論 文 審 査 の 結 果 の 要 旨

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（論文審査の結果の要旨）

Mutations in the surfactant protein C (SP-C) gene (*SFTPC*) have recently been linked to ILD. The SP-C encodes precursor SP-C (proSP-C) containing four distinct structural and functional domains, a short cytoplasmic N-terminal domain, a mature domain, a non-BRICHOS region, and a BRICHOS domain. The pathway and mechanism of mutations located within mature domain of proSP-C remain unclear. In the present study, we investigated the alteration of subcellular localization of proSP-C in human type II lung epithelial cell line (A549), which was associated with a novel heterozygous *SFTPC* mutation located in the mature domain of proSP-C in a Japanese girl with interstitial lung disease (ILD).

We found as follows:

1) Sequencing of *SFTPC* revealed a novel heterozygous mutation (c.163C > T) in mature domain of proSP-C, resulting in a leucine to phenylalanine substitution (p.L55F) in a Japanese girl associated with ILD. This mutation was not detected in her parents and 61 healthy volunteers.
2) Immunohistochemistry for proSP-C exhibited abnormal distribution in the patient's lung tissue.
3) Abnormal lamellar bodies were characterized in type II alveolar epithelial cell (AECs) by transmission electron microscopy (TEM) in the patient's lung tissue.
4) A549 cells stably expressing GFP/proSP-C^{WT}, GFP/proSP-C^{L55F} GFP/proSP-C^{I73T}, or GFP/proSP-C^{A116D} were successfully generated, respectively. *In vitro* assay, decreased band intensity of processing intermediate was displayed for proSP-C^{L55F} by Western blotting as compared with those of proSP-C^{WT}, proSP-C^{I73T}, and proSP-C^{A116D}.
5) In A549 cells expressing proSP-C^{L55F}, abnormal subcellular organelles were observed as compared with proSP-C^{WT} by TEM.
6) Subcellular localization of proSP-C^{L55F} was altered in A549 cells. ProSP-C^{L55F} partially colocalized in CD63-positive cytoplasmic vesicles, and partially trafficked towards the plasma membrane as compared with proSP-C^{WT} by immunofluorescence assay.

In conclusion: A novel mutation in *SFTPC* located in the mature domain of proSP-C was uncovered in a Japanese girl with ILD. The alteration of subcellular localization of proSP-C^{L55F} in A549 cells in comparison with wild type and the mutations in other domains was characterized. Thus, our findings may provide a new insight into the pathogenesis of ILD caused by the mutation in mature domain of proSP-C.

間質性肺疾患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た本研究は、間質性肺疾患の病態解明と診断の向上に大きく役立つことから、主査、副査は一致して本論文を学位論文として価値があるものと認めた。