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Antisense Bcl-2 Sensitizes Prostate Cancer Cells To Radiation

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Abstract

BACKGROUND—Bcl-2 is anti-apoptotic and overexpression is associated with prostate tumor aggressiveness. We hypothesized that Bcl-2 has a role in prostate cancer radiation (RT) response. The relationship of Bcl-2 expression in four prostate cancer cell lines, and the effect of modulating expression with a Bcl-2 antisense oligonucleotide (G3139, Genasense®, oblimersen sodium, Genta Incorporated), to RT was examined.

METHODS—The four cell lines studied were LNCaP (wild type p53), PC3 (p53 null), Bcl-2 stably transfected LNCaP (LNCaP-BST) and PC3 (PC3-BST) cells. Cells were treated with antisense Bcl-2 (AS) alone or with RT (2–6 Gy). Following RT, cells were processed at 3–6 hr for Western blots, 18 hr for Annexin V staining and flow cytometric analysis, 24 hr for caspases 3+7 quantification by fluorometric assay, and immediately for clonogenic survival..

RESULTS—AS caused a significant reduction in Bcl-2 expression in all cell lines. P53 expression was elevated following RT treatment in LNCaP and LNCaP-BST cells. P21 was increased by RT treatment in all cell lines. AS caused a significant increase in caspase 3+7 activity over the mismatch (MM) controls in all cell lines. When AS was combined with RT, caspase 3+7 activity was further increased significantly over all other groups in all cell lines. Moreover, AS+RT resulted in significantly reduced clonogenic survival over MM+RT, which was dampened in the bcl-2 overexpressing lines.

CONCLUSIONS—To our knowledge, these data demonstrate for the first time that a bcl-2 specific antisense oligonucleotide sensitizes prostate cancer cells to RT. p53 is not required for this effect.

Keywords

Antisense Bcl-2; prostate cancer; radiation; apoptosis

INTRODUCTION

One of the primary treatments for prostate cancer is radiation (RT) therapy. Although there has been a reduction in failure rates with the ability to increase radiation (RT) dose using sophisticated planning and delivery methods, local persistence of disease still remains in many cases (1). Cure rates should be improved by enhancing cell death in response to RT.

Bcl-2 belongs to a family of genes whose proteins are central to the regulation of programmed cell death (apoptosis) in both normal and abnormal cells (2,3). *Bcl-2* is overexpressed in a variety of human cancers, including prostate cancer (4–6). In prostate cancer, overexpression of *bcl-2* is associated with prostate tumor aggressiveness (7–9). Moreover, Bcl-2 protein is

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increased in the majority of tumors from men with androgen-refractory disease and is associated with resistance to androgen deprivation and RT (4,6,10–13).

Bcl-2 has been targeted to increase the efficacy of androgen deprivation and chemotherapy. Antisense oligonucleotides against Bcl-2 (AS) have been shown to inhibit Bcl-2, postpone the development of resistance to AD, and enhance the effects of chemotherapy (14–20). We hypothesized that AS would sensitize prostate cancer cells to RT. The effects of AS with RT were tested in four cell lines: LNCaP (wild type p53 and androgen sensitive), Bcl-2 stably transfected LNCaP (LNCaP-BST), PC3 (p53 null and androgen insensitive) and Bcl-2 stably transfected PC3 (PC3-BST) cells.

METHODS AND MATERIALS

Antisense Oligonucleotides

The antisense Bcl-2 (AS) molecule used here is an 18-mer phosphorothioate DNA oligonucleotide that is complementary to the first six codons of the human Bcl-2 open reading frame. Antisense Bcl-2 (Genasense®, G3139) was provided by Genta Incorporated (Berkeley Heights, NJ) as a concentrated solution in sterile saline. The sequences of the oligodeoxynucleotides (ODNs) used are as follows: Bcl-2 antisense G3139 (AS; 5′-TCTCCCAGCGTGCGCCAT-3′) and a two-base mismatch control G4126 (MM; 5′-TCTCCCAGCATGTGCCAT-3′). They were stored as frozen aliquots at −20°C.

Cell Culture and Transfection

LNCaP and PC3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)-F12 medium, containing 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin (complete medium [CM]) as described previously (21). Cells were typically cultured in complete medium for 2–3 days before the incubation with AS.

To establish stable transfectants of *bcl-2*, 1 x 10^6 LNCaP, or PC3, cells were seeded onto 10 cm dishes for 48 hours and transfected with the neomycin-selectable pSFFV/bcl-2 expression plasmid (kindly provided by Dr. Stanley Korsmeyer, Harvard Medical School, Boston, MA) by incubation in the presence of 7 μg/ml Lipofectin according to the manufacturer's procedure (Invitrogen, Carlsbad, CA). Neomycin-resistant cells were selected by incubation with 800 ug/ml Geneticin (G418, Life Technologies, Gaithersburg, MD) 48 hours after transfection. Expression of Bcl-2 was tested by Western blot analysis of cells selected from individual colonies.

