Antisense oligonucleotides directed at the *bcl-xl* gene product augment chemotherapy response in mesothelioma

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Abstract

Objective: Malignant pleural mesothelioma (MPM) is resistant to both conventional chemotherapy and apoptosis. The bcl-2 family proteins are major determinants of apoptotic homeostasis. MPM lines and tumors routinely overexpress the anti-apoptotic protein BCL-XL. We have previously shown that antisense inhibition of BCL-XL in MPM cells leads to apoptosis. We sought to determine whether antisense oligonucleotides directed at the *bcl-xl* gene product would augment response to a conventional chemotherapeutic agent in human mesothelioma cell lines. Methods: The human MPM cell lines REN and I-45 were exposed to two *bcl-xl* antisense oligonucleotides (15999, 16009) and one sense oligonucleotide (113529) construct at varying doses, followed by IC_{50} cisplatin. Cellular viability was assessed by a calorimetric assay, and apoptosis was evaluated by Hoechst staining, Annexin V staining, and sub-G_1 fluorescence-activated cell sorter analysis. Western blot analysis of BCL-2 family proteins was performed following single agent and combined treatment. Isobologram mathematical analysis was used to determine whether or not combination therapies were additive or synergistic. Results: Cell viability was most affected with the 15999 antisense oligonucleotides plus IC_{50} cisplatin combination (70% of I-45 and 90% of REN cells killed), and apoptosis was markedly increased with this combination by all measures. Western blot demonstrated 15999 antisense oligonucleotides construct down-regulation of BCL-XL, but no further effect on expression of BCL-2 proteins with cisplatin. Isobologram analysis demonstrated 15999 + cisplatin synergistic effect. Conclusions: Exposure of human MPM cells to *bcl-xl* antisense oligonucleotides sensitizes human mesothelioma cells to the conventional chemotherapeutic agent cisplatin. Similar approaches using a combination of molecular and conventional treatment may have clinical utility for this tumor. [Mol Cancer Ther 2004;3(5):545–50]

Introduction

Malignant pleural mesothelioma (MPM) is a tumor that is known to be clinically resistant to conventional therapies. Virtually every known chemotherapeutic agent has been investigated both as monotherapy and in various combinations, but the clinical response rates have rarely been greater than 20%, with minimal impact on survival (1). Recent reports suggest that surgery and radiation therapy may have a favorable impact on local control, and survival in selected patients (2, 3). However, metastatic and occult multi-cavity disease, once thought distinctly uncommon, is increasingly recognized, and tumor recurrence continues to be a major problem (4, 5).

A number of mechanisms have been proposed for solid and hematological tumor insensitivity to the effects of conventional chemotherapy agents, including multidrug resistance gene expression, membrane pump extrusion of chemotherapeutic agents, tumor hypoxia, decreased tumor angiogenesis, and unfavorable cell cycle kinetics (6–9). Resistance to apoptosis is another mechanism via which tumors may both avoid both programmed senescence and death as well as chemotherapy effects. Although some chemotherapeutic agents do not induce apoptosis directly, this program is often the final common pathway for cells that have been damaged in various ways by cytotoxic drugs. A growing body of literature supports that the development of apoptosis resistance, via any number of mechanisms, can lead to an impaired response to conventional chemotherapeutic agents. Although there are multiple pathways via which a cell can enter apoptosis, it appears that the majority of chemotherapy drugs induce apoptosis via effects on the mitochondrion (cytochrome *c* activation of cellular caspases) rather than cell surface death receptors such as FasL (10). The BCL-2 family of proteins and related phosphorylating enzymes are the major regulators of mitochondrial apoptotic homeostasis. The interplay between phosphorylating events, mediated largely by the phosphatidyl inositol 3 (P13) kinase system, as well as the balance achieved between pro-apoptotic and anti-apoptotic BCL-2 proteins determines whether or not the cell enters or avoids the process of programmed cell death. It has been previously demonstrated that MPM cells routinely overexpress the anti-apoptotic protein BCL-XL (11, 12). Others have demonstrated that MPM cells are relatively resistant to stringent apoptotic stimuli *in vitro*,

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a finding that is not surprising in light of the clinical resistance to treatment that this malignancy exhibits (13). We have postulated that this BCL-XL overexpression may render MPM relatively resistant to apoptosis, and have shown that both pharmacological and antisense oligonucleotide-mediated down-regulation of BCL-XL alone at the transcriptional and translational level in MPM cells leads to apoptotic cell death (11, 14).

In this study, we evaluate whether the reversal of apoptotic dysregulation in human MPM cells via down-regulation of BCL-XL can sensitize them to a conventional chemotherapy agent, cisplatin. We also evaluate whether cellular death engendered by this paradigm is related to changes in apoptosis. Finally, we determine if the combined administration of these agents leads to any interaction or augmentation of effect.

