学 位 論 文 の 要 旨

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学　位　論　文　名　Molecular Analysis of Lung Adenocarcinoma With or Without Chronic Obstructive Pulmonary Disease and Smoking Habit

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論 文 内 容 の 要 旨

INTRODUCTION

Lung cancer (LC) and Chronic obstructive pulmonary disease (COPD) are both attributable to the combined effects of smoking exposure and an underlying genetic susceptibility. Although smoking accounts for between 80-90% of all LC and COPD cases, these lung diseases only affect a minority of smokers. The recent discovery that LC synthesize and secrete acetylcholine (ACh) that acts as a growth factor in LC provides a potential new target to inhibit LC growth. It has been shown that small cell lung cancer (SCLC) and NSCLC express nicotinic and muscarinic receptors and cholineacetyltransferase (ChAT), activation of ACh receptors stimulates the growth of NSCLC cell lines. A role has been found for Signal transducer and activator of transcription (STAT)3 in the prevention of human tumor cell apoptosis, and it has also been shown to be important in angiogenesis. Survivin is known as a molecule downstream of STAT and is a recently identified protein of the inhibitor of apoptosis protein (IAP) family, which suppresses programmed cell death (apoptosis) and regulates cell division. We examined whether COPD and smoking can explain the difference in molecular expression in resected lung adenocarcinoma samples.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded blocks from 36 patients of lung adenocarcinoma that had undergone surgical resection from 2006 to 2009 were examined immunohistochemically for six proteins [AChR M3, ChAT, STAT3, survivin, hypoxia inducible factor-1α(HIF-1α), and

interleukin 6 (IL-6)] considered to modulate signal transduction pathways in cancer cells. All immunostained sections were evaluated by three authors who were blinded to the clinical diagnosis. DNA extracted from the tumor tissue was further analyzed using the peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR reaction for EGFR gene mutation. K-ras codon 12 mutations were analyzed using the PCR-restriction fragment length polymorphism (RFLP) method .

RESULTS AND DISCUSSION

We divided smokers into two groups, with COPD and without COPD, and evaluated the difference in degree of staining between these groups. The expression levels of AChR M3, and ChAT were significantly higher in smokers with COPD than those without COPD. These new findings implicated the cholinergic signal transduction pathway in the carcinogenesis of LC associated with COPD. It has also been shown that the phosphorylation of MAPK and Akt due to the activation of AChR in LC was inhibited by selective M3 receptor antagonists. Tiotropium is widely used clinically in the treatment of COPD and is known to increase long-term survival rate; it also acts as a selective M3 receptor antagonist. Tiotropium also suppresses the proliferation of LC cells *in vitro*.

We then divided all patients into the two groups of smokers and non-smokers, and similarly analyzed the immunostaining results. Of 23 smokers and 13 non-smokers, the non-smokers showed significantly higher expression of STAT3 compared to smokers. Although not a significant difference, the expression of survivin tended to be higher in non-smokers compared to smokers (P=0.068). Based on these findings, we propose that STAT3 can play a role in LC carcinogenesis of non-smokers. STAT3 is thought to act downstream of EGFR and ras signal transduction, and previous results indicated increased expression of STAT3 in cell lines expressing an EGFR mutation. In addition, the incidence of EGFR mutation is seemingly higher in non-smokers than smokers, and the expression of downstream STAT3 might be accentuated due to the activation of EGFR in non-smokers. However, this study found no significant correlation between the genetic mutations in EGFR/k-ras and the expression intensity of STAT3 (P=0.706, 0.654, respectively; data not shown). Similar studies in larger populations are clearly needed.

CONCLUSION

The results showed differences in the expression of cholinergic signaling pathway and JAK-STAT pathway components among patients with lung adenocarcinoma, in smokers and non-smokers and with/without COPD. These results could contribute to the treatment of recurrence, and also help to identify targets for cancer prevention strategies.