Western Blot Analysis

The protein levels of Bcl-2, p53, p21, Bax and β-actin were analyzed as described previously (21,22). Cells were cultured in complete medium for 2–3 days, incubated with 200 nM of AS or MM in 4.5 ml culture medium for 24–48 hours in the presence of 7 μg/ml Lipofectin, and then given 5 Gy of γ-irradiation (RT) using a Cesium-137 irradiator (Model 81-14R, J.L. Shepherd & Associates, San Fernando, CA). Cells were lysed at various times after RT in a lysis buffer (50 mM Tris-HCL, pH 6.8, 2% sodium dodecyl sulfate [SDS] with protease inhibitor cocktail set I [Calbiochem, San Diego, CA]), and were sonicated for 30 second on ice. Protein concentration was determined using the BCA protein assay reagent kit (Pierce, Rockford, IL). Identical amounts of protein were fractionated by SDS-PAGE electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). The membranes were then incubated in blocking buffer (PBS containing 0.1% Tween 20 and 5% non-fat milk) for 1 h at room temperature and were washed three times with the washing buffer (PBS containing 0.1% Tween 20) for 5 min.

The membranes were then incubated with the appropriate primary antibody; anti-Bcl-2 monoclonal antibody (mAb) at 1:1000 (DAKO A/S, Carpinteria, CA); anti-p53 mAb at 1:1000; anti-p21 mAb at 1:1000 and anti-β actin at 1:5000 dilution (all of the latter antibodies were from Calbiochem, San Diego, CA), or anti-Bax polyclonal IgG at 1:1000 dilution (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) overnight at 4°C. After washing, the membranes were then incubated with 1:1500 diluted sheep anti-mouse IgG or donkey-rabbit IgG horseradish peroxidase conjugated secondary antibody (Amersham Pharmacia Biotech, Piscataway, NJ) for 1 h at room temperature. After repeating the washes, the proteins of interest were detected by the enhanced chemiluminescence reagents according the manufacturer's directions (Amersham, Aylesbury, UK). The integrated density of protein band was quantified using IMAGE software from the National Institutes of Health.

Measurements of Apoptosis

Two apoptotic assays were performed to determine whether the inhibition of Bcl-2 protein expression in the presence or absence of RT enhanced apoptotic cell death. Apoptosis was measured by Annexin V staining and caspase 3+7 activity assays as described previously (21). LNCaP or LNCaP-BST (2 X 10^5), and PC3 or PC-BST cells (1.5 X 10^5), were seeded onto 60 mm dish for 48 hours and then treated with 200 nM AS or MM in the presence of Lipofectin (7 ng/mL) for 24 or 48 hr. Cells were then irradiated to 5 Gy. After 18 or 24 hr, cells floating in the culture were collected and those attached to the plates were harvested by trypsinization. The collected cells in the supernatant and trypsinized cells were centrifuged and incubated with Annexin V-Phycoerythrin (Annexin V-PE) and 7-amino-actinomycin D (7-AAD) (Guava Technologies Inc, Burlingame, CA) according to the manufacturer's instructions. The percentage of Annexin V-PE positive and 7-AAD negative cells were used as a measuew of early apoptosis. The samples were then analyzed by flow cytometry on a GuavaPC personal flow cytometer (Guava Technologies).

Caspase 3+7 activity was measured using a fluorometric substrate, Z-DEVD–Rhodamine (The Apo-ONE^{TM} Homogeneous Caspase-3/7 Assay kit; Promega, Madison, WI), as described previously (21). Cells were incubated with 200 nM AS or MM for 24–48 hr and then irradiated to 5 Gy. After 24 hr, a total of 5 X 10^4 cells in 50 nL culture medium were mixed with 50 nL of Homogeneous Caspase-3/7 reagent in 96 well plates and incubated at room temperature for 24 hours. Substrate cleavage was quantified fluorometrically at 485 nm excitation and 538 nm emission. Fluorescence was measured on a fluorescent plate reader (LabSystems Inc, Franklin, MA).

