Chemotherapy and Delayed Androgen-Independent Recurrence of Prostate Cancer With the Use of Antisense Bcl-2 Oligodeoxynucleotides

Hideaki Miayake, Anthony Tolcher, Martin E. Gleave

Background: Increased expression of the bcl-2 gene has been observed in prostate cancer cells after androgen withdrawal and has been associated with the development of androgen independence and chemoresistance. The objective of this study was to determine whether antisense Bcl-2 oligodeoxynucleotides could enhance paclitaxel cytotoxicity and delay androgen-independent progression. Methods: Northern and western blot analyses were used to measure changes in Bcl-2 expression in mouse Shionogi tumor cells after treatment with antisense Bcl-2 oligodeoxynucleotides and/or paclitaxel. Growth inhibition and induction of apoptotic cell death were assessed with the use of standard methods. All P values are two-sided. Results: Treatment of Shionogi tumor cells with 500 nM antisense Bcl-2 oligodeoxynucleotides decreased expression of Bcl-2 messenger RNA (mRNA) by approximately 85%. Paclitaxel treatment induced Bcl-2 protein phosphorylation but did not alter Bcl-2 mRNA expression. Antisense Bcl-2 oligodeoxynucleotide treatment substantially enhanced paclitaxel chemosensitivity in a dose-dependent manner. Characteristic apoptotic DNA laddering and cleavage of poly(adenosine diphosphate-ribose) polymerase were demonstrated only after combined treatment. Adjuvant in vivo administration of antisense Bcl-2 oligodeoxynucleotides and micellar paclitaxel following castration resulted in a statistically significant delay of androgen-independent, recurrent tumors compared with administration of either agent alone (P<0.001, Mantel–Cox log-rank test). Combination therapy also statistically significantly inhibited the growth of established hormone-refractory tumors compared with treatment with either agent alone (P<0.001, Student’s t test). Conclusions. Combined treatment with antisense Bcl-2 oligodeoxynucleotides and paclitaxel could be a novel and attractive strategy to inhibit progression to androgen-independent disease as well as growth of hormone-refractory prostate cancer through deprivation of Bcl-2 function. [J Natl Cancer Inst 2000;92:34–41]

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer-related deaths among men in Western industrialized countries. Androgen withdrawal remains the only effective form of systemic therapy for men with advanced disease, with symptomatic and/or objective response in 80% of patients. Unfortunately, progression to androgen independence occurs within a few years in the majority of these cases (1). Despite several hundred clinical studies of both experimental and approved chemotherapeutic agents, chemotherapy has limited antitumor activity, with an objective response rate of less than 10% and no demonstrated survival benefit (2). Therefore, androgen-independent disease is the main obstacle to improving the survival and quality of life in patients with advanced prostate cancer, and novel therapeutic strategies targeting the molecular basis of androgen resistance and chemoresistance of prostate cancer are required.

To date, controlled study of the complex molecular processes associated with progression to androgen independence has been difficult because of the lack of an ideal animal model that mimics the clinical course in men. The Shionogi tumor model is a mouse androgen-dependent mammary carcinoma that, like human prostate cancer, regresses after castration and later recurs as an androgen-independent tumor. In this model, androgen-dependent tumors in intact mice undergo complete regression following androgen ablation, but rapidly growing androgen-independent tumors recur after 1 month in a highly reproducible manner (3). Therefore, this model is particularly useful to evaluate the efficacy of agents targeting castration-induced apoptotic cell death and their effects on time to progression to androgen independence.

