Induction of Apoptosis in Mesothelioma Cells by Antisurvivin Oligonucleotides

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Abstract
Malignant pleural mesothelioma is a rare and aggressive tumor characterized by rapid progression, late metastases, and poor prognosis. In this study, we investigated the expression of survivin, a member of the inhibitors of apoptosis protein gene family, in mesothelioma and an antisense oligonucleotide-based gene therapy for mesothelioma using survivin as a target. Initially, we documented the expression of survivin in human mesothelioma cell lines and fresh tissues using reverse transcription-PCR and Western blot analysis. Our results showed that survivin was overexpressed in 7 of 8 (87.5%) mesothelioma cell lines assayed and in all (12 of 12; 100%) freshly resected mesothelioma tissues analyzed. To investigate the use of survivin as a therapeutic target on mesothelioma, we carried out transfections with antisurvivin oligonucleotides to induce apoptosis in mesothelioma cell lines MS-1 and H28. Results from cellular transfection and subsequent analysis using the flow cytometry demonstrated that antisurvivin oligonucleotides induced significantly greater apoptosis rates in the survivin-positive mesothelioma cell line H28 (42.5%) as compared with the control oligonucleotides (16.2%; P < 0.001). The survivin-negative cell line LRK1A (survivin−/−) did not apoptose with antisense oligonucleotides. Furthermore, time course evaluation by Western blot analysis showed that survivin was inhibited by antisurvivin oligonucleotides within 12 h after transfection. Our results show, for the first time, that survivin, an inhibitors of apoptosis protein family gene member, is highly overexpressed in malignant pleural mesothelioma. Down-regulation of survivin by a targeted antisense oligonucleotide appears to be an effective gene therapy approach to the treatment of mesothelioma.

Introduction
Apoptosis is the carefully regulated process of programmed cell death that is critical for maintaining normal cell and tissue homeostasis. Dysregulation of cell death pathways often results in tumor initiation, progression, and drug resistance in many human cancers. Survivin, a member of the IAP2 gene family, is of interest because it is specifically up-regulated in cancer cells and completely down-regulated and undetectable in normal adult tissues (1). Although its exact mechanism of action remains unclear, survivin has been implicated in the control of cell division and apoptotic cell death (2, 3). Recently, studies using a transgenic mouse model that selectively expresses survivin in the skin confirmed that survivin selectively inhibits the intrinsic, caspase-9-dependent apoptotic pathway (4).

The high prevalence of survivin in many human cancers has prompted studies using survivin as a therapeutic target in the treatment of cancer and as a prognostic marker for cancer. Previous studies have shown that reduction of survivin expression achieved by antisense strategies can cause apoptotic cell death and sensitization to anticancer drugs in several tumor cell lines (5). These results suggest that survivin expression is likely important for cell survival or resistance to chemotherapy in carcinomas.

Different approaches have been described for blocking survivin expression in tumor cells. Generation of survivin-specific CD8+ T effector cells pulsed with survivin peptides was reported to be effective in suppressing survivin expression (6). A survivin antisense cDNA was shown to down-regulate survivin action (5, 7, 8). Antisense oligonucleotides of 17-mer to 20-mer were shown to effectively down-regulate survivin expression (7, 9, 10) and to sensitize tumor cells to cytotoxic chemotherapy (10). Moreover, infection of cancer cell lines with a replication-deficient adenovirus encoding a survivin mutant (pAdT34A) resulted in a 2–3-fold increase in apoptosis and further enhanced the levels of apoptotic cell death in combination with chemotherapeutic drugs (11).

Malignant pleural mesothelioma is a rare (<4000 United States cases/year) and inexorably fatal tumor characterized by rapid local progression, late metastases, and poor prognosis. Standard cytotoxic chemotherapy and radiation therapy have had limited effectiveness (12, 13), although multidisciplinary therapy (surgery, radiation, and chemotherapy) may increase short-term survival. Molecular genetic changes in mesothelioma development and progression have been reviewed (14). Mutations in the p53, Ras, and pRB genes, which are found in the majority of human tumors, are uncommon in mesothelioma. Instead, homozygous deletion of the INK4a/ARF locus is detected in the majority of mesothelioma (15, 16). This deletion results in the loss of p14ARF, an increase in MDM2, and the functional inactivation of p53.

