学 位 論 文 の 要 旨

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学　位　論　文　名　Protein Tyrosine Kinase 2: A Novel Therapeutic Target to Overcome Acquired EGFR-TKI Resistance in Non-small Cell Lung Cancer

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論 文 内 容 の 要 旨

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide with non-small cell lung cancer (NSCLC) being the largest subgroup. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have been used to treat *EGFR* mutation-positive NSCLC. The response rate was ≤ 80% and progression-free survival was ~10–14 months. However, most tumors initially responding to EGFR-TKIs eventually recur as they acquire resistance to them. Overcoming EGFR-TKI resistance is important for prolonging overall survival. Though considerable effort has been made, ~18–30% of resistance mechanisms have not yet been elucidated.

Osimertinib, a third-generation EGFR-TKI, has been used for NSCLC harboring EGFR T790M mutation, which is a resistant mechanism to first- and second-generation EGFR-TKIs. Various acquired resistance mechanisms to EGFR-TKIs have been reported over the past decade, but most mechanisms of acquired resistance for osimertinib remain unclear and are largely responsible for treatment failure. Thus, the emergence of acquired resistance to EGFR-TKIs is a priority area for clinical research. Basic research to clarify the molecular mechanism of EGFR-TKI resistance may increase the number of potential clinical treatment options for tumors with acquired EGFR-TKI

resistance.

In this study, we used EGFR-TKI-resistant NSCLC cell lines to explore the molecular mechanisms of EGFR-TKI resistance. Here, we show that protein tyrosine kinase 2 (PTK2) is a new therapeutic target for acquired resistance to EGFR-TKIs and provide evidence that the combination of a PTK2 inhibitor and an EGFR-TKI is a potentially efficacious therapy for EGFR-TKI-resistant NSCLC.

MATERIALS AND METHODS

The EGFR-mutant lung adenocarcinoma cell line PC-9 was used. EGFR-TKI resistant cell lines PC-9/PEM and PC-9/ER-1–6 were established from PC-9 by exposing to pemetrexed or erlotinib. PC-9/PEM clone1 cell line is a monoclonal PC-9/PEM cell line. Cell viability in NSCLC cell lines was measured by a WST-8 assay. MET gene copy number and receptor tyrosine kinase gene expression were measured using quantitative PCR. Phosphorylation antibody array assay was used to detect the phosphorylation of 71 receptor tyrosine kinases. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (IZ29-63). PC-9 and PC-9/PEM clone1 cells (2×10^6) were injected subcutaneously into the left and right hind flanks respectively. Osimertinib (5 mg/kg/d) and/or defactinib (25 mg/kg/d) were administered by oral gavage twice daily for five days per week. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

We compared the cell viabilities of PC-9/PEM and PC-9 cells in response to exposures to pemetrexed and EGFR-TKIs. PC-9/PEM cells were more resistant to the drugs compared to parental PC-9 cells. To elucidate the underlying EGFR-TKI resistance mechanisms in PC-9/PEM, we assessed Akt and Erk signaling downstream of EGFR in both PC-9 and PC-9/PEM cells after treatment with erlotinib or DMSO. We found that erlotinib dramatically inhibited $pEGFR^{Y1068}$ in PC-9/PEM. Nevertheless, $pErk^{T202/Y204}$ and especially $pAkt^{S473}$ were less inhibited in PC-9/PEM than they were in PC-9. To clarify the Akt hyperphosphorylation mechanism, we measured gene expressions in the upstream signaling of the Akt pathway. Although only fibroblast growth factor receptor (FGFR) family FGFR1 and FGFR4 had upregulated gene expression in PC-9/PEM compared to PC-9, FGFR1 or FGFR4 total proteins were not detected in either PC-9 or PC-9/PEM. On the other hand, phosphorylation array analysis and immunoblotting showed that PTK2 was hyperphosphorylated in PC-9/PEM compared with PC-9. The PTK2 inhibitor defactinib inhibited $pPTK2^{Y576/577}$ in PC-9/PEM. In addition, co-treatment with defactinib and erlotinib or osimertinib had restored sensitivity to erlotinib and osimertinib on PC-9/PEM compared with either treatment alone. FGFR1 inhibitor PD173074, but not nintedanib or BLU-554, inhibited PTK2 as did defactinib.

We established a PTK2-activated monoclonal cell line PC-9/PEM clone1. Defactinib also

recovered EGFR-TKI sensitivity in PC-9/PEM clone1. Complete PTK2 inhibition reduced the Akt phosphorylation level in PC-9/PEM clone1 to that of parental PC-9. Osimertinib alone did not induce apoptosis, the combination treatment of defactinib and osimertinib significantly induced apoptosis in PC-9/PEM clone1. Moreover, the combination treatment showed improved in vivo therapeutic efficacy compared to the single-agent treatments, these data were consistent with those obtained from the in vitro experiments.

We established the erlotinib-resistant NSCLC cell lines PC-9/ER-1–6 derived from parental PC-9. No T790M mutation or MET amplification was detected in these EGFR-TKI-resistant cell lines. Several erlotinib-resistant NSCLC cell lines also expressed higher PTK2 phosphorylation than PC-9. Immunoblotting showed defactinib effectively inhibited PTK2 phosphorylation in PC-9/ER-4, which had the highest PTK2 phosphorylation level of all erlotinib-resistant cell lines and was also resistant to osimertinib, but the phosphorylation of EGFR, Akt and Erk was not inhibited. Furthermore, co-treatment with defactinib and osimertinib was more effectively decreased the cell viability of those PTK2 hyperphosphorylated erlotinib-resistant NSCLC cell lines than single osimertinib. Thus, PTK2 inhibition in the PTK2 hyperphosphorylated erlotinib-resistant cell lines also recovered EGFR-TKI sensitivity.

PTK2 upregulation and activation in tumors are linked to poor progression and aggressive disease. PTK2 protein levels may increase independently of *PTK2* gene expression. No differences in PTK2 protein level were identified between parental PC-9 and EGFR-TKI-resistant cells in this study.

The combination treatments of EGFR-TKI and other targeted agents to inhibit bypass signaling such as AXL, MEK, PI3K, Akt, and mTOR are under evaluation in clinical trials but no EGFR-TKI combination with defactinib at present. PTK2 and EGFR dual blockade should be considered for a clinical trial, especially involving EGFR-mutant NSCLC.

The mechanism of PTK2 activation remains unclear as no specific factor related to PTK2 activation was detected. Whole-genome sequencing may help to identify the genetic variants responsible for heritable PTK2 activation. We also plan to evaluate clinical specimens for PTK2 phosphorylation as a target of PTK2 inhibitor treatment in NSCLC and translate this basic research into clinical trials.

CONCLUSION

We provided evidence that PTK2 hyperphosphorylation is a critical factor in EGFR-TKI resistance in NSCLC. Moreover, we demonstrated that a combination of EGFR-TKI and PTK2 inhibitor is a potentially new therapeutic approach to overcome EGFR-TKI resistance.