The bcl-2 gene, initially recognized as the proto-oncogene translocated to the immunoglobulin (Ig) heavy-chain locus in human follicular B-cell lymphoma cells, is the prototype of a novel class of oncoproteins that contributes to neoplastic progression by enhancing tumor cell survival through inhibition of apoptotic cell death (4). Bcl-2 belongs to a growing family of apoptosis-regulatory gene products, which may act as either death antagonists (Bcl-2, Bcl-xL, and Mcl-1) or death agonists (Bax, Bak, Bcl-xS, Bad, and Bid). The selective and competitive dimerization between pairs of antagonists and agonists determines how a cell will respond to an apoptotic signal (5). For example, an increase in Bcl-2 levels increases Bcl-2:Bax heterodimer levels and stabilizes mitochondrial and microtubular integrity (6). Phosphorylation of Bcl-2 interferes with its dimerization to Bax, which consequently increases Bax homodimerization and enhances induction of apoptotic cell death (7). In prostate cancer, several experimental and clinical studies (8–13) report that increased expression of Bcl-2 confers both chemoresistance and androgen resistance and may facilitate progression to androgen independence. Accumulating evidence suggests that Bcl-2 overexpression protects prostate cancer cells from apoptotic cell death after androgen withdrawal and, therefore, represents a suitable molecular target with antisense technology.

Antisense oligodeoxynucleotides are chemically modified stretches of single-strand DNA that are complementary to mes-
senger RNA (mRNA) regions of a target gene that can inhibit gene expression by forming RNA–DNA duplexes, thereby reducing the activity of the target gene products (14). Phosphorothioate oligodeoxynucleotides are stabilized to resist nuclease digestion by substituting one of the nonbridging phosphoryl oxygens of DNA with a sulfur. Antisense oligodeoxynucleotides targeting several oncogenes have been reported to specifically inhibit expression of these genes and to delay tumor progression (15–18). However, since numerous genes mediate tumor progression, inhibition of a single target gene is likely insufficient to completely suppress tumor progression. Although there have been no reports demonstrating complete responses of established tumors in vivo with the use of antisense oligodeoxynucleotides alone, combined use of antisense oligodeoxynucleotides with chemotherapeutic agents has been demonstrated to improve response rates in some tumor model systems (17,18).

Conventional chemotherapy in advanced prostate cancer is ineffective for various reasons, including inherent chemoresistance, and the inability of elderly patients to tolerate its toxicity. Although paclitaxel has significant cytotoxicity in prostate cancer cells in vitro, results from clinical studies (2) in which paclitaxel is used as a single agent in hormone-refractory disease have been disappointing. However, paclitaxel is known to phosphorylate and to inactivate Bcl-2 (7); therefore, we undertook this study to test whether the cytotoxic effects of paclitaxel are enhanced by antisense Bcl-2 oligodeoxynucleotide treatment and to determine whether adjuvant use of antisense Bcl-2 oligodeoxynucleotide and paclitaxel after castration delays progression to androgen independence.

**Materials and Methods**

Shionogi tumor growth. The Toronto subline of the transplantable Shionogi SC-115 androgen-dependent mouse mammary carcinoma was used in all experiments (19). Shionogi tumor cells were maintained in Dulbecco's modified Eagle medium (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD) supplemented with 5% heat-inactivated fetal calf serum. For in vitro study, approximately 5 × 10^6 cells of the Shionogi carcinoma were injected subcutaneously into adult male DDS strain mice. When Shionogi tumors reached 1–2 cm in diameter, usually 2–3 weeks after injection, castration was performed through an abdominal incision in the animals while under methoxyflurane anesthesia. Details of the maintenance of mice, tumor stock, and surgical procedures have been described previously (20). Mice were maintained in accordance with institutional accredited guidelines of the University of British Columbia.