In our previous studies, we have showed that reintroduction of survivin expression can cause apoptosis of mesothelioma cells and sensitization to chemotherapy. Additionally, we have demonstrated that survivin expression inhibition can sensitize mesothelioma cells to various chemotherapeutic agents (17, 18). These results suggest that survivin expression is a potential target for the treatment of mesothelioma.

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2 The abbreviations used are: IAP, inhibitors of apoptosis protein; RT-PCR, reverse transcription-PCR.
p14ARF into mesothelioma cells leads to the overexpression of p14ARF, which results in G1-phase arrest and apoptotic cell death (16). Recently, using cDNA microarray hybridization, we have detected a number of IAPs, including Bcl-2 and survivin, that are overexpressed in primary freshly resected human mesothelioma. This has led us to analyze survivin expression and function in mesothelioma. In light of up-regulation in other human cancers, survivin may also play an important role in preventing apoptosis and cell proliferation in mesothelioma. In the present study, we examined survivin expression in multiple mesothelioma cell lines and freshly resected mesotheliomas. We also investigated the efficacy of antisurvivin oligonucleotides in inducing apoptosis in mesothelioma cell lines in which survivin was up-regulated.

Materials and Methods

Tumor Tissues and Cell Lines. Freshly resected mesothelioma tumor samples were obtained with consent from patients undergoing resection and snap-frozen in liquid nitrogen tank. Mesothelioma cell lines, including H28, H290, MSTO-211H (211H), MS-1, LRK1A, H513, Met5A, and H2052, breast carcinoma cell line MCF7, lung adenocarcinoma cell line A549, and glioma cell lines U87 and CF210 were obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI 1640 or MEM complete medium supplemented with 2 mM L-glutamine and 10% FCS at 37°C.

Materials and Methods

RNA Isolation. Total RNA was isolated from cell cultures and mesotheliomas using Trizol reagents according to the manufacturer’s instructions (Life Technologies, Inc.). Human normal lung total RNA was obtained from Ambion Inc.

cDNA Microarray Hybridization. Incorporation of aminomethyl-dUTP into cDNA was conducted with the FairPlay Microarray Labeling kit (Stratagene, La Jolla, CA) using total RNA (10 μg each) from mesothelioma and normal pleura. Fluorescent dyes (Amersham Pharmacia Biotech) were coupled to aminomethyl-dUTP-labeled tumor (Cy3) and normal pleural cDNAs (Cy5), respectively, and cohybridized to the cDNA microarray slide (HPLower9k.7) according to DeRisi et al. Slides were scanned with the GenePix 4000A scanner (Axon), and acquired images were analyzed with the software GenePix Pro3.0 and Microsoft Excel.

RT-PCR. RT-PCR was performed in a GeneAmp PCR System 9700 using a one-step RT-PCR kit (Life Technologies, Inc.) according to the manufacturer’s instructions. Briefly, 1 μg of total RNA was used as template that was mixed with reaction buffer, 10 pmol of sense and antisense survivin gene primers, and 1 μl of reverse transcriptase/platinum Taq mixture in a PCR tube, respectively, in a 50-μl volume. The cDNA synthesis and preadnatanation were performed as follows: 1 cycle of 50°C for 30 min and 94°C for 2 min. PCR amplification was continued for 30–35 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 1 min. A final extension was performed for 7 min at 72°C. RT-PCR primers were ordered from Life Technologies, Inc. The primer sequences for a 436-bp fragment of survivin were as follows: survivin-S, 5′-ATGGGTGCCGCAGGTG-3′; and survivin-A, 5′-AGAGGCTCAAATCGATG-3′. A 395-bp fragment of the L19 ribosomal protein gene was used as an internal control (L19-S, 5′-GAATCGCAATTGCAACT-3′; L19-A, 5′-TCTTGAACCTGCCAGGCTCA-3′).