Radiation Treatment and Clonogenic Assay

Cells were incubated with 500 nM AS or MM in presence of Lipofectin (7 μg/ml). After 48 hr, cells were irradiated to 2, 4 and 6 Gy. Immediately following irradiation, the cells were trypsinized, serially diluted, and known numbers replated onto 100-mm dishes. The plates were incubated for 14 days and stained with 0.25% methylene blue. Colonies were counted using an automated counter (Imaging products Internation, Inc, Chantilly, VA). Clonogenic survival results were corrected for differences in plating efficiency ([number of colonies/number of colonies of unirradiated control] x 100) for the various culture conditions. Dilutions for clonogenic assay were done in triplicate and the results were averaged together (intra-experiment averages). The data shown in the clonogenic survival table represent the average from multiple experiments (inter-experiment average), as delineated.

RESULTS

Effect of Antisense Bcl-2 ± RT on the Expression of Multiple Proteins

As shown by Western blot analysis in Figure 1A, Bcl-2 expression was inhibited by AS (200 nM) in wild-type LNCaP cells. The inhibition of Bcl-2 expression by AS was dose-dependent (data not shown). There was no change in Bcl-2 expression at 3 and 6 hr after RT (5 Gy) exposure, although as expected, the level of p53 increased at 3 hr after RT. A relative increase in p53 from RT was also seen when AS was present, although the level of p53 at 0 hr RT was higher and the relative increase from RT less than without AS present. The level of p21 was increased by RT treatment alone at 3 hr and 6 hr, which was slightly blunted by AS treatment. There was no substantive change in Bax protein levels by either AS or RT treatment.

Figure 1B shows the effect of AS and/or RT on the expression of these proteins in LNCaP-BST cells. Although it is not apparent in the figure because of differences in exposure times between panels A and B, LNCaP-BST cells had a 13.6 fold higher level of Bcl-2 expression over wild-type LNCaP cells, as determined by integrated density measurements of the Western blot bands. AS caused a reduction in the Bcl-2 protein level. In the presence of AS, the relative changes in p53 and p21 were blunted, like that seen in wild-type LNCaP cells. Bax protein expression was altered little by AS and/or RT.

The expression of Bcl-2, p21 and Bax were also determined in PC3 (p53 null and androgen insensitive) cells and Bcl-2 overexpressing PC3 (PC3-BST) cells. Although it is not apparent in the figure because of differences in exposure times between panels C and D, Bcl-2 expression was increased 15 fold in PC3-BST cells over wild-type PC3 cells. As illustrated in Figures 1C and 1D, Bcl-2 expression was significantly inhibited by AS treatment in both PC3 and PC3-BST cells. P21 was elevated to a greater degree after RT treatment in PC3, in contrast to PC3-BST cells. No change on Bax protein levels was observed in both cell lines following Bcl-2 inhibition by AS and/or RT.

Effect of AS±RT on Apoptotic Cell Death

The ability of AS to enhance the apoptotic response of prostate cancer cells to RT was measured by Annexin V binding and Caspase 3+7 production assays. Cells were cultured in complete medium for 2–3 days and then incubated with 200 nM AS or MM for 24 h with LNCaP and LNCaP-BST cells, and 48 h with PC3 and PC3-BST cells, before 5 Gy γ-irradiation was administered. Twenty-four hours after irradiation, cells were prepared for caspase 3+7 activity.

A summary of seven experiments measuring caspase 3+7 activity in LNCaP and LNCaP-BST cells is shown in Table 1. AS exposure resulted in a significant increase in caspase 3+7 activity over that of MM or the lipofectin controls. When AS was combined with RT, caspase 3+7 activity was further increased significantly over all of the other groups in both cell lines. AS +RT also resulted in a similar pattern for early apoptosis by Annexin V staining in LNCaP cells. Table 2 shows that early apoptosis (Annexin-V-PE-positive and 7-AAD-negative) was significantly higher from AS+RT (32.2%) over either AS alone (23.1%), RT alone (8.4%) or MM+RT (18%). However, for LNCaP-BST cells there was no significant difference between AS and AS+RT.

In PC3 and PC3-BST cells, the pattern of apoptotic cell death observed by the caspase 3+7 assay was very similar to that seen in both LNCaP and LNCaP-BST cells. As shown in Table 3, caspase 3+7 activity was increased from AS+RT as compared to AS or RT treatment individually for both PC3 and PC3-BST cell lines. Table 4 shows that AS caused a significant increase in the percentage of Annexin V positive cells compared to MM and the lipofectin controls. The combination of AS+RT did not result in a further increase in early apoptosis by

Annexin V staining in PC3 cells. There was no difference between AS and MM or AS and AS +RT by Annexin V binding assay in PC3-BST cells (Table 4).