Antisense Bcl-2 oligodeoxynucleotide. Phosphorothioate oligodeoxynucleotides used in this study were supplied by Dr. Brett P. Monia (Isis Pharmaceuticals, Carlsbad, CA). The sequence of antisense Bcl-2 oligodeoxynucleotides corresponding to the mouse bcl-2 translation initiation site was 5'-TCTCCCGGTTTGCCCAT-3'. A two-base mismatch Bcl-2 oligodeoxynucleotide (5'-TCTCCCGGGCATGTCATCAT-3') was used as control. The expression of Bcl-2 and poly(adenosine diphosphate-ribose) polymerase (PARP) protein in cultured Shionogi cells and/or Shionogi tumor tissues was determined by western blot analysis as described previously (21). Briefly, samples containing equal amounts of protein (15 μg) were subjected to electrophoresis on a sodium dodecyl sulfate (SDS)–polyacrylamide gel and transferred to a nitrocellulose filter. The filters were blocked in PBS containing 5% nonfat milk powder at 4 °C overnight and then incubated for 1 hour with a 1:200-diluted anti-human Bcl-2 mouse monoclonal antibody that reacts with mouse Bcl-2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) or anti-human PARP mouse monoclonal antibody that reacts with mouse PARP (Pharmingen, Mississauga, Canada). The filters were then incubated for 30 minutes with horseradish peroxidase-conjugated anti-mouse IgG antibody (Amersham Life Science Inc.), and specific proteins were detected with the use of an enhanced chemiluminescence system (Amersham Life Science Inc.).

**In vitro cell growth assay.** The in vitro growth-inhibitory effects of antisense Bcl-2 oligodeoxynucleotide and/or paclitaxel on Shionogi tumor cells were assessed by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay as described previously (22). Briefly, 1 × 10^6 cells were seeded in each well of 96-well microtiter plates and allowed to attach overnight. The cells were then treated once daily with various concentrations of oligodeoxynucleotide for 2 days. After oligodeoxynucleotide treatment, the cells were treated with various concentrations of paclitaxel. After 48 hours of incubation, 20 μL of 5 mg/mL MTT (Sigma Chemical Co.) in PBS was added to each well, followed by incubation for 4 hours at 37 °C. The formazan crystals were dissolved in DMSO. The optical density was determined with a microculture plate reader (Becton Dickinson Labware, Lincoln Park, NJ) at 540 nm. Absorbance values were normalized to the values obtained for the vehicle-treated cells to determine the percent survival. Each assay was performed in triplicate.

**DNA fragmentation analysis.** The nucleosomal DNA degradation was analyzed as described previously with a minor modification (22). Briefly, 1 × 10^5 Shionogi tumor cells were seeded in 6-cm culture dishes and allowed to adhere overnight. After the treatment with oligodeoxynucleotide and/or paclitaxel under the same schedule as described above, cells were harvested and then lysed in a solution containing 100 mM NaCl, 10 mM Tris (pH 7.4), 25 mM EDTA, and 0.5% SDS. After centrifugation at 10 000g for 10 minutes at 4 °C, the supernatants were incubated with 300 μg/mL proteinase K for 5 hours at 65 °C and extracted with phenol–chloroform. The aqueous layer was treated with 0.1 volume of 3 M sodium acetate, and the DNA was precipitated with 2.5 volumes of 95% ethanol. After treatment with 100 μg/mL ribonuclease A for 1 hour at 37 °C, the sample was subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide.

**Assessment of in vivo tumor growth.** For the determination of whether the combined treatment with antisense Bcl-2 oligodeoxynucleotide and paclitaxel delays the time to androgen-independent recurrence after castration compared with treatment with either agent alone, male DDS mice bearing the Shionogi tumor were castrated and randomly selected for treatment with antisense Bcl-2 oligodeoxynucleotide alone (group 1), antisense Bcl-2 oligodeoxynucleotide plus paclitaxel (group 2), or mismatch control oligodeoxynucleotide plus paclitaxel (group 3). Each experimental group consisted of six mice. Beginning 1 day after castration, 12.5 mg/kg antisense Bcl-2 or mismatch control oligodeoxynucleotide was injected intraperitoneally once daily into each mouse for 14 days.
RESULTS

Effects of Antisense Bcl-2 Oligodeoxynucleotide and Paclitaxel Treatment on Bcl-2 mRNA and Protein Expression

