論 文 の 内 容 の 要 旨

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論 文 題 目

A novel surfactant protein C L55F mutation associated with interstitial lung disease alters subcellular localization of proSP-C in A549 cells

（間質性肺病変患者に認められたサーファクタント蛋白質 C 遺伝子の新規変異 L55F は、A549 細胞において proSP-C の細胞質内での局在を変化させる）

（論文の内容の要旨）

Background: Surfactant protein C (SP-C) is a hydrophobic protein produced exclusively by type II alveolar epithelial cells (AECs) and plays an important role in the modulation of lung mechanics by its direct effects on alveolar surface tension. The SP-C gene (*SFTPC*) encodes precursor SP-C (proSP-C) containing four distinct structural and functional domains, a short cytoplasmic N-terminal domain (residues 1-23), a mature domain (residues 24-58), a non-BRICHOS region (residues 59-89), and a BRICHOS domain (residues 90-197).

Interstitial lung disease (ILD) in infants and children are mostly chronic and associated with high morbidity and mortality. Mutations in the *SFTPC* have recently been linked to ILD associated with abnormal expression of SP-C. Previous studies showed that mutations involving non-BRICHOS and BRICHOS domains of proSP-C may represent different pathogenesis of ILD. However, the pathway and mechanism of mutations located within mature domain of proSP-C remain unclear.

In the present study, we described a novel heterozygous *SFTPC* mutation located in the mature domain of proSP-C in a Japanese girl associated with ILD, and investigated whether the novel proSP-C^{L55F} showed alteration of subcellular localization in human type II lung epithelial cell line (A549).

Patient and methods: A Japanese young girl patient diagnosed as ILD, patient's parents, and 61 healthy Japanese volunteers participated in this study. Sequencing of *SFTPC* was employed on the patient, patient's parents, and all healthy volunteers. Histopathology and transmission electron microscopy (TEM) of lung tissue from the patient were assessed. We constructed a model of A549 cells stably transfected with GFP/proSP-C^{WT}, GFP/proSP-C^{L55F} located in the mature domain, GFP/proSP-C^{I73T} in the non-BRICHOS, and GFP/proSP-C^{A116D} in the BRICHOS domains of proSP-C. The differences of the wild type and mutant proSP-C isoforms were evaluated by Western blotting, immunofluorescence, and TEM.

Results: *SFTPC* genetic analysis revealed that only this patient had a novel c.163C＞T in exon 2 located in the mature transmembrane domain, resulting in a leucine to phenylalanine substitution

(p.L55F). First, we examined proSP-C on the patient's lung tissue by immunohistochemistry and found abnormal localization as compared with normal control. Additionally, TEM findings of her lung tissue and the A549 cells expressing proSP-C^{L55F} stably displayed abnormal cytoplasmic organelles. Although proSP-C^{L55F} exhibited a band pattern similar to that of proSP-C^{WT} for processed intermediates by Western blotting, the two lower molecular weight intermediates were observed at a reduced expression level as compared with those of proSP-C^{WT}, proSP-C^{I73T}, and proSP-C^{A116D}, indicating a loss in proSP-C^{L55F} expression in the processing pathway. Interestingly, in contrast to proSP-C^{WT}, immunofluorescence studies demonstrated that proSP-C^{L55F} partially colocalized in CD63-positive cytoplasmic vesicles of A549 cells, and partially trafficked towards the plasma membrane, suggesting that this novel mutation induced abnormal trafficking of proSP-C.

Conclusion: (1) We uncovered a novel mutation in *SFTPC* located in the mature domain of proSP-C in a Japanese girl with ILD. (2) We characterized the alteration of subcellular localization of proSP-C^{L55F} in A549 cells in comparison with wild type and the mutations in other domains. Thus, in addition to previously reported *SFTPC* mutations in non-BRICHOS, and BRICHOS domains associated with ILD, our findings may provide a new insight into the pathogenesis of ILD caused by the mutation in mature domain of proSP-C.