Materials and Methods

Cell Lines and Growth Conditions

To study the effect of *bcl-xl* antisense oligonucleotide and chemotherapy exposure in MPM, the two well-characterized human cell lines I-45 and REN were used. I-45 is a human sarcomatous type MPM and p53 wild type (Dr. J. Testa, Fox Chase Institute, Philadelphia, PA). REN is a human epithelial type MPM and p53 mutant (developed by author W.R.S.) (15). Cell lines were maintained in RPMI containing 10% (vol/vol) fetal bovine serum, 1% nonessential amino acids, and 100 units/ml penicillin and 100 μg/ml of streptomycin. Cells were housed in a humidified incubator with 5% CO_2 at 37°C. At a plating density of 2000 cells/well in a 96-well plate, both cell lines reach confluence at 72 h.

Oligonucleotides and Cisplatin

20 mer 2′-*O*-methoxyethyl (MOE) terminal modified chimeric oligonucleotides were used in these experiments. These oligonucleotides have 2′-*O*-methoxyethyl/phosphorothioate residues which flank on either side a central 2′-deoxynucleotide/phosphorothioate central region that encourages RNase cleavage of RNA duplexes within cells (16). The *bcl-xl* antisense oligonucleotides 15999 (sequence tcccggttgctctgagacat—targeting *bcl-xl* sequence 135–154) and 16009 (sequence ctacgctttccacgcacagt—targeting *bcl-xl* sequence 581–601) as well as 113529 (sense complementary oligonucleotide) were obtained from ISIS Pharmaceuticals, Inc. (Carlsbad, CA) (16). Both antisense and sense oligonucleotides were diluted with PBS before use. Oligofectamine, a cationic lipid (Invitrogen, Inc., Carlsbad, CA), was used as a delivery vehicle. Human MPM cells were treated with various concentrations of antisense oligonucleotides and sense oligonucleotides after a preincubation for 15 min with 0.6 μl oligofectamine in serum-free OPTI-MEM (Invitrogen). Four hours following initiation of incubation, the medium containing antisense oligonucleotides or sense oligonucleotides and oligofectamine was replaced with standard culture medium described above. Cisplatin (Platinol—AQ 1 mg/ml, Bristol-Myers Squibb Laboratories, New York, NY) was placed in solution with 10% RPMI fresh before each use. The inhibitory concentration required to kill 50% of cells (IC_{50}) was determined by a dose-response exposure of human mesothelioma cells and the cell viability (XTT) assay described below.

Cell Viability Assays

Cell viability was tested using the calorimetric XTT assay (Roche Diagnostics Corp., Indianapolis, IN). REN and I-45 were plated at 2000 cells/well in a 96-well plate, and then exposed to (16009, 15999) bcl-X antisense oligonucleotides or (113529) sense oligonucleotides at varying doses from 0 to 200 nM for 24 h once near confluence was noted. Next, IC_{50} cisplatin was administered. The XTT assay evaluated surviving cells at 48 h post-drug dose. The labeling reagent was added to the electron-coupling reagent in a 1:50 ratio. Fifty milliliters of the XTT reagent were then added to each well in a 96-well plate. Plates were than incubated at 37°C for 4 h. The plates were then analyzed using a calorimetric microplate reader at a wavelength of 490 nm (Dynatech Laboratories, Chantilly, VA). Cell viability experiments were performed on three wells per experiment on three separate occasions.

Western Blot Analysis

Mesothelioma cells were first treated with 200 nM (15999) *bcl-xl* antisense oligonucleotides or 113529 sense oligonucleotides and then treated 24 h later with an IC_{50} dose of the drug cisplatin (50 μM for REN, 1 μM for I-45). Forty-eight hours later, cell lysates were prepared by treating plated cell monolayers with SDS-PAGE sample buffer. The protein content of the lysates was then determined by BCA protein assay (Pierce, Rockford, IL). Next, each lane on a SDS-polyacrylamide (12%) gel was loaded with 60 μg of cell lysate and electrophoresed to separate proteins under reducing conditions for the protein of interest. After being electrophoresed at 120 V for 2 h, the proteins were transferred to high bond-ECL membranes (Amersham Corp., Arlington Heights, IL). The membranes were than incubated with the primary and secondary antibodies, and developed according to the Amersham ECL protocol. Actin was used as a control. Antibody to actin (monoclonal) was obtained from Sigma-Aldrich (St. Louis, MO). BCL-XL, BAK, and BAX (polyclonal) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibody to Bcl-2 (monoclonal) was obtained from Dako Corporation (Carpinteria, CA).