Northern blot analysis was used to determine the effect of treatment with antisense Bcl-2 oligodeoxynucleotide and paclitaxel on Bcl-2 mRNA expression in Shionogi tumor cells. As shown in Fig. 1, A, treatment of Shionogi tumor cells with 500 nM antisense Bcl-2 oligodeoxynucleotide decreased Bcl-2 mRNA by approximately 85% compared with those cells treated with 500 nM mismatch control oligodeoxynucleotide; however, Bcl-2 mRNA expression was not affected by paclitaxel treatment. Western blotting was then used to analyze changes in Bcl-2 protein expression in Shionogi tumor cells after treatment with antisense Bcl-2 oligodeoxynucleotide, paclitaxel, or both agents. Fig. 1, B, shows that treatment of Shionogi tumor cells with antisense Bcl-2 oligodeoxynucleotide resulted in a substantial decrease in Bcl-2 protein and that paclitaxel treatment induced the expression of the slow-migrating (i.e., phosphorylated) form of the Bcl-2 protein. In addition, incubation of the cell lysates after paclitaxel treatment with A protein phosphatase, which has specificity for cleavage of phosphate groups appended to the amino acids serine, threonine, or tyrosine (24), resulted in the absence of the slowly migrating form of the Bcl-2 protein. These findings confirm that paclitaxel treatment results in Bcl-2 phosphorylation, which has been reported to interfere with Bcl-2 dimerization to Bax in several cell types and consequently to increase Bax homodimerization, resulting in enhanced induction of apoptotic cell death (7).

Synergistic Cytotoxicity of Antisense Bcl-2 Oligodeoxynucleotide and Paclitaxel Treatment in Shionogi Tumor Cell Growth In Vitro

To determine whether treatment with antisense Bcl-2 oligodeoxynucleotide enhances the cytotoxic effect of paclitaxel, we treated Shionogi tumor cells with various concentrations of antisense Bcl-2 or mismatch control oligodeoxynucleotide once daily for 2 days and then incubated them with various concentrations of paclitaxel for 2 days. The MTT assay was then performed to determine cell viability. As shown in Fig. 2, A, treatment with antisense Bcl-2 oligodeoxynucleotide and paclitaxel were synergistic, as determined by an analysis that utilized the fractional product method (23). We also observed synergistic cytotoxic effects between antisense Bcl-2 oligodeoxynucleotide and paclitaxel by increasing the concentration of antisense Bcl-2 oligodeoxynucleotide while keeping the concentration of paclitaxel constant at 10 nM (two-sided P<.044, ANOVA) (Fig. 2, B).

A DNA fragmentation assay was performed to compare the effects of combined treatment with antisense Bcl-2 oligodeoxynucleotide (500 nM) and paclitaxel (10 nM) on induction of apoptotic cell death. With the use of the same treatment schedule described above, the characteristic apoptotic DNA ladder was observed only after combined treatment with antisense Bcl-2 oligodeoxynucleotide plus paclitaxel (Fig. 3, A). Furthermore,
Fig. 2. Effect of combined treatment with antisense Bcl-2 oligodeoxynucleotide (ODN) and paclitaxel (Taxol) on Shionogi tumor cell growth. A) Shionogi tumor cells were treated daily with 500 nM antisense Bcl-2 or mismatch control ODN for 2 days. After ODN treatment, the medium was replaced with medium containing various concentrations of paclitaxel. After 48 hours of incubation, cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. Each data point represents the mean of three independent experiments with standard deviations. The cytotoxic effects of paclitaxel on Shionogi cells were statistically significantly enhanced by antisense Bcl-2 ODN treatment (two-sided \( P = .018 \), analysis of variance). B) Shionogi tumor cells were treated with various concentrations of antisense Bcl-2 ODN or mismatch control ODN and then incubated for 48 hours with medium alone or medium containing 10 nM paclitaxel, and the cell viability was determined by MTT assay. Each data point represents the mean of three independent experiments with standard deviations. Treatment of Shionogi tumor cells with antisense Bcl-2 ODN statistically significantly enhanced the sensitivity to paclitaxel (two-sided \( P < .044 \), analysis of variance). * indicates a synergistic effect between antisense Bcl-2 ODN and paclitaxel, as determined by the fractional product method.

