学 位 論 文 の 要 旨

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学 位 論 文 名　Picropodophyllin Inhibits the Growth of Pemetrexed-Resistant Malignant Pleural Mesothelioma via Microtubule Inhibition and IGF-1R-, Caspase-Independent Pathways

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論 文 内 容 の 要 旨

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a rare but aggressive cancer with a long latent period after asbestos exposure. Most patients with MPM are diagnosed at an advanced stage with a poor prognosis. The standard first-line chemotherapy, pemetrexed (PEM) plus platinum, continues to be used with other therapies for advanced MPM. Although PEM continuation maintenance therapy is also standard, long-term PEM therapy promotes acquired PEM resistance. Thus, additional therapeutic options are needed for MPMs with acquired PEM resistance.

However, preclinical targeted drug studies have focused on the fibroblast growth factor receptor, focal adhesion kinase, mesothelin, and poly(ADP-ribose) polymerase in MPM cells; molecular target therapy is not recommended for MPM due to its lack of efficacy in clinical trials. IGF-1R inhibitors, including monoclonal antibodies and tyrosine kinase inhibitors (TKIs), are also being evaluated as candidate molecules against MPM. However, studies targeting molecules such as IGF-1R in MPMs with acquired PEM resistance are lacking.

In this study, we established two MPM cell lines with acquired PEM resistance to identify effective anticancer drugs, including the IGF-1R inhibitor picropodophyllin (PPP), against acquired PEM-resistant MPMs. Our findings may provide a novel therapeutic approach for MPMs with acquired PEM resistance.

MATERIALS AND METHODS

Two human MPM lines, H2452 and MSTO-211H, were used to establish acquired PEM-resistant MPM lines. H2452 and 211H were exposed to PEM *in vitro* during the culture, and the PEM

concentration in the culture medium was gradually increased from 0.01 μM to 3μM. Then, established resistant lines were named H2452/PEM and 211H/PEM, respectively. Two human non-small cell lung cancer PEM-resistant lines, PC-9/PEM and A549/PEM, were also used with their parental PC-9 and A549, respectively. The cell viability was evaluated by WST-8 assay. Quantitative reverse-transcription PCR was performed to evaluate the relative amount of mRNA expression. A phosphorylation antibody array was used to determine the phosphorylation status of receptor tyrosine kinases (RTKs). Immunoblot analysis was used to determine the amount of protein expression. Apoptotic and necrotic cells were detected by staining with annexin V and propidium iodide. Cell cycles were analyzed using bromodeoxyuridine and 7-aminoactinomycine D. Senescence-associated beta-galactosidase (SA-βGal) staining was used to evaluate cellular senescence. DNA damage was determined by the phosphorylation status of Chk2. PPP and siRNA against *IGF1R* were used to evaluate the efficacy of IGF-1R inhibition. Immunofluorescence was used to evaluate microtubule localization. The efficacy of PPP was also evaluated in 3-dimensional (3D) MPM models.

RESULTS AND DISCUSSION

To confirm the constitutive PEM resistance of the PEM-resistant lines, we compared their parental lines and the PEM-resistant lines. PEM decreased the cell viability of PEM-naïve parental lines but not of PEM-resistant lines. Moreover, PEM exerted effects on the cell cycle in the parental lines, while PEM did not affect the cell cycle in both PEM-resistant lines. In addition, PEM induced apoptosis in both parental lines but not in PEM-resistant lines. Although PEM induced SA-βGal activity in parental and resistant lines, SA-βGal activity was higher in PEM-treated surviving parental MPM lines. PEM primarily promoted apoptosis in H2452 cells and induced the cell cycle arrest with cellular senescence in 211H cells but not in the PEM-resistant lines. These results consistently indicate that H2452/PEM and 211H/PEM cells are resistant to PEM with different characteristics.

TYMS expression was increased in H2452/PEM and 211H/PEM compared to the respective parental line. However, TYMS knockdown did not resensitize the PEM-resistant lines to PEM. To identify a molecular target protein from RTKs in MPM lines with acquired PEM resistance, we evaluated the gene expression and phosphorylation of RTKs. The gene expression and phosphorylation of IGF-1R were increased in H2452/PEM than those of the H2452. However, IGF-1R knockdown did not resensitize H2452/PEM cells to PEM, which indicated IGF-1R activation is not the mechanism of PEM resistance of H2452/PEM. On the contrary, an IGF-1R non-ATP competitive TKI, PPP, decreased the viability of H2452/PEM cells and 211H/PEM cells more than it decreased the viability of the parental lines. Moreover, PPP decreased the viability of a PEM-resistant lung cancer line PC-9/PEM to a greater extent than it did for PC-9. However, PPP was resistant in another line, A549/PEM. PPP increased the proportion of H2452/PEM cells in the sub-G1 phase, and the proportion of 211H/PEM cells in sub-G1 and G2/M was greater than that of the parental lines. In addition, PPP induced more senescent cells in

both PEM-resistant MPM lines than in their respective parental lines. PPP treatment increased apoptotic cells in 211H/PEM and H2452/PEM. Moreover, PPP induced necrosis in H2452/PEM and 211H/PEM cells. However, PPP did not induce DNA damage in PPP-sensitive PEM-resistant MPM lines. Additionally, the pan-caspase inhibitor, Q-VD-OPh, did not rescue the effect of PPP in H2452/PEM and 211H/PEM cells. Those results indicated that PPP induced caspase-independent cell death without causing DNA damage in MPM cells with acquired PEM resistance.

Although PPP inhibited the phosphorylation of IGF-1R in MPM cells with acquired PEM resistance, knockdown of IGF-1R did not decrease the cell viability of both H2452/PEM and 211H/PEM. Moreover, IGF-1R knockdown had no efficacy on PEM-resistant MPM cells. Those results indicated that IGF-1R inhibition did not lead to cytotoxicity in PEM-resistant MPM lines and was not the mechanism of PPP efficacy on PEM-resistant MPMs.

Due to reports that PPP inhibits microtubules, we examined the inhibition of microtubules after PPP treatment. PPP induced the multipolar spindles or spindle collapse in H2452/PEM cells and induced multinucleation and increased proportion of cytoplasmic microtubules in 211H/PEM cells. Furthermore, we found that PPP plus vinorelbine had a synergistic effect on MPM lines with acquired PEM resistance. These data suggest that the mechanism of action of PPP differs from that of vinorelbine. Therefore, microtubules may be a critical factor for PEM-resistant MPMs. In addition, PPP was also effective against the 3D MPM tumor model.

To our knowledge, this is the first report on PPP being an effective drug for MPM cell lines with acquired PEM resistance. Furthermore, the synergistic effect of PPP plus vinorelbine against MPM cell lines with acquired PEM resistance was revealed in this study.

CONCLUSION

PPP may be more effective against MPMs with acquired PEM resistance than against PEM-naïve MPMs. We found that vinorelbine can potentially be combined with PPP to treat MPMs with acquired PEM resistance.