Sub-G_1 FACS Analysis for Apoptosis

Following exposure to antisense oligonucleotides or sense oligonucleotides with cisplatin, apoptotic cell death was evaluated by changes in cell cycle flow cytometry. Fluorescence-activated cell sorter (FACS) analysis was performed as follows: cells were first treated with 200 nM (15999) *bcl-xl* antisense oligonucleotides or 113529 sense oligonucleotides and then treated 24 h later with an IC_{50} dose of the drug cisplatin (100 μM for REN, 10 μM for 145). Forty-eight hours later, cells were trypsinized, collected by centrifugation, resuspended in PBS, and fixed in 70% ethanol at −20°C for 1 day. After centrifugation, the cells were washed in PBS and resuspended in potassium iodide (PI) staining solution (PBS, PI, RNase) (Boehringer Mannheim

Co., Indianapolis, IN). Specimens were incubated in the dark for 30 min at 37°C and then analyzed with the use of an EPICS Profile II flow cytometer (Coulter Corp., Hialeah, FL). All experiments were performed in triplicate.

Annexin V Analysis for Apoptosis

Annexin V-FITC staining analysis was performed as follows: the I-45 and REN cell lines were exposed to 200 nM antisense oligonucleotides (15999) or sense oligonucleotides (113529) and 24 h later to an IC_{50} dose of cisplatin (50 μM for REN, 1 μM for I-45). Cells were trypsinized and collected by centrifugation 24 h following cisplatin exposure and resuspended in PBS. The cells were then washed in PBS and resuspended in 1× binding buffer (BD Biosciences PharMingen Co., San Diego, CA) at a concentration of 1×10^6 cells/ml. One hundred microliters of the solution were then transferred (1×10^5 cells) to a 5-ml culture tube and 5 μl of Annexin V-FITC (BD Biosciences PharMingen) were added, as well as 5 μl of PI solution (BD Biosciences PharMingen). The solution was then incubated for 15 min at room temperature in the dark, and 400 μl 1× binding buffer were added. In 1 h, the specimens were analyzed with the use of an EPICS Profile II flow cytometer (Coulter). Experiments were performed in triplicate.

Statistical Analysis

Continuous variables were compared using Student's *t* test. To determine the therapeutic relationship between *bcl-xl* antisense oligonucleotides and cisplatin, two-dimensional isobolograms were constructed using averages from three independent XTT assays for each combination of therapies in both cell lines. Three isoeffect curves (modes I, IIa, and IIb) were created according to the method described previously by Steel and Peckham (17). The area enclosed by all three lines represent the ''envelope of additivity.'' Experimental data points falling to the left of the envelope signify synergy. Those data points falling to the right of the envelope signify a sub-additive relationship.

Results

The Combination of *bcl-xl* Antisense Oligonucleotide and Cisplatin Exposure Effectively Kills Human Mesothelioma Cells

Cellular viability was assayed following bcl-xl antisense oligonucleotides administration at varying doses followed by IC50 cisplatin exposure 24 h later. The IC50 was found to be approximately 1 mM for the I-45 cell line and 50 mM for the REN cell line. The cell viability curves following mono- and combined therapy are presented in [Fig. 1, A and B]. The 15999 antisense oligonucleotides construct was demonstrated to more effectively kill both human mesothelioma cell types than the other therapeutic antisense oligonucleotides construct 16009 as well as the control sense oligonucleotides 113529. The combination of the 15999 antisense oligonucleotides construct and cisplatin led to a more significant reduction in cellular viability than other combinations ($P < 0.05$). At the 200-nM dose of 15999 and IC_{50} cisplatin, approximately 70% of I-45 (Fig. 1A) and 90% of REN (Fig. 1B) cells were killed.

A Significant Increase in Apoptosis Is Noted in Cells Treated with a Combination of *bcl-xl* Antisense Oligonucleotide and Cisplatin Exposure

To determine whether or not the primary cause for a decrease in cellular viability noted in the 15999 *bcl-xl* antisense oligonucleotides plus cisplatin group might be related to an increase in apoptosis, Annexin V analysis and sub-G_1 FACS analyses of apoptosis were performed. Sub-G_1 FACS analysis demonstrates a significant increase in cells exhibiting apoptosis in the 15999 antisense oligonucleotides plus cisplatin group (REN—36.9 ± 9.7%,