Western Blotting. After medium was removed, cells were rinsed once with PBS solution at room temperature. After that, all of the following steps were performed on ice. Appropriate amounts of cold radioimmunoprecipitation assay buffer containing proteinase inhibitors were added to the cell culture plate. Cells were removed from the plate and transferred to a 1.5-ml microcentrifuge tube. The cell lysate was passed through a 21-gauge needle to shear the DNA. After centrifugation at 10,000 × g for 10 min, protein concentration was measured using Bio-Rad Protein Assay reagent. Whole cell lysate protein (30 μg) was boiled for 5 min and separated by 10–20% SDS-PAGE. Proteins were transferred to an Immobilon-P membrane (Millipore Corp., Bedford, MA) using semi-dry transfer cell (Bio-Rad). The membrane was blocked with 5% nonfat milk powder and 0.1% Tween 20 in Tris-buffer saline overnight at 4°C and then incubated with primary antibody for 1 h at room temperature. Membrane was washed in 5% nonfat milk powder and 0.1% Tween 20 in Tris-buffer saline for three 10-min periods. Primary antibodies for survivin and actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated goat antirabbit or donkey antigoat antibodies were used as secondary antibodies. Proteins were visualized with chemiluminescence luminol reagents (Santa Cruz Biotechnology).

Antisurvivin and Control Oligonucleotides. Antisurvivin 20-mer phosphorothioate antisense oligonucleotide targeting nucleotides 232–251 of survivin mRNA was used based on the oligonucleotide 4003 sequence (10). The control oligonucleotide was the reverse of the antisense sequence. The antisurvivin oligonucleotide sequence was 5′-CCCAGCCTTCCAGCTCTTG-3′, and the control primer was 5′-GGTCCTCGACCTTCCGACCC-3′. The primers were ordered from Oligos Etc Inc. (Wilsonville, OR) using the second-generation processing procedure and purified by level 1 high-performance liquid chromatography.

Treatment of Cells with Antisense and Control Oligonucleotides. Two survivin-positive mesothelioma cell lines, H28 and MS-1, and one survivin-negative cell line, LRK1A, were used for transfection with oligonucleotides. One day before transfection, 2 × 105 cells/well were plated in 6-well tissue culture plates. Cells were rinsed with 2 ml of Opti-MEM I medium before transfection. Oligonucleotides were delivered in the form of complexes with Lipofectin (Life Technologies, Inc.) as follows: 20 μl of Lipofectin were mixed with oligonucleotides (100–600 nM) in 2 ml of Opti-MEM I reduced serum medium and added to pretrained cells. After culturing for 5 h, the oligonucleotide-Lipofectin mixture was replaced with 2 ml of RPMI 1640, and cells were cultured for an additional 19 h or longer.
Dose-dependent Analysis. To evaluate the effects of oligonucleotides or Lipofectin on cells after transfection, we assessed cell viability using an inverted phase-contrast microscope (Leica) and trypan blue exclusion assays. Cells were treated with different concentrations of oligonucleotides (100–600 nm) in 6-well cell culture plates for 5 h. After the medium was changed, cells were continued in culture to 12–36 h. Cells were harvested after trypsinization and resuspended in PBS. An equal volume of 0.4% trypan blue solution (Sigma, St. Louis, MO) was added to the cell suspension. Viable and dead cells were counted with a hemocytometer. All cell counts were done on triplicate samples.