Fig. 3. Effect of combined treatment with antisense Bcl-2 oligodeoxynucleotide (ODN) and paclitaxel (Taxol) on apoptotic death of Shionogi tumor cells. A) Shionogi tumor cells were treated daily with 500 nM antisense Bcl-2 ODN or mismatch control ODN for 2 days. After ODN treatment, the medium was replaced with medium containing 10 nM paclitaxel. After 48 hours of incubation, DNA was extracted from culture cells, subjected to electrophoresis in a 2% agarose gel, and visualized by ethidium bromide staining. M = molecular weight markers (1-kilobase ladder; Life Technologies, Inc.). B) Proteins were extracted from Shionogi tumor cells after the same treatment as described in panel A and analyzed by western blotting with an antipoly(adenosine diphosphate-ribose) polymerase (PARP) protein antibody. Uncleaved intact PARP, molecular weight \(( M_f ) = 116 \) kilodalton (kD); cleaved PARP, \( M_c = 85 \) kD.
cleavage of the PARP protein, a substrate of the caspase activated during the process of apoptotic execution (25), was examined by western blotting. The 116-kilodalton (kD) intact form of PARP was observed in all of the samples examined, whereas the 85-kD PARP cleavage fragment was detected only after combined treatment with antisense Bcl-2 oligodeoxynucleotide plus paclitaxel (Fig. 3, B).

Delayed Progression to Androgen Independence of Shionogi Tumors In Vivo by Combined Treatment With Antisense Bcl-2 Oligodeoxynucleotide Plus Paclitaxel

Male mice with Shionogi tumors that were between 1 and 2 cm in diameter were randomly selected for treatment with either antisense Bcl-2 oligodeoxynucleotide alone, antisense Bcl-2 oligodeoxynucleotide plus paclitaxel, or mismatch control oligodeoxynucleotide plus paclitaxel. The mean tumor volume was similar at the beginning of treatment in the three treatment groups. Beginning 1 day after castration, 12.5 mg/kg antisense Bcl-2 or mismatch control oligodeoxynucleotide, diluted with PBS, was injected intraperitoneally once daily for 14 days. Beginning 10 days after castration, 0.5 mg of polymeric micellar paclitaxel was administered intravenously once daily for 5 days. Fig. 4, A, illustrates the changes in the mean tumor volume after castration and adjuvant therapy. By 40 days after castration, the mean tumor volume in the group treated with antisense Bcl-2 oligodeoxynucleotide plus paclitaxel was 91% and 86% lower than that of the group treated with antisense Bcl-2 oligodeoxynucleotide or that of the group treated with mismatch control oligodeoxynucleotide and micellar paclitaxel, respectively (two-sided \( P < .001 \), Student’s \( t \)-test). Fig. 4, B, illustrates the differences in recurrence-free survival after castration and adjuvant therapy. Androgen-independent tumors recurred in three of six mice after a median of 37 days in the group treated with antisense Bcl-2 oligodeoxynucleotide plus micellar paclitaxel, while androgen-independent tumors recurred in all mice after a median of 23 or 28 days in the group treated with antisense Bcl-2 oligodeoxynucleotide or in the group treated with...
mismatch control oligodeoxynucleotide and micellar paclitaxel, respectively (two-sided $P<.001$, Mantel–Cox log-rank test). These data demonstrate that antisense Bcl-2 oligodeoxynucleotide and paclitaxel prolong time to progression to androgen independence when combined in an adjuvant manner with androgen ablation.

**Efficacy of Combined Antisense Bcl-2 Oligodeoxynucleotide plus Paclitaxel in Treatment of Established Androgen-Independent, Recurrent Shionogi Tumors**

