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審査研究科	人間総合科学研究科
学位論文題目	Exploring the function and regulation of 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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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Zeinab Kosibaty describes the function and regulation of epithelial cell transforming 2 in lung adenocarcinoma cells.

The content is summarized as follows:

（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. A previous study revealed that the *ECT2* was amplified in early invasive lung adenocarcinoma. In this dissertation, the author 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）

In this dissertation, the author determined the expression of ECT2 by western blot and RT-PCR and analyzed the function of ECT2 by using cell proliferation, migration, and invasion assays after applying small interfering RNA targeting *ECT2* (siECT2). A cell fractionation 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 were used to assess the effects of ECT2 on cell-matrix adhesion behavior. The regulation mechanism of ECT2

on cell-matrix adhesion was demonstrated by using RNA-sequencing, functional enrichment analysis, immunoprecipitation, and western blot analysis.

（結果　Results）

The author first confirmed the correlation between *ECT2* amplification and overexpression and explored the oncogenic functions of ECT2 in lung adenocarcinoma cells. The author's data showed that the suppression of ECT2 causes 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, the author then aimed to examine the potential role of ECT2 in the cancer cell-matrix adhesion process and its underlying molecular mechanism. The author 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 analysis showed that 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 showed significant enrichment in focal adhesion pathway. Concordant with this observation, focal adhesion proteins were decreased in siECT2 treatment. Interestingly, the author 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）

In this dissertation, the author provides evidence about how ECT2 modulates lung pathophysiology. The author's data indicate 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, the author's results indicate that cytoplasmic ECT2 could acquire oncogenic function and facilitate the progression of lung adenocarcinoma. The question that arises from the author's observations is how ECT2 facilitates tumor progression. The author shows that the suppression of ECT2 impairs cell adhesion and the spreading of lung adenocarcinoma cells and suggests that the mechanism of ECT2 regulation in cell adhesion and spreading may involve an abnormal focal adhesion-signaling pathway. Based on the facts that ECT2 is bound to FAK and the suppression of ECT2 reduces the formation of focal adhesion complex, the author suggests that the overexpression of ECT2 functionally induces the formation of focal adhesion, which may result in an acceleration of the focal adhesion-signaling axis.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

In this dissertation, the author investigates the role of epithelial cell transforming sequence 2 (ECT2) in lung adenocarcinoma cells and shows that the abnormal expression and localization of ECT2 play an essential role in the pathological steps of lung adenocarcinoma progression. The author's results not only demonstrate the importance and molecular mechanism of ECT2 for progression of lung adenocarcinoma but also indicate that ECT2 could be a potential molecular target for cancer therapy.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on December 25, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.