Caspase-3 Activity Assay. The caspase-3 colorimetric activity assay kit was used for measurement of caspase activation essentially according to the manufacturer’s instructions (Chemicon International, Inc., Temecula, CA). Briefly, approximately 1 × 10^6 cells/10-cm dish were treated with antisurvivin oligonucleotides and controls. Cells were harvested at 26 h after the start of treatment and lysed in 200 μl of cell lysis buffer. After incubation for 5 min on ice, lysates were centrifuged at 10,000 g at 4°C for 5 min. Cytosolic protein (200 μg) was mixed with 30 μg of caspase-3 substrate (ac-DEVD-pNA) and incubated at 37°C for 4–5 h. The reaction was monitored at 405 nm using a SPECTRAmax microplate reader and analyzed using Softmax PRO software (Molecular Devices). Human caspase-3 (active) recombinant protein was used as a positive control, and a caspase-3-specific inhibitor (ac-DEVD-CHO) was used as a negative control according to the instructions provided. Fold increase in caspase-3 activity was determined by comparing the absorbance readings from the induced samples with those of untreated controls.

Annexin V Apoptosis Analysis. Approximately 1 × 10^6 H28 cells were plated in 10-cm dishes and incubated overnight (16 h) at 37°C. Cells were rinsed with Opti-MEM I medium, transfected with 500 nm antisurvivin or control oligonucleotide-Lipofectin complex in 5 ml of serum reduced Opti-MEM I medium for 5 h, and continued to grow for 19 h after changing the medium. H28 cells without oligonucleotide treatment were used as mock control. Cells were harvested after trypsinization, and apoptotic cells were assayed with an annexin V-FITC apoptosis detection kit (Oncogene, Cambridge, MA). Briefly, 5 × 10^5 cells in 0.5 ml of PBS were incubated with 10 μl of medium-binding reagent and 1.25 μl of annexin V-FITC for 30 min at room temperature in the dark. After centrifugation at 1000 × g for 5 min, the medium was removed, and cells were gently suspended in 0.5 ml of ice-cold 1× binding buffer, and then 10 μl of propidium iodide were added immediately before the flow cytometry analysis (FACScan; Becton Dickinson, Franklin Lake, NJ). Early apoptotic cells with exposed phosphatidylserine but intact cell membranes bound to annexin V-FITC but excluded propidium iodide. Cells in necrotic or late apoptotic stages were labeled with both annexin V-FITC and propidium iodide.

Statistical Analysis. Results were expressed as means ± SD. All statistical analyses were made with a two-sided Student’s t test. P < 0.05 was considered to be statistically significant.

Results
Detection of Survivin Gene Expression in Mesothelioma Cell Lines and Tissues. High levels of survivin expression have been detected in multiple human cancers, including cancers of the lung, colon, pancreas, prostate, and breast. Survivin expression has not been described in human mesothelioma. Using cDNA microarray hybridization, we have recently detected overexpression of the Bcl-2 and survivin genes in a number of mesothelioma tissues. A representative cDNA microarray in Fig. 1 showed that Bcl-2 and survivin genes were both up-regulated 5.1- and 4.6-fold, respectively, in mesothelioma as compared with normal pleura. Considering the overwhelming expression of the IAP gene family member survivin in many common cancers, we then extended our screening for survivin expression in mesothelioma cells.

We screened 8 human mesothelioma cell lines and 12 fresh human mesothelioma tissues using RT-PCR and Western blot analysis. Our results showed that survivin was expressed in both mesothelioma cell lines and freshly resected mesothelioma tissues. As shown in Fig. 2a, the survivin gene was detected in all but the LRK1A mesothelioma cell line (seven of eight cell lines). It was reported previously that the LRK1A cell line did not express Bcl-2 (17). Expression of the survivin gene in LRK1A was not detected in this study by RT-PCR, and these results were confirmed by Western blot analysis (Fig. 2a). This cell line was therefore used as a negative control for antisurvivin oligonucleotide transfection experiments in our study.

