

Increased Activation of TAK1/NF-kappaB Activity is Associated With the Aggressiveness of Ovarian Cancers

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INTRODUCTION: TGF-beta-activated kinase-1 (TAK1), a mitogen-activated protein kinase, exerts a variety of biophysiological functions through governing numerous downstream signalings such as TGF beta, JNK and NFkb depending on the cell types and stimulus. Emerging evidences have revealed that TAK1 is significantly involved in human cancer progression. However, the functional roles and molecular mechanisms of TAK1 in ovarian cancers remain totally obscure.

MATERIALS AND METHODS: Quantitative RT-PCR (Q-PCR) and Immunohistochemistry (IHC) analyses were used to evaluate TAK1 expression level in human ovarian cancer samples. Stable overexpressing TAK1 was generated by transfection of pCMV-HA-TAK and pCMV-FLAG-MAT-Tag-1-TAK1 in ovarian cancer cell lines, while knockdown of endogenous TAK1 was established using vector-based RNAi constructs in TAK1 overexpressing cell lines. (5Z)-7-Oxozeaenol was used to inhibit TAK1 activity. *In vitro* tumorigenic assays such as cell proliferation, colony formation, wound healing, soft agar and cell invasion assays were performed. Western blotting was performed to study the molecular mechanism of TAK1 using antibodies p-TAK1 (Ser412; Thr184/187), TAK1, p-IKK, p-AMPK, AMPK and b-actin.

RESULTS AND DISCUSSION: Q-PCR results showed that *TAK1* was significantly upregulated ($P = 0.005$) in clinical ovarian cancer samples ($n = 87$) as compared with normal controls ($n = 47$). IHC analysis on an ovarian cancer tissue array further proved that TAK1 was remarkably upregulated in ovarian cancer samples. Importantly, clinical-pathological correlation analysis revealed that the overexpressed TAK1 was significantly associated with high-grade ($P = 0.08$), lymph node and distant metastasis ($P = 0.025$), as well as a tendency toward advanced stage ovarian cancers. Functionally, enforced expression of TAK1 was able to augment cell proliferation, colony formation, anchorage independent growth and migration/invasion in ovarian cancer cells. Conversely, depletion of endogenous TAK1 in ovarian cancer cells noticeably abrogated these tumorigenic capacities. Similar results were observed by specific inhibition of TAK1 activity by (5Z)-7-Oxozeaenol. In addition, cotreatment of (5Z)-7-Oxozeaenol could sensitize ovarian cancer cells to cisplatin-induced cell apoptosis. Moreover, our data showed that there was an increase of Ser412 but not Thr184/187 phosphorylation of TAK1 in ovarian cancer cells. Importantly, the increased phosphorylated TAK1 was accompanied by elevated expression of phospho-IKK, indicating the activated TAK1 may upregulate NFkB signaling activity in ovarian cancer cells.

CONCLUSION: Our findings suggest that the aberrant activation of TAK1 is associated with ovarian cancer cell growth, migration/invasion, and chemoresistance and maybe through enhancing NFkB signaling activity. Further biochemical studies in delineating

the functional importance of TAK1 phosphorylation at Ser412 in governing NFκB signaling are warranted.