Effect of AS±RT on Overall Cell Death by Clonogenic Cell Survival Assay

Clonogenic cell survival experiments were performed to determine whether the increase in apoptotic cell death translates into reduced overall cell survival. Figures 2 and 3 show the clonogenic assay results for LNCaP, LNCaP-BST and PC3, PC3-BST cells grown in vitro for 2–3 days and then treated with Lipofectin alone, AS, or MM for 24hr prior to RT. The cells were replated immediately after 2, 4 or 6 Gy RT. Overall cell survival was significantly reduced over the LC and MM controls by AS when 4 and 6 Gy RT was given to LNCaP cells (Table 5). A similar trend was observed for PC3 cells (Table 6), although AS + 4 Gy RT was not significantly different from MM + 4 Gy RT. There were no significant differences in the survival between AS+RT and MM+RT in LNCaP-BST or PC3-BST cells.

DISCUSSION

Bcl-2 is implicated in the response to, and progression after, radiotherapy for prostate cancer. Clinical studies have shown that *bcl-2* overexpression, and/or abnormal *bax* expression, is associated with an increased risk of biochemical failure after radiotherapy (12,13,23). Moreover, Bcl-2 is overexpressed in recurrent tumors after RT (24,25). Radiation may also affect Bcl-2 expression, which seems to be dependent in part on p53. Some studies have shown that RT decreases Bcl-2 protein expression in p53 wild-type cell lines, in conjunction with apoptotic cell death (26); p53 appears to down-regulate the expression of *bcl-2* and transactivate *bax* (27). Moreover, Bcl-2 appears to inhibit the nuclear transport of p53 in response to genotoxic stress (28). In cell lines lacking wild-type p53 protein, *bcl-2* expression may be up-regulated by RT (29). In a recent study (30), *bcl-2* expression was elevated initially (1 hr) and then decreased after RT in LNCaP cells; there was no significant change of Bcl-2 levels in PC3 cells. We have not observed a consistent change in *bcl-2* expression in response to RT in LNCaP ($p53^{wild\text{-}type}$) or PC3 ($p53^{null}$) cells (21) (Figure 1).

Bcl-2 overexpression has been associated with a reduction in the apoptotic response of prostate cancer cells to chemotherapy, androgen deprivation and RT (11,18,31–33). In one example, Coffey et al. (33) found that diethyl-maleate (DEM) restored sensitivity to radiation induced apoptosis in *bcl-2* overexpressing LNCaP cells by inhibiting Bcl-2 protein expression. Although prior studies have indicated that bcl-2 expression affects response to RT, in only one prior study has the interaction of AS and RT been investigated (34).

The down-regulation of Bcl-2 expression via an antisense strategy has been shown to delay the development of insensitivity to androgen deprivation and sensitize cells to the effects of chemotherapeutic agents in prostate tumor models (14–20,35). We hypothesized that AS would sensitize prostate cancer cells to RT and that high Bcl-2 protein levels would reduce this sensitization. The data we describe support this hypothesis in part. When AS was added to RT, there was a significant enhancement in apoptosis by caspase 3+7 activity in parental LNCaP and PC3 cells, as well as in *bcl-2* overexpressing LNCaP-BST and PC3-BST cells. The results based on Annexin V binding were less consistent. The combination of AS+RT was superior to AS alone and MM+RT for LNCaP, but not for PC3, LNCaP-BST or PC3-BST cells.

In the lines overexpressing Bcl-2, the measurement of cell death effects by Annexin V staining and clonogenic assay were blunted, but not by the caspase 3+7 assay. One explanation is that the quantity of endogenous Bcl-2 may not be a critical determinant of AS efficacy (36–38). However, it is also possible that the early apoptotic response measured represents a point in time which may be discordant with overall cell death (31). The clonogenic survival results in Figures 2 and 3 are representative of cumulative cell death and are consistent with the

mechanism outlined, that AS sensitizes prostate cancer cells to RT and that *bcl-2* overexpression reduces this effect. The present study is the first to demonstrate that antisense-Bcl-2 sensitizes prostate cancer cells, independent of p53 status.

More recently, Hara et al (34) found that the inhibition of Bcl-2 function and transcription by Tetracarcin A (TC-A) and antisense-Bcl-2 in *bcl-2* overexpressing HeLa cells enhanced the response to radiation. Their results are notably different from ours. They observed essentially no effect on clonogenic survival by Tetracarcin A or antisense Bcl-2 on wild-type HeLa cells, while we found significant radiosensitivity of both wild-type LNCaP and PC3 cells. Hara et al (34) found significant radiosensitization by clonogenic assay only in *bcl-2* overexpressing HeLa cells, while we found reduced clonogenic responses in *bcl-2* overexpressing LNCaP-BST and PC3-BST cells. However, we did observe an increase in early apoptosis, mainly by caspases 3+7 activity, in LNCaP-BST and PC3-BST cells, which did not ultimately translate into a significant reduction in clonogenic survival. The differences between Hara et al (34) and our results may be related to downstream factors in the cell lines used.