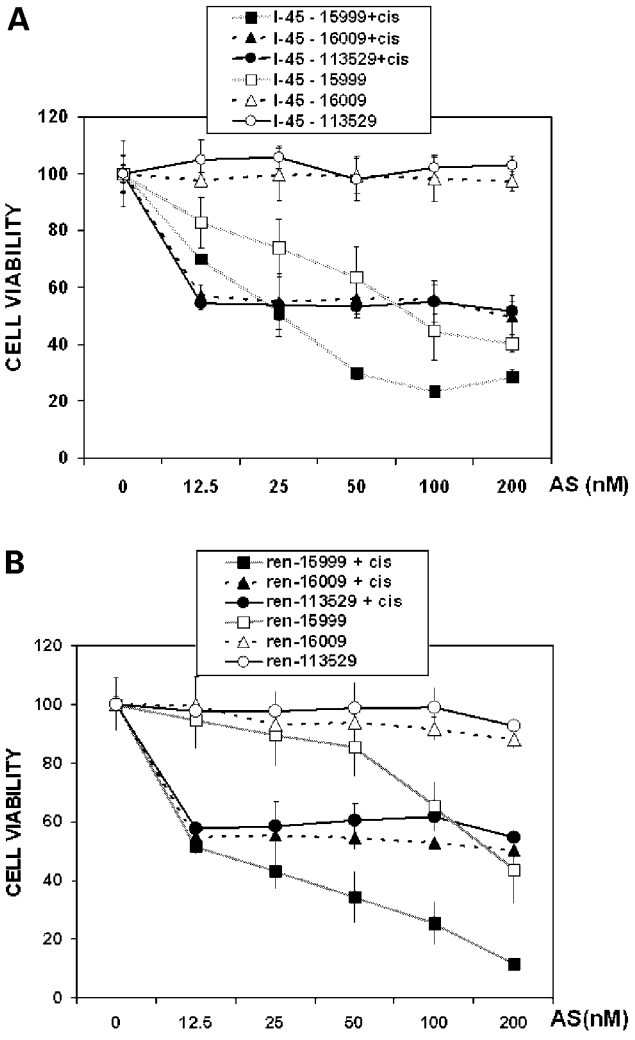

Figure 1. A, XTT calorimetric cell viability assay for the I-45 mesothelioma cell line treated with either antisense (*16009*, *15999*) or sense (*113529*) *bcl-xl* oligonucleotide constructs followed by an IC_{50} dose of cisplatin (1 μM), or oligonucleotide constructs alone. The combination of 15999 plus IC_{50} cisplatin is most effective ($P = 0.008$, 15999 *versus* 15999 plus cisplatin). **B,** XTT calorimetric cell viability assay for the REN mesothelioma cell line treated with either antisense (*16009*, *15999*) or sense (*113529*) *bcl-xl* oligonucleotide constructs followed by an IC_{50} dose of cisplatin (50 μM), or oligonucleotide constructs alone. The combination of 15999 plus IC_{50} cisplatin is most effective ($P = 0.003$, 15999 *versus* 15999 plus cisplatin).

I-45—35.8 ± 8.7%), while combination 16009 antisense oligonucleotides and 113529 sense oligonucleotides plus cisplatin do not differ greatly from cisplatin treatment alone (Fig. 2, A and B, $P < 0.05$).

Annexin V staining demonstrated a significant increase in apoptotic early membrane changes in the cells treated with cisplatin and 15999 compared to 16009 or the control sense oligonucleotides 113529 (Fig. 3).

Treatment with Cisplatin Does Not Further Alter BCL-2 Family Protein Expression following Down-Regulation of BCL-XL with Antisense Oligonucleotides

We have previously demonstrated that *bcl-xl* antisense oligonucleotides can effectively down-regulate *bcl-xl* expression in mesothelioma cells, but used different oligonucleotide constructs (14). Figure 4 demonstrates BCL-XL protein down-regulation by Western blot analysis in both human mesothelioma cell lines with the 15999 antisense oligonucleotides construct. No further down-regulation of BCL-XL is noted with cisplatin administration, nor up-regulation of pro-apoptotic proteins BAK and BAX. Interestingly, BCL-2 protein expression is normally low to absent in untreated mesothelioma cells; however, following BCL-XL down-regulation with 15999, BCL-2 is markedly up-regulated by Western blot. This suggests an apoptosis ''defense mechanism'' in tumor cells as one anti-apoptotic protein is up-regulated following down-regulation of another. This change was not noted following administration of 16009 antisense oligonucleotides with less impressive concomitant BCL-XL down-regulation.

