学 位 論 文 の 要 旨

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学　位　論　文　名　Bcl-xL Inhibition by Molecular-Targeting Drugs Sensitizes Human Pancreatic Cancer Cells to TRAIL

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論 文 内 容 の 要 旨

INTRODUCTION

Many molecules are involved in apoptosis. Among them, Bcl-2 family molecules participate in intrinsic apoptosis via mitochondria. The family of Bcl-2-related anti-apoptotic proteins includes Bcl-2, Bcl-xL, Bcl-w, and Mcl-1. An increase in Bcl-2 expression protects cancer cells from apoptosis, and the elevated expression of Bcl-2 and Bcl-xL has been frequently observed in a variety of cancers. Thus, the inhibition of Bcl-2 and/or Bcl-xL is hypothesized to potentiate the effect of chemotherapy and, consequently, several Bcl-2 family inhibitors have been developed. ABT-737 is a small molecule inhibitor of Bcl-2, Bcl-xL, and Bcl-w. ABT-263 is a clinically approved orally bioavailable inhibitor with the same specificity as ABT-737. ABT-199 is a new, orally bioavailable inhibitor that inhibits Bcl-2 and Bcl-w, but not Bcl-xL.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) can provide a death signal via the extrinsic apoptotic pathway. It is therapeutically important that TRAIL can induce cancer cell death while causing almost no cytotoxicity to normal cells. In this study, we investigated the effects of the Bcl-2 family inhibitors on TRAIL sensitivity using a panel of

human pancreatic cancer cell lines and found that Bcl-xL is responsible for TRAIL resistance in human pancreatic cancer cells.

MATERIALS AND METHODS

Nine human pancreatic cancer cell lines (BxPC-3, SW1990, CAPAN-2, CFPAC-1, Panc10.05, AsPC-1, MiaPaCa-2, Panc-1, and HPAF-II) were used. Cell viability was analyzed using the WST-8 assay. Cell death was assessed using the Annexin V-FITC Apoptosis Detection kit and propidium iodide and a FACSCalibur flow cytometer. Immunoblot was performed using the following primary antibodies: anti-Bcl-2, anti-Bcl-$X_{S/L}$, anti-Mcl-1, anti-FLIP $_{S/L}$, anti-DR5, anti-CHOP, anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-Bid, anti-β-actin and anti-α-tubulin. Goat anti-rabbit or goat anti-mouse alkaline phosphatase-conjugated secondary antibodies were used to detect the primary antibodies. To knockdown Bcl-2 family molecules, specific siRNAs were transfected using Lipofectamine^{TM} RNAiMAX. To examine localization of Bax, treated cancer cells fixed on round cover glasses were stained with Hoechst 33342, MitoTracker Red, and anti-Bax antibody followed by Alexa Fluor 488-conjugated anti-rabbit IgG F(ab')$_2$ fragment. Confocal imaging was performed using an Olympus FV1000-D laser scanning microscope. In xenograft mouse models, BALB *nu/nu* mice were subcutaneously inoculated in the right flank with cancer cells with Matrigel. On the indicated days, these cancer-bearing mice were administered an intratumoral injection of TRAIL (1 μg) and/or an intraperitoneal injection of ABT-737 (75 mg/kg). All experiments with animals were approved by the Ethics Committee for Animal Experimentation of Shimane University (IZ26-103). Data were evaluated statistically using an unpaired two-tailed Student's *t*-test or an ANOVA together with Bartlett's test. A P-value < 0.05 was considered to indicate significance.

RESULTS AND DISCUSSION

Initially, we examined the sensitivity of nine human pancreatic cancer cell lines to TRAIL

and their expression of DRs, and found that DR5 expression did not reflect the TRAIL sensitivity of the human pancreatic cancer cell lines. We also examined the expression of anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-xL, and Mcl-1) in nine cancer cell lines, and found that a higher expression of Bcl-xL was detected in the six TRAIL-insensitive cell lines. Selective knockdown of Bcl-xL by siRNA transfection revealed that Bcl-xL was responsible for TRAIL resistance of cancer cells. We further examined the antitumor effect of TRAIL on the TRAIL-insensitive pancreatic cancer cell lines when combined with ABT-199 or ABT-263, and found that ABT-263 significantly augmented the TRAIL sensitivity of TRAIL-insensitive cancer cell lines. These lines of evidence indicate that Bcl-xL is responsible for TRAIL resistance in human pancreatic cancer cells. Flow cytometric analysis revealed that the combination of TRAIL and ABT-263 induced caspase-dependent apoptosis in human pancreatic cancer cells. Additional experiments of immunoblot and flow cytometric analysis revealed that the NF-κB pathway, but not endoplasmic reticulum stress, was involved in an increased expression of DR5 on ABT-263-treated Panc-1 cells. Alternatively, in *in vivo* xenograft models of AsPC-1 and Panc-1, the combination of TRAIL and ABT-737 significantly suppressed the tumor growth compared with the groups treated with either drug separately.

CONCLUSION

In this study, we found that Bcl-xL is responsible for TRAIL resistance in human pancreatic cancer cells and that the Bcl-2 family inhibitors, including ABT-263 and ABT-737, can restore the TRAIL sensitivity of pancreatic cancer cell lines both *in vitro* and *in vivo*. The Bcl-2 family of inhibitors could be promising reagents to sensitize human pancreatic cancer cells in DR-targeting therapy.