CONCLUSIONS

Bcl-2 is a regulatory component in the cell death response of prostate cancer cells to RT. Antisense Bcl-2 resulted in a reduction in Bcl-2 protein levels and a significant reduction in clonogenic survival in $p53^{wild\text{-}type}$ LNCaP and $p53^{null}$ PC-3 cells; indicating that this effect is independent of p53. Our results have broad treatment implications since radiotherapy is an important option for men with prostate cancer and antisense-Bcl-2 is a significant radiosensitizer. Moreover, radiotherapy is often combined with androgen deprivation and, as Gleave and colleagues have demonstrated (15), AS prolongs response to androgen deprivation. Antisense-bcl-2 has the potential to substantially improve the outcome of men with intermediate-to-high risk prostate cancer treated with radiotherapy ± androgen deprivation.

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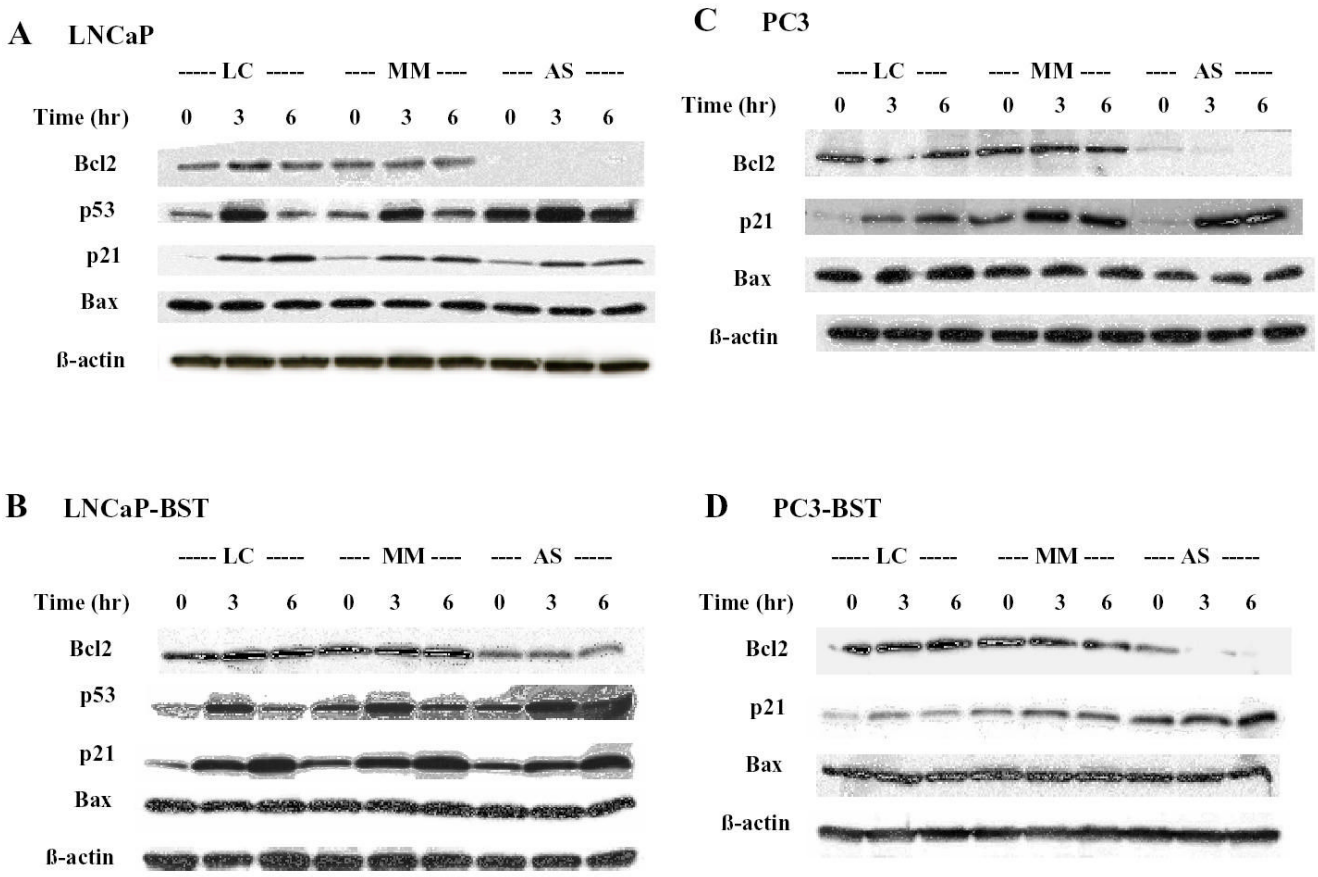

Figure 1.