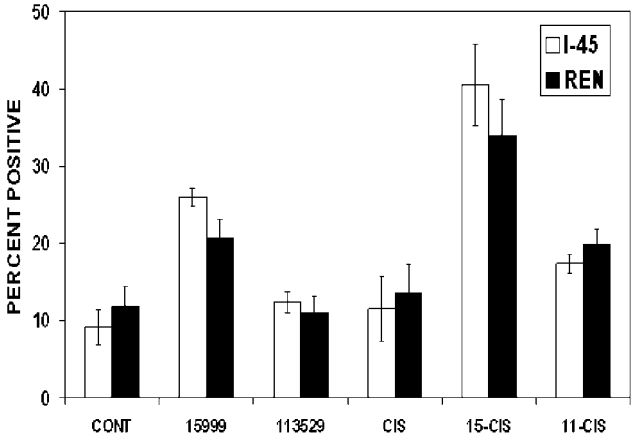

Figure 3. Annexin V staining assay for apoptotic membrane changes following exposure of human mesothelioma cells to antisense oligonucleotides and cisplatin. The most significant increase in Annexin V staining is noted in the antisense oligonucleotides (*15999*) plus cisplatin group for both the I-45 and REN cell lines (*Percent Positive,* percentage of Annexin V-positive cells).

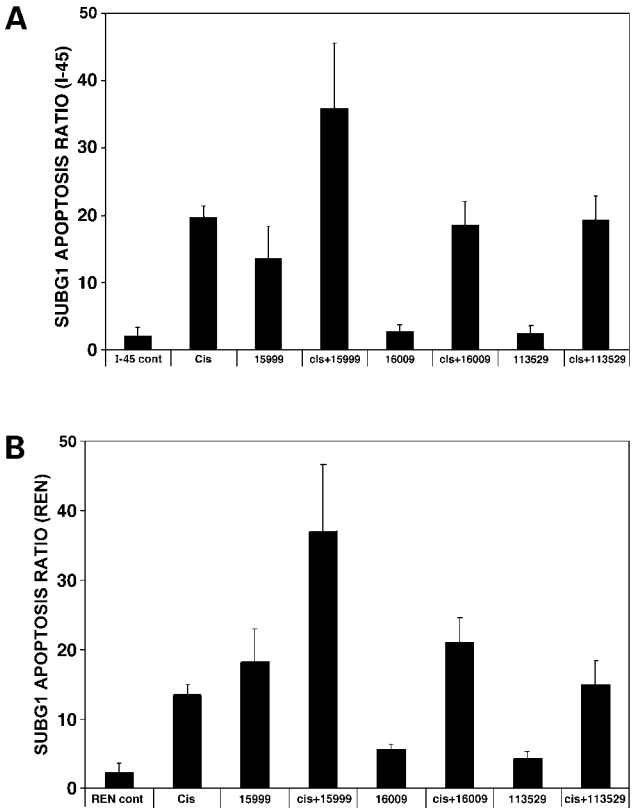

Figure 2. **A,** sub-G_1 FACS apoptosis assay for I-45 mesothelioma cells treated with either antisense (*16009*, *15999*) or sense (*113529*) *bcl-xl* oligonucleotide constructs followed by an IC_{50} dose of cisplatin (1 μM). Numerical FACS data are presented as a *bar graph*. The combination of 15999 + cisplatin led to the greatest increase in sub-G_1 fraction, or apoptosis (*SubG1 Apoptosis Ratio,* percentage of sub-G_1 population *versus* total population; *error bars,* SE). **B,** sub-G_1 FACS apoptosis assay for REN mesothelioma cells treated with either antisense (*16009*, *15999*) or sense (*113529*) *bcl-xl* oligonucleotide constructs followed by an IC_{50} dose of cisplatin (50 μM). Numerical FACS data are presented as a *bar graph*. The combination of 15999 + cisplatin led to the greatest increase in sub-G_1 fraction, or apoptosis (*SubG1 Apoptosis Ratio,* percentage of sub-G_1 population *versus* total population; *error bars,* SE).

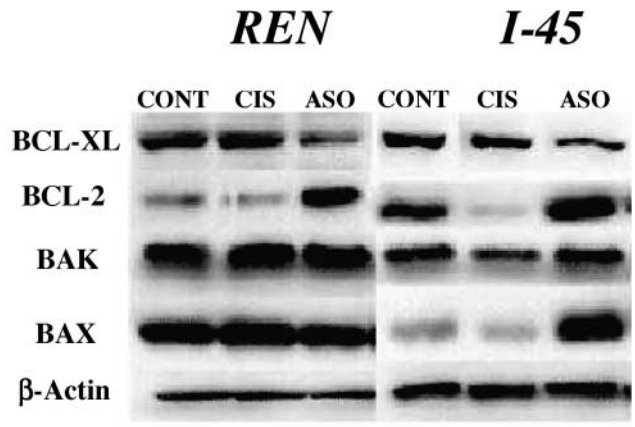

Figure 4. Western blot analysis of *bcl-2* family protein expression in human mesothelioma cell lines following either IC_{50} cisplatin or *bcl-xl* antisense oligonucleotides exposure (*CONT,* control, no treatment; *CIS,* IC_{50} cisplatin exposure; *ASO,* 15999 *bcl-xl* antisense oligonucleotide exposure). 15999 exposure led to down-regulation of BCL-XL protein, and cisplatin had no effect alone on expression of BAR, BAX, BCL-2, or BCL-XL. An increase in BCL-2 expression is consistently noted following *bcl-xl* antisense oligonucleotides 15999 exposure.