Approximately 3–4 weeks after castration, androgen-independent Shionogi tumors recur and grow rapidly, with a doubling time of 72 hours (19). When androgen-independent tumors reached 1 cm in diameter, the mice were randomly selected for treatment with either antisense Bcl-2 oligodeoxynucleotide alone, antisense Bcl-2 oligodeoxynucleotide plus micellar paclitaxel, or mismatch control oligodeoxynucleotide plus micellar paclitaxel, and the treatment was administered under the same schedule as described above. The mean tumor volume was similar at the beginning of treatment in the three treatment groups. Untreated mice with androgen-independent Shionogi tumors require sacrifice within 2–3 weeks after recurrence because their tumor mass became larger than 10% of their body weight or because of weight loss, tumor ulceration, or gait disturbance (data not shown). Hence, time to sacrifice was delayed in all three treatment groups; however, combined treatment with antisense Bcl-2 oligodeoxynucleotide plus paclitaxel resulted in the most statistically significant delay in tumor progression of the three treatment groups, producing a mean tumor volume that was 50%–70% lower at day 38 than that in the other two treatment groups (two-sided $P<.001$, Student’s $t$ test) (Fig. 5). During a 38-day observation period, the mice treated with antisense Bcl-2 oligodeoxynucleotide plus micellar paclitaxel averaged a 1.6-fold increase in tumor volume compared with a 2.9-fold or 2.6-fold increase in the mice treated with antisense Bcl-2 oligodeoxynucleotide or control oligodeoxynucleotide plus micellar paclitaxel, respectively.

![Fig. 5. Effects of combined treatment with antisense Bcl-2 oligodeoxynucleotide (ODN) and polymeric micellar paclitaxel (Taxol) on androgen-independent Shionogi tumors.](https://academic.oup.com/jnci/article-abstract/92/1/34/2905751)
The effects of combined in vivo treatment with antisense Bcl-2 oligodeoxynucleotide and paclitaxel on Bcl-2 mRNA expression, Bcl-2 phosphorylation, and cleavage of PARP protein in Shionogi tumors were examined with the use of northern or western blot analysis. Androgen-independent tumors were harvested after completion of the same treatment schedule described above. Treatment with antisense Bcl-2 oligodeoxynucleotide resulted in a substantial reduction in Bcl-2 mRNA and protein levels, while micellar paclitaxel induced Bcl-2 phosphorylation in androgen-independent Shionogi tumors (Figs. 5, B and C). Furthermore, the 85-kD PARP cleavage fragment was detectable in androgen-independent Shionogi tumors only after combined treatment with antisense Bcl-2 oligodeoxynucleotide and micellar paclitaxel (Fig. 5, D).

DISCUSSION

Although Bcl-2 expression in normal prostatic epithelial cells is low or absent, Bcl-2 is highly elevated in prostate cancer cells after androgen withdrawal and during progression to androgen independence (8, 9). Accumulating evidence strongly suggests that Bcl-2 overexpression protects prostate cancer cells from apoptotic cell death induced by several therapies, including androgen withdrawal and cytotoxic chemotherapy, thereby accelerating progression to androgen independence and conferring chemoresistance. For example, introduction of Bcl-2 cDNA into LNCaP human prostate cancer cells increases in vivo tumorigenic potential and renders the cells highly resistant to androgen ablation (11). Repression of Bcl-2 expression in LNCaP cells blocks the protective effect of androgens on etoposide cytotoxicity (10). Taken together, these findings suggest that inhibition of increased Bcl-2 expression precipitated by androgen withdrawal may enhance castration-induced apoptotic cell death and delay androgen-independent progression of prostate cancer and may also render cells more sensitive to cytotoxic chemotherapy. This hypothesis has not, however, been confirmed in in vivo models, which is critical when evaluating the mechanism of castration-induced apoptotic cell death and androgen resistance, which are complicated processes that are lost in cells in vitro culture.

Antisense oligodeoxynucleotide therapy offers one strategy to specifically target bcl-2 gene expression. Phosphorothioate oligodeoxynucleotides are water-soluble, stable agents manufactured to resist nuclease digestion. After parenteral administration, phosphorothioate oligodeoxynucleotides become associated with high-capacity, low-affinity, serum-binding proteins (26). Various reports have shown that antisense Bcl-2 oligodeoxynucleotides induce apoptotic cell death in various types of malignant cell lines in vitro, including small-cell lung cancer (16), myeloma (27), leukemia (28), lymphoma (29), and cholangiocarcinoma (30). Furthermore, combined use of antisense Bcl-2 oligodeoxynucleotide with chemotherapeutic agents resulted in a more than additive inhibition of small-cell lung cancer cells in vitro (18) and melanoma cells in vitro and in vivo (17).