Effects of AS-Bcl-2 and radiation on protein levels of Bcl-2, p53, p21 and Bax in LNCaP, LNCaP-BST, PC3 and PC3-BST cells. AS or MM was administered at 200 nM and 48 hr later RT at 5 Gy was given. The cells were harvested at 0, 3 and 6 hr later and the protein levels were examined by Western blotting. LC, Lipofectin control.

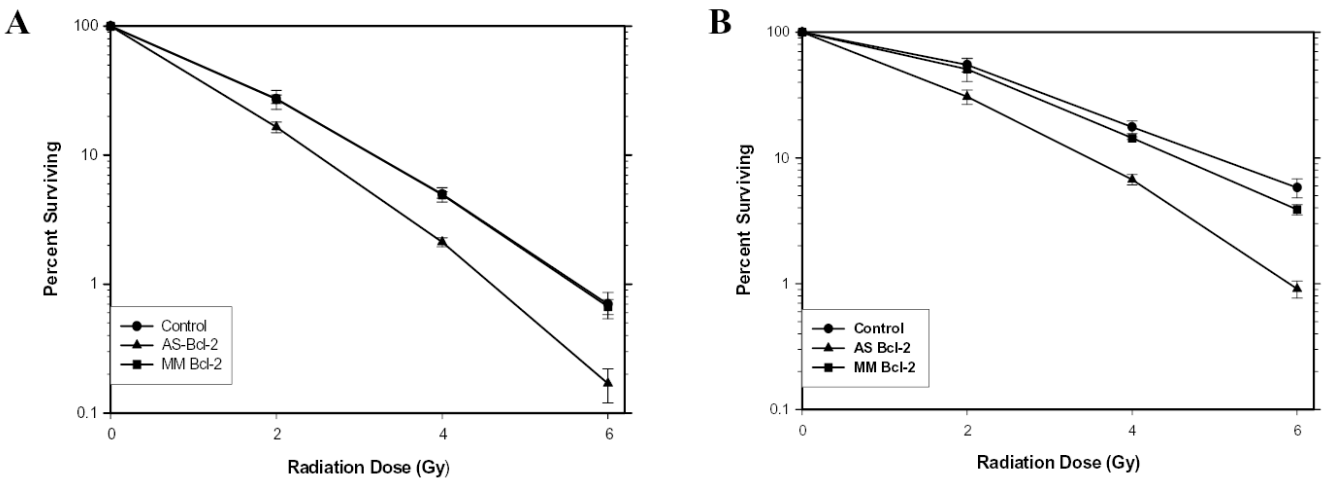

Figure 2.

Clonogenic assays of LNCaP (A) and PC3 (B) cells cultured in CM with LC, AS or MM (500 nM) added for 24 hr before RT at 2, 4 or 6 Gy. Values were expressed as a percent surviving (Mean±SEM) from five separate experiments for LNCaP cells and three separate experiments for PC3 cells.

