effect of fibroblast α11 was reproduced in 2 other NSCLC cell lines: NCI-H460 and -H520. Gene expression profiling indicated that IGF2 mRNA expression level was tightly regulated by alpha-11 in the fibroblasts, and A549+KO tumors expressed >200-fold lower IGF2 compared to A549+WT tumors. The shRNA downregulation of IGF2 in WT (WTsh-IGF2) fibroblasts resulted in decreased growth rate of A549+WTsh-IGF2 compared to A549+WT tumors. In the orthotopic NCI-H460 human lung cancer model, host stromal α11 expression was significantly higher in metastatic compared to the primary tumors.

Conclusion: Integrin α11 expressed on tumor stromal fibroblasts provide a novel and alternate mechanism for collagen to modulate the growth of NSCLC cells, and IGF2 is one of the mediators for such activity.