High expression of Fas-associated factor 1 indicates a poor prognosis in non-small-cell lung cancer

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論文題目

High expression of Fas-associated factor 1 indicates a poor prognosis in non-small-cell lung cancer

（Fas-associated factor 1 の高発現は非小細胞肺がんの予後不良を示す）

論文の内容の要旨

[Introduction]

Lung cancer is the most common malignant tumor disease in the world. In recent decades, the discovery of different molecular targets has allowed for the development of targeted therapies against non-small-cell lung cancer (NSCLC). However, the 5-year survival rate of lung cancer is still not optimistic due to untimely diagnosis, limited beneficiary population, and drug resistance of patients. Therefore, it is necessary to explore potential biomarkers and therapeutic targets for lung cancer. Fas-associated factor 1 (FAF1) is a death-promoting protein identified as an interaction partner of the death receptor Fas. The downregulation and mutation of FAF1 have been reported in a variety of human tumors, but there have been few studies on lung cancer. In this paper, we semi-quantified FAF1 in NSCLC for the first time, investigated the clinical significance and the prognostic significance of FAF1, and researched whether aberrant FAF1 expression may be involved in the pathogenesis and prognosis in NSCLC.

[Materials and Methods]

We collected 609 resected NSCLC tumor specimens from who had received curative surgical treatment at Hamamatsu University Hospital [Institutional Review Board (IRB) 20-011] and Seirei Mikatahara General Hospital (IRB 20-36). Clinical and pathological data were retrospectively obtained from the patients' medical records. Tissue microarray (TMA) blocks composed of representative lung cancer tissue cores and their corresponding normal lung tissue cores were made, and their sections were subjected to a standard immunohistochemistry (IHC) protocol, using anti-FAF1, anti-epidermal growth factor receptor (EGFR) E746-750 deletion-specific, and anti-EGFR L858R mutant-specific antibodies and also subjected to TdT-mediated dUTP Nick End Labeling (TUNEL) assay. FAF1 mRNA expression data of NSCLC were obtained from the Gene Expression Omnibus (GEO). Survival analysis was performed in both TMA cases and GEO cases. FAF1 expression in human lung cancer cell lines (A549, H460, PC9, H1299) and normal human bronchial epithelial (NHBE) cell line was examined by western blotting and quantitative real-time PCR (qPCR). Cell viability assay and apoptosis assay were performed to investigate the effect of FAF1 expression in human lung cancer cell lines transfected with FAF1 expression plasmid or FAF1 siRNA.

[Results]

Immunohistochemically, 408 of 609 NSCLC specimens (67.0%) had positive FAF1 expression in a different degree, negative FAF1 expression was found in their corresponding normal lung tissues. Among 125 EGFR mutation specimens, 94 (75.2%) cases were found along with significant positive FAF1 expression. At the same time, apoptotic cells presented in FAF1 negative specimens apparently rather than FAF1 positive ones. FAF1 expression level was statistically higher in patients with an advanced pathological stage, an advanced extension of primary tumor size, and subsets with worse lymph node metastasis. Survival analysis in TMA cases showed that patients with positive FAF1 protein expression had a significantly worse prognosis in overall survival, recurrence-free survival and disease-specific survival in NSCLC, particularly in adenocarcinoma. Consistently, in GEO cases, positive FAF1 mRNA expression also indicated poor prognosis in NSCLC.

Deficient FAF1 protein expression was found in H460 cell line by western blotting. In the H460 cells transfected with the FAF1 expression plasmid, neither cell proliferation nor cell apoptosis changed considerably, compared to cells with empty plasmid transfection. qPCR result showed that FAF1 mRNA expression was higher in the A549 cell line than in the other cell lines. In A549 cells transfected with FAF1 siRNA, cell growth was inhibited significantly, compared to cells with negative control siRNA transfection, and cells in early apoptosis remarkably increased.

[Discussion]

In this study, FAF1 protein expression was semi-quantified using IHC analysis for the first time. Positive FAF1 expression was found in NSCLC specimens along with worse prognosis. Consistently, GEO dataset suggests that the upregulation of FAF1 mRNA is also associated with poor prognosis in NSCLC. Previous research found that functional loss, rather than the overexpression of FAF1, was frequently found in cancers of various origins. However, the manifestation found here demonstrated that FAF1 reverses its role as a tumor suppressor for unknown reasons in NSCLC. It was reported that FAF1 enhanced apoptosis by interacting with Fas in the cytoplasm, on the other hand, it modulated a variety of biological processes by interacting with diverse molecules in the nucleus. However, apoptosis was not induced in our NSCLC cohort, although FAF1 was detected in both the nucleus and cytoplasm. Our findings suggest that FAF1 plays a variety of roles with complex mechanism in NSCLC progression, which need further study to be figured out.

[Conclusion]

Our findings show that FAF1 is overexpressed in NSCLC, which is associated with a poor prognosis. It appears that FAF1 has a variety of bio-functions in NSCLC in

comparison to the presumption that it enhances or initiates apoptosis.