Recently, a novel polymeric micellar paclitaxel, which is characterized by high drug payload and long circulation time in the blood compared with conventional Cremophor paclitaxel, has been developed (31,32) and has been demonstrated to induce complete responses in androgen-independent LNCaP tumors (Gleave ME: unpublished data). We have previously shown that the antisense Bcl-2 oligodeoxynucleotides used in these experiments decrease Bcl-2 expression levels in Shionogi tumor cells in a dose-dependent manner, enhance castration-induced apoptotic cell death, and delay time to androgen-independent progression (33). The objective of this study was to determine whether combined treatment with antisense Bcl-2 oligodeoxynucleotide plus paclitaxel after castration delays androgen-independent progression beyond that achieved with either agent alone. Because of its androgen-dependent behavior, the Shionogi tumor model is particularly useful to study the androgen action, the molecular mechanism regulating castration-induced apoptotic cell death, and the progression to androgen independence, as well as therapeutic approaches to delay or arrest tumor progression (3).

In this study, phosphorothioate antisense Bcl-2 oligodeoxynucleotides, corresponding to the mouse bcl-2 translation initiation site, inhibited expression of Bcl-2 mRNA and protein in Shionogi tumor cells, whereas two-base mismatch Bcl-2 oligodeoxynucleotides had no effects on Bcl-2 expression levels. Although paclitaxel did not affect Bcl-2 expression levels, it did induce Bcl-2 phosphorylation in Shionogi tumor cells in a dose-dependent manner. Bcl-2 phosphorylation has been demonstrated to result in the decreased ability to form heterodimers with Bax protein (7). These findings suggest that combined treatment with antisense Bcl-2 oligodeoxynucleotides and paclitaxel cooperatively inhibits Bcl-2 function. Indeed, antisense Bcl-2 oligodeoxynucleotide enhanced paclitaxel-induced apoptotic cell death and decreased the IC_{50} of paclitaxel by one order of magnitude. In vivo administration of antisense Bcl-2 oligodeoxynucleotides plus micellar paclitaxel delayed the time to progression to androgen independence compared with either agent alone and cooperatively inhibited established androgen-independent Shionogi tumor growth. We also documented an in vivo decrease in Bcl-2 mRNA expression and phosphorylation of Bcl-2 protein by antisense Bcl-2 oligodeoxynucleotides and micellar paclitaxel, respectively. These findings illustrate that systemic administration of antisense Bcl-2 oligodeoxynucleotides and micellar paclitaxel cooperatively inhibits Bcl-2 function in tumor cells. Enhanced cleavage of the PARP protein in androgen-independent Shionogi tumors by combined treatment suggests that inhibition of Bcl-2 function results in increased apoptotic cell death in tumor tissues.

Several hundred nonhormonal therapies for prostate cancer have been traditionally evaluated in patients with advanced hormone-refractory disease; when used in this end-stage setting, none has demonstrated improved survival (2). A more rational strategy to improve survival would be to combine antisense agents earlier with androgen ablation to target adaptive changes in gene expression precipitated by androgen withdrawal in order to enhance castration-induced apoptotic cell death and delay emergence of hormone-refractory disease. A second strategy would be to try to enhance the sensitivity to conventional chemotherapeutic agents by use of antisense agents that target cell survival genes mediating chemoresistance. Our study confirms that the inhibition of Bcl-2 function with the use of antisense Bcl-2 oligodeoxynucleotides plus paclitaxel causes a delay in progression to androgen independence as well as inhibition of established androgen-independent tumor growth in the Shionogi tumor model. These preclinical data provide support for clinical studies with antisense Bcl-2 oligodeoxynucleotides plus paclitaxel for patients with prostate cancer.
REFERENCES


NOTES

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