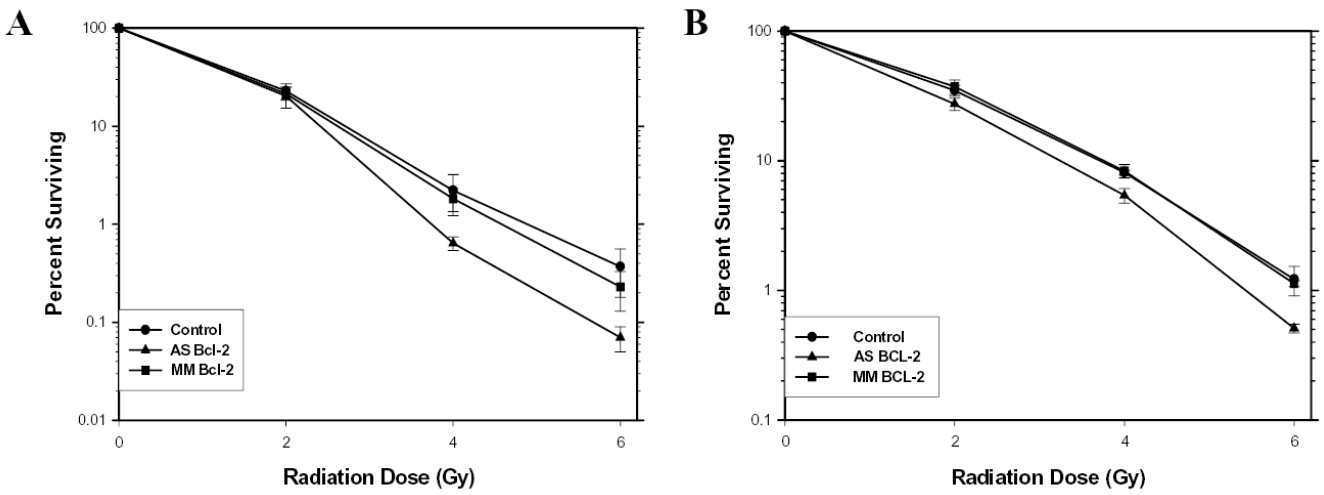

Figure 3.

Clonogenic assays of LNCaP-BST (A) and PC3-BST (B) cells cultured in CM with LC, AS or MM (500 nM) added for 24 hr before RT at 2, 4 or 6 Gy. Values were expressed as a percent surviving (Mean±SEM) from six separate experiments for LNCaP-BST cells and three separate experiments for PC3-BST cells.

Table 1

Effects of AS±RT on LNCaP and LNCaP-BST Cell Caspase-3 + 7 Activity

Treatment	Caspase-3 + 7 activity (RFLUs)			
	LNCaP		LNCaP-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC	91 ± 15	-----	117 ± 14	-----
MM	196 ± 28	.976	193 ± 27	1.000
AS	493 ± 58	<0.0001	593 ± 51	<0.00001
LC+RT	123 ± 14	<0.0001	117 ± 8	<0.00001
MM+RT	257 ± 19	0.308	206 ± 22	1.000
AS+RT**	819 ± 42	<0.0001	773 ± 57	<0.0001

LNCaP and LNCaP-BST cells were treated with AS-Bcl-2 (200 nM) alone or in combination with RT (5 Gy). Caspase-3+7 activity was measured by fluorometric assay.

Abbreviations: RFLUs = relative fluorescence units; LC = lipofectin control; AS = antisense Bcl-2; MM = antisense mismatch; RT = radiation.

* Compared to group above, One way ANOVA, Bonferroni test. The data shown represent the mean values (M±SEM) from seven independent experiments for each cell line.

** Other LNCaP ANOVA comparisons (n = 6 treatment groups): AS + RT versus AS alone ($p < 0.0001$). Other LNCaP-BST comparisons (n = 6 treatment groups): AS + RT versus AS alone ($p = 0.013$).

Table 2

Effects of AS+RT on LNCaP and LNCaP-BST Cell Annexin V Staining

Treatment	% Annexin V positive			
	LNCaP		LNCaP-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC	5.3 ± 0.7	-----	2.6 ± 0.6	-----
MM	14.0 ± 0.5	0.001	12.4 ± 2.2	0.037
AS	23.1 ± 1.9	<0.0001	15.6 ± 2.4	1.000
LC+RT	8.4 ± 0.7	<0.0001	3.7 ± 0.7	0.006
MM+RT	18.0 ± 1.4	<0.0001	13.3 ± 3.0	0.044
AS+RT**	32.2 ± 1.3	<0.0001	17.1 ± 2.2	1.000

LNCaP and LNCaP-BST cells were treated with AS-Bcl-2 (200 nM) alone or in combination with RT (5 Gy). The percentage of early apoptotic cells was measured by flow cytometric analysis of the percentage of Annexin V-PE positive and 7-AAD negative stained cells.

Abbreviations: LC = lipofectin control; AS = antisense Bcl-2; MM = antisense mismatch; RT = radiation.

* Compared to group above, One way ANOVA, Bonferroni test. The data shown represent the mean values (±SEM) from five independent experiments.

** Other LNCaP ANOVA comparisons (n = 6 treatment groups): AS + RT versus AS alone ($p<0.0001$). Other LNCaP-BST comparisons (n = 6 treatment groups): AS + RT versus AS alone ($p=1.000$).

Table 3

Effects of AS+RT on PC3 and PC3-BST Cell Caspase-3 + 7 Activity

Treatment	Caspase-3 + 7 activity (RFLUs)			
	PC3		PC3-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC	75 ± 6	-----	88 ± 16	-----
MM	231 ± 45	0.873	214 ± 35	1.000
AS	435 ± 43	0.221	464 ± 77	0.011
LC+RT	97 ± 7	0.003	115 ± 32	<0.0001
MM+RT	287 ± 58	0.331	330 ± 45	0.046
AS+RT**	781 ± 106	<0.0001	757 ± 58	<0.0001

PC3 and PC3-BST cells were treated with AS-Bcl-2 (200 nM) alone or in combination with RT (5 Gy). Caspase-3+7 activity was measured by fluorometric assay.

