学 位 論 文 の 要 旨

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学 位 論 文 名　Novel Drug-resistance Mechanisms of Pemetrexed-treated Non-small Cell Lung Cancer

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論 文 内 容 の 要 旨

INTRODUCTION

Pemetrexed (PEM), a drug that inhibits several intracellular molecules and the main target is thymidylate synthase (TYMS). PEM exhibits anti-cancer effects by blocking synthesis of deoxythymidine monophosphate. After first-line PEM-based chemotherapy to treat advanced non-small cell lung cancer (NSCLC), PEM alone is continuously prescribed to improves the overall survival of patients when administered as maintenance therapy. However, PEM resistance often appears during long-term therapy. Although TYMS is known to be responsible for PEM resistance in several basic studies, two clinical reports suggested no significant correlation between TYMS expression and PEM resistance of NSCLC. Also, no other mechanisms have been investigated in detail. In this study, we explored new drug resistance mechanism of PEM and we found SLC19A1 and AKT as important PEM-resistant factors in PEM-resistant NSCLC cell lines.

MATERIALS AND METHODS

Two human lung adenocarcinoma cell lines were used: PC-9, a differentiated human lung adenocarcinoma cell line; and A549, a human lung adenocarcinoma cell line derived from

epithelial cells. PC-9 has an epidermal-growth-factor-receptor (EGFR) exon 19 deletion mutation (p.E746_A750del), and A549 has a KRAS proto-oncogene, GTPase mutation (p.G12S). To establish PEM-resistant NSCLC cell lines, cancer cells were continuously cultured in the presence of PEM. PEM concentration was gradually increased from ineffective concentration to more than 1 μM. These cell lines were designated PC-9/PEM and A549/PEM. The cell viability was determined by WST-8 assay. Quantitative RT-PCR and immunoblotting were performed to measure relative number of mRNAs and amount of proteins respectively. Cell cycles were examined by flow cytometry with fluorescently labeled bromodeoxyuridine and 7-amino-actinomycin D. To determine the relative number of senescent cells, SA-β-Gal staining was used and quantified the fluorescence intensity by flow cytometry. siRNA transfection was performed to induce mRNA knockdown. To evaluate PEM resistance in in vivo condition, human tumor xenograft mouse model was used with immunodeficient BALB/c nu/nu mice. Parental and PEM-resistant human NSCLC cell lines were transplanted subcutaneously and treated with PEM. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSSION

We established two PEM-resistant NSCLC cell lines to confirm mechanisms of PEM resistance. Growth inhibition were observed after PEM treatment in parental two NSCLC cells but not in PEM-resistant NSCLC cells. Next, we evaluated drug functions of PEM for those PEM-sensitive parental NSCLC cells. Apoptotic PC-9 cells were drastically increased by PEM treatment. Surprisingly, PEM had induced A549 cells arrested in S-phase rather than apoptotic. Moreover, we found that those senescent A549 cells were increased after 96 h treatment of PEM. We next compared the mRNA expression of several genes considered to be involved in PEM resistance between the parental and PEM-resistant cell lines. The mRNA expression of *FOLR1* and *SLC19A1* in A549/PEM cells significantly decreased in comparison to the parental A549 cells. We also confirm to becoming PEM-resistant by siRNA-mediated knockdown of *SLC19A1* in parental NSCLC cell lines. On the other hand, PC-9/PEM cells increased TYMS expression and AKT activation. We evaluated drug sensitivity of a EGFR tyrosine kinase inhibitor erlotinib because of AKT is a downstream protein of EGFR. Erlotinib inhibited the activation of EGFR in both PC-9 and PC-9/PEM cells. While erlotinib also inhibited the AKT in PC-9 cells, the inhibition of AKT was insufficient in PC-9/PEM cells. Supporting this notion was the fact that PC-9/PEM cells had lower erlotinib sensitivity than parental PC-9 cells. Finally, we compared the PEM sensitivity between those parental and PEM-resistant cells in tumor-bearing mice. Although tumor growth of PEM-resistant cell was slower than each parental cell of vehicle

treatment group, there are no significantly differences between PEM-treatment and vehicle-treatment group in PEM-resistant cells.

The decreased expression of SLC19A1 would reduce the amount of folate, which is necessary for cell proliferation. However, there are compensatory systems to take up folate. Without SLC19A1, cells can take up folate via other transport molecules, including FOLR1 and SLC46A1. FOLR1 captures folates and transports them into cells via receptor-mediated endocytosis. Acidic conditions in endosomes help SLC46A1 function. SLC46A1 and FOLR1 are thought to work in cooperation. The decreased expression of SLC19A1 may be convenient for the survival of cancer cells by decreasing the uptake of PEM.

Whereas PEM decreased the activation of AKT in PC-9, the activation of AKT was significantly increased in PC-9/PEM to resist inhibition by PEM. We consider PEM resistance of PC-9/PEM bestowed EGFR-TKI resistance to PC-9/PEM simultaneously because the increased activation of AKT is a known convergent feature of EGFR-TKI resistance.

CONCLUSION

Our findings showed that SLC19A1 negatively regulates PEM resistance in NSCLC cells. In addition, EGFR-TKI inhibitor resistance was observed to occur with PEM resistance in EGFR mutation-carrying NSCLC cells via an up-regulated AKT activation.