

学位論文の要旨

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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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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 LipofectamineTM 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.

論文審査及び最終試験又は学力の確認の結果の要旨

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<p>学位論文名</p>	<p>Bcl-2 Family Inhibition Sensitizes Human Prostate Cancer Cells to Docetaxel and Promotes Unexpected Apoptosis Under Caspase-9 Inhibition.</p>	
<p>学位論文審査委員</p>	<p>主査 副査 副査</p>	<p>浦野 健 鈴宮 淳司 関根 浄治</p> 
<p>論文審査の結果の要旨</p> <p>わが国の前立腺がんの罹患数（2010年）は約 6.5 万人で、男性がん罹患全体に占める割合は 2020 年には、胃がんを抜き一位になると予想されている。再発前立腺がんにはドセタキセル（以下、DTX）が使用されるが、薬剤耐性はその治療を困難にしている。申請者らは、抗アポトーシス作用を有する Bcl-2 ファミリーに対する阻害剤を併用することで、DTX の抗腫瘍効果の増強について詳細に検討した。</p> <ol style="list-style-type: none"> 1) 前立腺がん細胞株 PC3 および同細胞株を用いた担がんマウスモデルにおいて、Bcl-xL に対する阻害効果を有する Bcl-2 ファミリー阻害剤 (ABT-263 および ABT-737) が DTX の抗腫瘍効果を増強した。さらに PC3 由来の DTX 耐性細胞株 DR-PC3 に対しても、ABT-263 は DTX の抗腫瘍効果を増強した。 2) DTX と ABT-263 の併用により PC3 細胞株において、カスパーゼ 9 およびカスパーゼ 3 の活性化を伴うアポトーシスが誘導された。 3) DTX と ABT-263 の併用に対して、さらにカスパーゼ 9 阻害剤を追加したところ、PC3 細胞株のアポトーシスがさらに増強するという予期せぬ結果を得た。ABT-263 とカスパーゼ 9 阻害剤との併用でも同じ結果であり、カスパーゼ 8 を介したアポトーシスが誘導された。前立腺がん細胞株 LNCaP でも同様の結果であったが、前立腺がん細胞株 DU145 には、ABT-263 とカスパーゼ 9 阻害剤との併用効果は認められなかった。 <p>DTX 耐性の前立腺がんの治療に対して、Bcl-2 ファミリー阻害剤の可能性を示したばかりではなく、Bcl-2 ファミリー阻害剤とカスパーゼ 9 阻害剤との併用による新たなアポトーシス誘導の一端を明らかにした研究と考えられる。</p> <p>最終試験又は学力の確認の結果の要旨</p> <p>申請者は、DTX と Bcl-xL に対する阻害効果を有する Bcl-2 ファミリー阻害剤との併用効果を詳細に検討し、DTX 耐性の前立腺がんの治療に対して Bcl-2 ファミリー阻害剤の有効性を始めて示した。さらに、カスパーゼ 9 阻害剤による新たなアポトーシス誘導の一端を明らかにした。この研究成果は DTX 耐性の前立腺がんの薬物療法の可能性を示すもので、質疑応答も的確で、関連分野の知識も豊富であり、学位授与に値する。（主査：浦野 健）</p> <p>申請者は、前立腺がんに対する Bcl-2 ファミリー阻害剤と DTX との併用が有効であることを、<i>in vitro</i> およびマウスモデルを用いて明らかにした。DTX 耐性細胞に対しても効果があることを示し、難治性前立腺がんに対する新たな薬物療法の可能性を示した。発展性の高い研究であり、関連知識も豊富で、学位授与に値すると判断した。（副査：鈴宮 淳司）</p> <p>申請者は、<i>in vitro</i> ならびに <i>in vivo</i> にて前立腺がんにおける Bcl-2 ファミリー阻害剤と DTX の併用効果を明らかにした。また、実験の途中で明らかとなったカスパーゼ 9 の抑制がアポトーシスを増強する原因の解明を試みた。今後、小胞体ストレスに関する検討を行う予定で極めて発展性が高い研究である。解析方法も確かで、関連分野の知識も豊富であり学位授与に値すると判断した。（副査：関根 浄治）</p>		

（備考）要旨は、それぞれ 400 字程度とする。