Abbreviations: RFLUs = relative fluorescence units; LC = lipofectin control; AS = antisense Bcl-2; MM = antisense mismatch; RT = radiation.

* Compared to group above, one way ANOVA, Bonferroni test. The data shown represent the average values (±SEM) from six independent PC3 experiments and seven independent PC3-BST experiments.

** Other PC3 ANOVA comparisons (n = 6 treatment groups): AS + RT versus AS alone (p=0.002). Other PC3-BST comparisons (n = 6 treatment groups): AS + RT versus AS alone (p =0.002).

Table 4

Effects of AS+RT on PC3 and PC3-BST Cell Annexin V Staining

Treatment	% Annexin V positive			
	PC3		PC3-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC	2.4 ± 0.6	-----	1.2 ± 0.2	-----
MM	10.6 ± 2.3	0.092	7.2 ± 1.8	0.09
AS	20.5 ± 7.9	0.022	12.2 ± 1.9	0.30
LC+RT	3.5 ± 1.1	<0.0001	2.5 ± 0.6	0.001
MM+RT	13.4 ± 0.9	0.022	8.0 ± 0.7	0.182
AS+RT**	19.2 ± 1.6	0.684	14.0 ± 2.1	0.092

PC3 and PC3-BST cells were treated with AS-Bcl-2 (200 nM) alone or in combination with RT (5 Gy) and the percentage of apoptotic cells was measured by flow cytometric analysis of the percentage of Annexin V-PE positive and 7-AAD negative stained cells.

Abbreviations: LC = lipofectin control; AS = antisense Bcl-2; MM = antisense mismatch; RT = radiation therapy.

* Compared to group above, One way ANOVA, Bonferroni test. The data shown represent the average values (±SEM) from five independent experiments.

** Other PC3 ANOVA comparisons (n = 6 treatment groups): AS + RT versus AS alone (p=1.000). Other PC3-BST comparisons (n = 6 treatment groups): AS + RT versus AS alone (p=1.000).

Table 5

Effects of AS±RT on LNCaP and LNCaP-BST Cell Clonogenic Survival

Treatment	Percent Surviving			
	LNCaP		LNCaP-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC + 2 Gy	27.2 ± 4.5	-----	22.9 ± 4.2	-----
AS + 2 Gy	16.5 ± 1.6	0.082	20.2 ± 5.0	1.00
MM + 2 Gy	27.2 ± 2.1	0.081	21.5 ± 3.0	1.00
LC + 4 Gy	4.9 ± 0.63	-----	2.2 ± 1.0	-----
AS + 4 Gy	2.1 ± 0.17	0.001	0.64 ± 0.1	0.307
MM + 4 Gy	4.9 ± 0.28	0.001	1.82 ± 0.48	0.635
LC + 6 Gy	0.70 ± 0.16	-----	0.37 ± 0.19	-----
AS + 6 Gy	0.17 ± 0.05	0.013	0.07 ± 0.02	0.304
MM + 6 Gy	0.67 ± 0.086	0.020	0.23 ± 0.1	1.00

*Compared to group above, One way ANOVA, Bonferroni test (n=3 treatment groups per test). The data shown represent the average values (± SEM) from five independent LNCaP experiments and six independent LNCaP-BST experiments.

Table 6

Effects of AS±RT on PC3 and PC3-BST Cell Clonogenic Survival

Treatment	Percent Surviving			
	PC3		PC3-BST	
	Mean ± SEM	p*	Mean ± SEM	p*
LC + 2 Gy	55.0 ± 6.9	-----	34.8 ± 3.3	-----
AS + 2 Gy	30.6 ± 4.0	0.19	27.4 ± 3.0	0.621
MM + 2 Gy	50.9 ± 10.5	1.00	37.2 ± 4.7	0.335
LC + 4 Gy	17.6 ± 2.0	-----	8.1 ± 0.4	-----
AS + 4 Gy	6.7 ± 0.6	0.002	5.4 ± 0.7	0.112
MM + 4 Gy	14.4 ± 0.3	0.338	8.3 ± 1.0	0.082
LC + 6 Gy	5.8 ± 1.0	-----	1.2 ± 0.3	-----
AS + 6 Gy	0.9 ± 0.1	0.004	0.5 ± 0.04	0.095
MM + 6 Gy	3.9 ± 0.4	0.043	1.1 ± 0.06	0.155

*Compared to group above, One way ANOVA, Bonferroni test (n=3 treatment groups per test). The data shown represent the average values (± SEM) from three independent PC3 experiments and three independent PC3-BST experiments.