論　文　概　要

Abstract

論 文 題 目　　(Theme)

Exploring the function and regulation of the epithelial cell transforming sequence 2 in lung adenocarcinoma cells

(肺腺癌細胞における epithelial cell transforming sequence 2 の機能と制御機構の探索)

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Abstract

(目 的 Purpose)

Epithelial cell transforming sequence 2 (ECT2) is a guanine nucleotide exchange factor especially for Rac1, RhoA, and Cdc42. ECT2 is predominantly localized in the nucleus and regulates cytokinesis. ECT2 is also localized in the cytoplasm of cancer cells. Previous study in our research group revealed that the *ECT2* was amplified in early invasive lung adenocarcinoma. In the present studies, I aimed to investigate alterations of the expression of ECT2 and its underlying molecular mechanism in the progression of lung adenocarcinoma.

(対象と方法　Materials and Methods)

The expression of ECT2 was determined by western blot and RT-PCR. Functional analysis of ECT2 was examined by using cell proliferation, migration, and invasion assays after applying small interfering RNA targeting *ECT2* (siECT2). A cellular fractions assay was performed to determine the subcellular localization of ECT2 in lung adenocarcinoma cell lines. The cytoplasmic expression of ECT2 in 167 cases was evaluated by immunohistochemistry and its clinical significance was examined using Kaplan-Meier curves and Cox regression analysis. Immunocytochemistry of 13 scraping cytology specimens were used to assess the subcellular localization of ECT2 and its phosphorylation at Thr790 (P-ECT2(T790)). Cell adhesion, spreading, and immunofluorescence assays assessed the effects of ECT2 on cell-matrix adhesion behavior. The regulation mechanism of ECT2 on cell-matrix adhesion was

demonstrated using RNA-sequencing, functional enrichment analysis, immunoprecipitation, and western blot analysis.

(結果 Results)

First, I confirmed the correlation between *ECT2* amplification and overexpression. I also explored the oncogenic functions of ECT2 in lung adenocarcinoma cells. The data showed that the suppression of ECT2 caused a reduction in cell growth, migration, and invasion. Moreover, ECT2 was localized in both the nucleus and the cytoplasm of lung adenocarcinoma cell lines and tumor tissues. Aberrant cytoplasmic expression of ECT2 was detected in 83 (50%) out of the 167 cases and was found to increase during cancer progression. Cytoplasmic positivity for ECT2 was associated with poor outcomes and was an independent prognostic factor. Consequently, P-ECT2(T790) positivity in the cytoplasm and membrane, but not in the nucleus, was detected in Calu-3 cells and scraping cytology specimens. Positive P-ECT2(T790) staining was correlated with cytoplasmic ECT2 expression in 6 of the 13 scraped cytology specimens tested.

Based on the roles of ECT2 in cancer progression, my next aim was to examine the potential role of ECT2 in the cancer cell-matrix adhesion process and its underlying molecular mechanism. I found that ECT2 suppression reduced the adhesion and the spreading of lung adenocarcinoma cells. In terms of morphological changes, cells transfected with siECT2 showed a clear rounded shape with actin cytoskeleton defects. RNA-seq transcriptome analysis showed a total of 1569 genes and 828 genes were altered after applying siECT2 (absolute fold change and difference >2) in Calu-3 and NCI-H2342 cells, respectively, with 298 genes common to both cell lines. Functional enrichment analysis of common altered genes was significantly enriched in focal

adhesion. Concordant with this observation, focal adhesion proteins were decreased in siECT2 treatment. Interestingly, I found that ECT2 bound to FAK in lung adenocarcinoma cells and suppression of ECT2 led to a reduced formation of the focal adhesion complex.

(考察 Discussion)

My current research provides evidence in how ECT2 modulates lung pathophysiology. The data indicates that ECT2 is localized to the cytoplasm of lung adenocarcinoma cells, but not in normal cells, in early invasive adenocarcinoma and positive cytoplasmic expression of ECT2 is correlated with a poor prognosis. Therefore, cytoplasmic ECT2 could acquire oncogenic function and facilitate the progression of lung adenocarcinoma. The question that arises from these observations is how ECT2 facilitates tumor progression. I showed that the suppression of ECT2 impairs cell adhesion and the spreading of lung adenocarcinoma cells. The mechanism of ECT2 regulation in cell adhesion and spreading may involve in an abnormal focal adhesion signaling pathway. Based on the facts that ECT2 bound to FAK and the suppression of ECT2 reduces the formation of focal adhesion complex, the overexpression of ECT2 appears to functionally induce the formation of focal adhesion that may result in an acceleration of the focal adhesion signaling axis.

(結 論 Conclusion)

Taken together, these findings further demonstrate that the abnormal expression and localization of ECT2 play an essential role in the pathological steps of lung adenocarcinoma progression and could be a potential molecular target for cancer therapy.