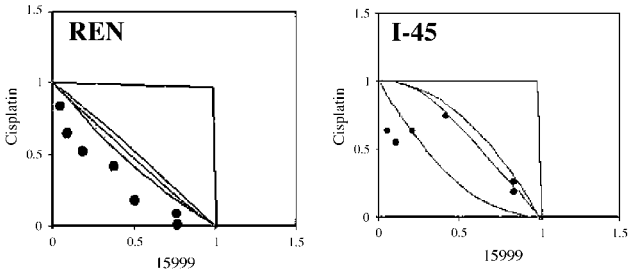

Figure 5. Isobologram analysis of human mesothelioma cell lines treated with the *bcl-xl* antisense oligonucleotides 15999 followed by cisplatin in a dose-response fashion for both. Primarily synergistic effect is noted with this combination treatment.

Isobologram Analysis Demonstrates Synergism of Mesothelioma Cellular Toxicity following Combination *bcl-xl* Antisense Oligonucleotides and Cisplatin Treatment

Isobologram analysis assists in the determination of whether or not combination therapies are additive, synergistic, or sub-additive (antagonistic). Points that fall within the isobologram curves are additive, those below indicate synergism, and points falling above the curves indicate a sub-additive, or antagonistic relationship (17). The two isobolograms presented in Fig. 5 demonstrate that for all doses examined, the 15999 antisense oligonucleotides and cisplatin combination was synergistic in the REN cell line, and synergistic to additive in the I-45 cell line.

Conclusions

We and others have previously demonstrated that *bcl-xl* is overexpressed in mesothelioma cell lines and tumors, and that apoptosis can be engendered in mesothelioma by down-regulation of the expression of the BCL-XL protein (11, 14). In this report, we demonstrate that down-regulation of the *bcl-xl* gene product using antisense oligonucleotides can sensitize human MPM cells to the effects of a conventional chemotherapy agent, cisplatin.

A relationship between *bcl-xl* expression and chemotherapy sensitivity has been noted in other tumor types. Investigators have noted a relationship between *bcl-xl* expression and chemotherapy sensitivity. U-937 cells were stably transfected to overexpress *bcl-xl*, and apoptosis was decreased following exposure to vinblastine, paclitaxel, and *cis*-platinum by more than 50% for all combinations (18). In a system designed to provide lymphoma cells with microenvironment survival adjuvants (CD40 antibody, IL4, VCAM-1 fusion protein) followed by etoposide, the most consistent change which correlated with chemotherapy resistance was BCL-XL expression up-regulation (19). Similarly, Luo *et al*. (20) demonstrated that hepatoblastoma cells were rendered more sensitive to taxol and doxorubicin by stable transfection of a *bcl-xl* antisense plasmid construct. When colonic adenocarcinoma cells noted to be deficient in *bcl-2* but overexpress *bcl-xl* were treated with *bcl-xl* antisense oligonucleotides followed by 5-fluorouracil, they exhibited a 40% decrease in cell viability and a 55% increase in apoptosis compared to 5-fluorouracil alone (21). Finally, stable *bcl-xl* overexpression in a murine tumor cell line (FK5.12) has been shown to engender a form of multidrug resistance phenotype, as these cells are dramatically more resistant than parental cells to a panel of several chemotherapeutic agents (bleomycin, vincristine, cisplatinum, etoposide) and metabolic poisons (hygromycin B, mycophenolate) (22). One group has recently demonstrated that down-regulation of *bcl-xl* with antisense oligonucleotides can sensitize bladder carcinoma cells to a number of cytotoxic chemotherapeutic agents, and that further overexpression of *bcl-xl* can abrogate this effect (23). Finally, *bcl-xl* antisense oligonucleotides have been shown to effectively facilitate VP-16 chemotherapy-induced tumor cell death in a mouse solid tumor model of leukemia (24).

A large number of clinical trials have evaluated the efficacy of conventional platinum-based chemotherapy for MPM. The overall results have been relatively disappointing, with response rates between 5% and 14% (1). The newer agents carboplatin and oxaliplatin have not demonstrated improvement over cisplatin-based regimens (25, 26). It has been suggested that as DNA adduct formation is the initial event in cisplatin toxicity, that in cells with defective or aberrant apoptotic machinery, a higher level of DNA damage would be necessary to engender cell death. In support of these theories, it has been shown that cisplatin-induced apoptosis in ovarian carcinoma cells is related directly to the levels of pro-apoptotic BAX and BAK proteins (less expression = less apoptosis), which counter the effects of anti-apoptotic proteins such as BCL-XL (27). It is logical to assume that overexpression of an anti-apoptotic *bcl-2* family protein (*i.e.*, BCL-XL) could have the same effect as decreased levels of pro-apoptotic proteins with regard to cisplatin sensitivity.

