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学位論文題目　Exploration of natural products as potential enhancers for cancer therapy targeting TRAIL-induced cancer cell death.
（TRAIL誘導性細胞死を増強する新規天然物由来化合物の探索）

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論 文 内 容 の 要 旨

Introduction

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been known to selectively show its cytotoxic function against various types of cancer cells without harming normal healthy cells. TRAIL triggers target cell death by inducing apoptosis through its specific death receptors, DR4/TRAIL-R1 or DR5/TRAIL-R2. Recently, several clinical trials have been conducted to test the application of TRAIL in cancer therapy by using recombinant TRAIL or agonistic monoclonal antibodies against TRAIL death receptors either as a mono-therapy or as a combination therapy with other anti-cancer drugs. Despite the specificity of TRAIL-induced cell death in cancer cells, cell intrinsic and extrinsic TRAIL resistance has been often observed in many cancer cell types. Such TRAIL-resistance could associate with defects in TRAIL death receptor signalling or dys-regulation of anti-apoptotic proteins controlled by pro-survival machinery often over-activated in cancer cells. In this study, I aim to explore the utility of natural products to design the effective cancer therapy targeting TRAIL-induced cancer cell death by facilitating the TRAIL-mediated cytotoxicity or by overcoming TRAIL resistance in cancer cells.

1. Identification of natural products enhancing TRAIL-targeted therapy [1,2]

To identify natural products that can enhance the efficacy of TRAIL-targeted therapy, I first screened 55 chemical structure-defined compounds and 138

water extracts from natural products in their efficacy combined with TRAILinduced cell death against human breast cancer cell lines (TNBC). MDA-MB-231 and MDA-MB-468 cells were used as a TRAIL-sensitive and TRAILresistant cell line, respectively. Among the screened compounds, I found that piperine showed highly synergistic effect to TRAIL-induced apoptosis against human breast cancer cells *in vitro*. In addition to its effect on TRAIL-induced apoptosis, piperine also suppressed the expression survivin and the phosphorylation of p65, both has been known to associate with anti-apoptotic machinery of cancer cells. Importantly, I further demonstrated that *in vivo* piperine treatment potentiated tumor suppressive effect of TRAIL-targeted therapy by using agonistic monoclonal antibodies against TRAIL death receptor 5 (anti-DR5 mAb) in murine 4T1 breast cancer model. Of note, the combination treatment of anti-DR5 mAb and piperine inhibited spontaneous lung metastasis of 4T1 tumor along with the inhibition of primary tumor growth,and further prolonged survival rates of tumor-bearing mice. In addition to piperine, I have also identified the extracts of *Uvaria dac* (Ud) and *Artemisia vulgaris* (Av) strongly enhanced TRAIL-induced apoptosis. Both Ud and Av extracts suppressed the phosphorylation of p65 and further the oral administration of Ud enhanced the efficacy of TRAIL-targeted therapy by anti-DR5 mAb in 4T1 breast cancer model.

2. Chrysin as an active component of the *Propolis* extract to enhance TRAIL-targeted therapy [3,4]

It has been known that the ability of *Propolis* extract (bee glue) to potentiate TRAIL-induced apoptosis, however the active ingredient of the *Propolis* extract responsible for enhancing TRAIL effect was not explored. Therefore, Iinvestigated the efficacy of chrysin and tectochrysin that are the major isolated constituents of *Propolis* extract, in combination with TRAIL-induced cell death in human cancer cell lines. By testing those two major compounds of *Propolis* extract, I found that chrysin showed a potent effect on enhancing TRAILinduced apoptosis in both A549 lung cancer cells and HeLa cervical cancer cells. In accordance with its pro-apoptotic effect in combination with TRAIL,chrysin down-regulated the protein and the gene expression of MCL-1 antiapoptotic molecule. The contribution of MCL-1 in TRAIL resistance was confirmed by using siRNA knockdown of MCL-1. Additionally, the suppression of MCL-1 by chrysin may be through the suppression of up-stream STAT3 phosphorylation because the treatment of STAT3-specific chemical inhibitor,cucurbitacin-I, also decreased the MCL-1 levels and enhanced TRAIL-induced apoptosis. In addition to its effect on TRAIL-induced apoptosis, I also found that chrysin inhibits hypoxia-induced STAT3 phosphorylation and VEGF gene expression in 4T1 cells. Finally, I showed the *in vivo* treatment of chrysin significantly suppressed primary 4T1 tumor growth and further enhanced the anti-metastatic activity of TRAIL-targeted therapy by using anti-DR5 mAb in 4T1 model.

Conclusion

In this study, I identified piperine and the extract of Ud and Av as novel

enhancers for cancer therapy targeting TRAIL-induced cancer cell death in accordance with the inhibition of anti-apoptotic machinery. Furthermore, I also identified chrysin as an active ingredient of the *Propolis* extract responsible for enhancing TRAIL effect possibly through the inhibition of MCL-1/STAT3 axis. Collectively, the present results clearly indicate the utility of those natural products to develop novel TRAIL-targeted combination therapies against cancer.

References

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