学 位 論 文 の 要 旨

氏名　　玉木　宏樹

学位論文名　Bcl-2 Family Inhibition Sensitizes Human Prostate Cancer Cells to Docetaxel and Promotes Unexpected Apoptosis Under Caspase-9 Inhibition

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著者名　Hiroki Tamaki, Nanae Harashima, Miho Hiraki, Naoko Arichi, Nobuhiro Nishimura, Hiroaki Shiina, Kohji Naora, Mamoru Harada

論 文 内 容 の 要 旨

INTRODUCTION

Recurrent prostate cancer after surgery, radiotherapy, and hormone therapy increases its malignancy, and docetaxel (DTX) has been used as a chemotherapeutic drug to combat it; however, recurrent cancer cells frequently acquire DTX resistance, and efficient treatment modalities to overcome this resistance are required.

Bcl-2 family molecules play a crucial role in mitochondria-mediated apoptosis. The family of Bcl-2-related anti-apoptotic proteins includes Bcl-2, Bcl-xL, Bcl-w, and Mcl-1. Inhibition of Bcl-2 and/or Bcl-xL is hypothesized to potentiate the effect of chemotherapy, and several Bcl-2 family inhibitors/antagonists have been developed. ABT-737 is a small molecule inhibitor of Bcl-2, Bcl-xL, and Bcl-w. ABT-263 is an orally bioavailable inhibitor with the same specificity as ABT-737. Alternatively, ABT-199 is a new, orally bioavailable inhibitor that inhibits Bcl-2 and Bcl-w, but not Bcl-xL. Several reports have shown the efficacy of these inhibitors against hematological malignancies as well as several types of solid tumors.

In this study, we investigated the effect of combining DTX with Bcl-2 family inhibitors on

human prostate cancer cell lines and the underlying mechanisms of apoptosis when DTX less sensitive human prostate PC3 cancer cells were treated with both Bcl-2 family inhibitors and DTX.

MATERIALS AND METHODS

Three human prostate cancer cell lines (LNCaP, PC3, and DU145) were used. DR-PC3 is a DTX-resistant PC3 cell line. To knockdown Bcl-2 family molecules, specific siRNAs were transfected using Lipofectamine™ RNAiMAX. Cell death was assessed using the Annexin V-FITC Apoptosis Detection kit and propidium iodide. Analysis was performed using a FACSCalibur flow cytometer. Immunoblot was performed using the following primary antibodies: anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-caspase-2, 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. In a xenograft mouse model, male BALB *nu/nu* mice were subcutaneously inoculated in the right flank with PC3 cells and Matrigel. On the indicated days, these PC3-bearing mice were treated with DTX and/or ABT-263 or ABT-737. 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. 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, the cytotoxic effect of combining DTX with either of two Bcl-2 family inhibitors, ABT-263 and ABT-199, was assessed. Among three cell lines, PC3 cells were relatively resistant to DTX and DU145 cells were less sensitive to both inhibitors compared with the other two cell lines. Of note, ABT-263 decreased the viability of PC3 cells more drastically than did ABT-199 with suboptimal doses of DTX. Such a synergistic effect was not observed in LNCaP or DU145 cells. Given the difference in specificity of inhibition between ABT-263 and ABT-199, we examined whether the augmenting effect of ABT-263 was due to its inhibition of Bcl-xL alone versus the inhibition of both Bcl-xL and Bcl-2. RNA interfering experiments revealed that the

augmented antitumor effect induced by ABT-263 in PC3 cells treated with low-dose DTX was primarily due to inhibition of Bcl-xL. Additionally, ABT-263 sensitized DR-PC3 cells to DTX-induced cytotoxicity. ABT-737 showed a similar synergistic effect on PC3 cells as ABT-263 and, in a xenograft mouse model, intraperitoneal administration of ABT-737 sensitized PC3 cells to DTX significantly.

We further examined the mechanism underlying the synergistic antitumor effect of DTX and ABT-263. Flow cytometry and immunoblot analysis revealed that co-treatment with ABT-263 and DTX induced apoptosis in PC3 cells in a caspase-9-dependent manner. However, the addition of a caspase-9 inhibitor unexpectedly increased apoptosis of ABT-236-treated PC3 cells. Augmentation of apoptosis of ABT-263-treated PC3 cells induced by the caspase-9 inhibitor was blocked by caspase-8 inhibition. Caspase-9 inhibition was found to significantly increase apoptosis in ABT-263-treated LNCaP cells, and inhibition of caspase-8 blocked this augmentation. In contrast, the addition of the caspase-9 inhibitor significantly inhibited apoptosis of ABT-263-treated DU145 cells.

In *in vivo* experiments, we combined ABT-263 or ABT-737 with DTX. However, no significant tumor growth suppression was observed when oral administration of ABT-263 was combined with DTX, despite that ABT-263 and ABT-737 showed similar effects *in vitro*. Although we have no clear answer regarding this result at present, we suppose that this discrepancy in therapeutic efficacy could result from the difference in the administration routes of these reagents. We have no idea regarding the quantity of orally administered ABT-263 that would be absorbed in the intestine and have no information about its pharmacokinetics.

CONCLUSION

Our data indicate that these Bcl-2 inhibitors effectively enhance DTX-induced antitumor effects on DTX less sensitive human prostate cancer cells both *in vitro* and *in vivo*, suggesting that they may be promising agents for restoring DTX sensitivity of human prostate cancers. Additionally, we uncovered a unique apoptotic pathway in which ABT-263 and caspase-9 inhibition paradoxically promote caspase-8-dependent apoptosis in human prostate cancer cells.