The suggestion that down-regulation of expression of *bcl-xl* might render cells more sensitive to conventional chemotherapeutic agents such as cisplatin is supported by the fact that (*a*) the *bcl-2* family of proteins is the major regulator of mitochondrial permeability and mitochondrial pathway apoptosis, and (*b*) the mitochondrial pathway, rather than the membrane death receptor pathway, is primarily responsible for chemotherapy-induced apoptotic cancer cell death (10). It has been demonstrated that Fas receptor and Fas ligand expression can be up-regulated by chemotherapeutic drug exposure, including cisplatin (28). However, other reports suggest that the concentrations of chemotherapeutic agents required are unusually high, and that cancer cells are killed at lower chemotherapy doses that do not affect the Fas/FasL system (29). More convincingly, multiple reports suggest that direct blockade of the Fas/FasL system (soluble Fas, anti-FasL antibodies) as well as interference with its downstream effectors (overexpression of FLIP or dominant-negative FADD) do not abrogate chemotherapy-induced apoptotic cancer cell death (10).

What about the potential clinical efficacy of systemic administration of *bcl-2* family member antisense oligonucleotides in MPM, perhaps as a means to sensitize relatively non-responsive tumors to conventional chemotherapeutic agents such as cisplatin? Two recent clinical trials have been reported using treatment with *bcl-2* family

antisense oligonucleotides in patients with refractory lymphoma and melanoma as early ''proof of principle'' of the use of antisense targeting of anti-apoptotic proteins in solid tumors. A dose-escalation protocol using *bcl-2* antisense oligonucleotides in addition to dacarbazine in patients with advanced melanoma who had previously failed chemotherapy alone was recently reported (30). All patients expressed BCL-2 at baseline, and received i.v., and in some cases, s.c. oligonucleotides. Toxicity was minimal (lymphopenia, fever, and transient liver function abnormalities). Six patients had a tumor response (one complete), and all patients were surviving at median follow-up of 12 months. BCL-2 down-regulation was noted in tumor biopsies in 10 of 12 patients achieving adequate serum levels of antisense, and apoptosis was increased in tumor specimens as well. In a similar Phase I study, 21 patients with BCL-2-positive expressing relapsed non-Hodgkin's lymphoma were treated with *bcl-2* antisense alone. Following treatment, there were three objective tumor responses and nine patients with stable disease.

The future results of genomic, proteomic, and other discrete molecular pathway research will provide us with a number of targets on which to base biological therapies for malignancy in the future. However, these tumor-specific biological targets may also allow for the administration of treatments that can significantly augment our older, established drugs, rendering them more effective and less toxic. Means by which the dysregulation of apoptosis in MPM can be reversed and capitalized on, perhaps related to BCL-XL overexpression, warrants further basic and clinical investigation.

References

1. Baas P. Chemotherapy for malignant mesothelioma: from doxorubicin to vinorelbine. Semin Oncol 2002;29(1):62 – 9.

2. Sugarbaker DJ, Flores RM, Jaklitsch MT, et al. Resection margins, extrapleural nodal status, and cell type determine postoperative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. J Thorac Cardiovasc Surg 1999;117(1):54 – 63.

3. Rusch VW, Rosenzweig K, Venkatraman E, et al. A phase II trial of surgical resection and adjuvant high-dose hemithoracic radiation for malignant pleural mesothelioma. J Thorac Cardiovasc Surg 2001;122(4): 788 – 95.

4. Baldini EH, Recht A, Strauss GM, et al. Patterns of failure after trimodality therapy for malignant pleural mesothelioma. Ann Thorac Surg 1997;63(2):334 – 8.

5. Kawai A, Nagasaka Y, Muraki M, et al. Brain metastasis in malignant pleural mesothelioma. Intern Med 1997;36(8):591 – 4.

6. Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000; 92(16):1295 – 302.

7. Mattem J. Role of angiogenesis in drug resistance. Anticancer Res 2001;21(6B):4265 – 70.

8. Vaupel P, Kelleher DK, Hockel M. Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin Oncol 2001;28(2 Suppl 8):29 – 35.

9. Shah MA, Schwartz GK. Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. Clin Cancer Res 2001;7(8): 2168 – 81.

10. Kaufmann SH, Eamshaw WC. Induction of apoptosis by cancer chemotherapy. Exp Cell Res 2000;256(1):42 – 9.

11. Cao XX, Mohiuddin I, Ece F, McConkey DJ, Smythe WR. Histone deacetylase inhibitor down-regulation of bcl-xl gene expression leads to apoptotic cell death in mesothelioma. Am J Respir Cell Mol Biol 2001;25(5):562 – 8.

12. Soini Y, Kimrula V, Kaarteenaho-Wiik R, Kurttila E, Linnainmaa K, Paakko P. Apoptosis and expression of apoptosis regulating proteins bcl-2, mcl-1, bcl-X, and bax in malignant mesothelioma. Clin Cancer Res 1999;5(11):3508 – 15.

13. Narasimhan SR, Yang L, Gerwin BI, Broaddus VC. Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and Bax. Am J Physiol 1998;275(1 Pt 1):L165 – 71.

14. Smythe WR, Mohuiddin I, Ozveran M, Cao XX. Antisense therapy for malignant mesothelioma with oligonucleotides targeting the bcl-xl gene product. J Thorac Cardiovasc Surg 2002;123(6):1191 – 98.

15. Smythe WR, Hwang HC, Amin KM, et al. Use of recombinant adenovirus to transfer the herpes simplex virus thymidine kinase (HSVtk) gene to thoracic neoplasms: an effective *in vitro* drug sensitization system. Cancer Res 1994;54(8):2055 – 9.

16. Taylor JK, Zhang QQ, Monia BP, Marcusson EG, Dean NM. Inhibition of Bcl-xL expression sensitizes normal human keratinocytes and epithelial cells to apoptotic stimuli. Oncogene 1999;18(31):4495 – 504.

17. Steel GG, Peckham MJ. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys 1979;5:85 – 91.

18. Schmitt E, Sane AT, Steyaert A, Cimoli G, Bertrand R. The Bcl-xL and Bax-α control points: modulation of apoptosis induced by cancer chemotherapy and relation to TPCK-sensitive protease and caspase activation. Biochem Cell Biol 1997;75(4):301 – 4.

19. Taylor ST, Hickman JA, Dive C. Epigenetic determinants of resistance to etoposide regulation of Bcl-X(L) and Bax by tumor microenvironmental factors. J Natl Cancer Inst 2000;92(1):18 – 23.

20. Luo D, Cheng SC, Xie H, Xie Y. Effects of Bcl-2 and Bcl-XL protein levels on chemoresistance of hepatoblastoma HepG2 cell line. Biochem Cell Biol 2000;78(2):119 – 26.

21. Nita ME, Ono-Nita SK, Tsuno N, et al. Bcl-X(L) antisense sensitizes human colon cancer cell line to 5-fluorouracil. Jpn J Cancer Res 2000; 91(8):825 – 32.

22. Minn AJ, Rudin CM, Boise LH, Thompson CB. Expression of bcl-xL can confer a multidrug resistance phenotype. Blood 1995;86(5): 1903 – 10.

23. Lebedeva I, Raffo A, Rando R, Ojwang J, Cossum P, Stein CA. Chemosensitization of bladder carcinoma cells by bcl-xL antisense oligonucleotides. J Urol 2001;166(2):461 – 9.

24. Fennell DA, Corbo MV, Dean NM, Monia BP, Cotter FE. *In vivo* suppression of Bcl-XL expression facilitates chemotherapy-induced leukaemia cell death in a SCID/NOD-Hu model. Br J Haematol 2001; 112(3):706 – 13.

25. Raghavan D, Gianoutsos P, Bishop J, et al. Phase II trial of carboplatin in the management of malignant mesothelioma. J Clin Oncol 1990;8(1): 151 – 4.

26. Steele JP, John W, Vogelzang NJ. Phase II trial of vinorelbine and oxaliplatin in malignant pleural mesothelioma [Abstract]. Proc Am Soc Clin Oncol 1999;18:1844A.

27. Jones NA, Turner J, Mcilwrath AJ, Brown R, Dive C. Cisplatin- and paclitaxel-induced apoptosis of ovarian carcinoma cells and the relationship between bax and bak up-regulation and the functional status of p53. Mol Pharmacol 1998;53(5):819 – 26.

28. Fulda S, Sieverts H, Friesen C, Herr I, Debatin KM. The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. Cancer Res 1997;57(17):3823 – 9.

29. Adjei AA, Charron M, Rowinsky EK, et al. Effect of pyrazoloacridine (NSC 366140) on DNA topoisomerases I and II. Clin Cancer Res 1998; 4(3):683 – 91.

30. Jansen B, Wacheck V, Heere-Ress E, et al. Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet 2000; 356(9243):1728 – 33.