Results from screening of freshly resected mesothelioma tissues showed that survivin was expressed in all samples screened (12 of 12 samples; Fig. 2b). With RT-PCR, we also detected survivin gene expression in four other cell lines including breast carcinoma MCF7, lung carcinoma A549, and glioma cells CF210 and U87. Survivin expression was not detected in normal human lung RNA, which is consistent with the notion that survivin is not expressed in normal and differentiated tissues.
Induction of Apoptosis in Mesothelioma treated with 500 nM antisurvivin oligonucleotides and con-

positive mesothelioma cell lines, H28 and MS-1, were (A). Lines.

b, equal sample loadings. L19- and 10799T, 4WTT, 237, 10, 12, 32,
isolated from 12 mesothelioma tissues (179, 242, 2120, 9120T, 0390T, sected human mesothelioma tissues.
soluble proteins were analyzed by Western blot and detected by antisur-
vivin oligonucleotides on cell growth. Two survivin

at the top. The H28 cell line was used as a positive control for equal sample loadings. b, detection of survivin expression in freshly re-
sected human mesothelioma tissues. A, RT-PCR analyses. Total RNA was isolated from 12 mesothelioma tissues (179, 242, 2120, 9120T, 0390T, 10799T, 4WTT, 237, 10, 12, 32, and 246) and analyzed by RT-PCR using survivin- and L19-specific primers. Four cell lines (MCF7, A549, CF210, and U87) were used for comparison. A normal human lung total RNA was used as a negative control for PCR. B, Western blot analysis. Soluble proteins (30 µg) from each mesothelioma tissue were analyzed by Western blot and immunochemistry as described above. The mesothelioma sample case numbers are shown on the top. The H28 cell line was used as a positive control. β-Actin antibody was used as a control for equal sample loading (data not shown).

Effects of Antisurvivin Oligonucleotides on Mesothelioma Cell Growth. We first examined the effects of antisurvivin oligonucleotides on cell growth. Two survivin-positive mesothelioma cell lines, H28 and MS-1, were treated with 500 nM antisurvivin oligonucleotides and control oligonucleotides. The LRK1A cell line (survivin nega-
tive) was used as control. As shown in Fig. 3, transfection of antisurvivin oligonucleotide caused cell death in both H28 and MS-1 cells 24 h after transfection (Fig. 3, A and C). Transfection using control oligonucleotides did not significantly affect cell growth in these two cell lines (Fig. 3, B and D). In contrast, the LRK1A cell line was not susceptible to apoptotic cell death by antisurvivin oligonucleotides (Fig. 3E).

Dose-dependent Analysis of the Effect of Antisurvivin Oligonucleotides on Cell Viability. To investigate the biological effect on cell viability of down-regulation of survivin, we treated H28 cells (survivin positive) with antisurvivin oligonucleotide 4003 (100–600 nM). The percentage of dead cells was counted using the trypan blue staining method 24 h after the start of transfection. Untreated cells, cells treated with Lipofectin alone, and cells treated with the control oligonucleotide were used for comparison. The apoptotic effects of antisurvivin oligonucleotides on H28 cells appeared from 200 nM and exhibited a dose-dependent manner until 600 nM (Fig. 4). Treatment of cells with antisurvivin oligonucleotides at concentrations higher than 600 nM resulted in severe cell death.

Increased Caspase-3 Activity by Treatment with Antisurvivin Oligonucleotides. We tested whether cell death in MS-1 and H28 cells was due to the induction of apoptosis by antisurvivin oligonucleotide treatment using a caspase-3 activity assay (Fig. 5). Lysates from antisurvivin oligonucleotide-treated cells showed increased caspase-3 activity (7–9-fold higher than that of control groups). These results suggest that the observed cell death in mesothelioma cell lines after treatment with antisurvivin oligonucleotide is likely caused by apoptosis signaling pathways.

Apoptosis Analysis by Flow Cytometry. To further verify that apoptotic cell death resulted from transfection of antisurvivin oligonucleotides, we analyzed H28 cells 24 h after transfection with 500 nM oligonucleotides with an annexin V-FITC apoptosis detection kit. The results showed that antisurvivin oligonucleotides induced significantly greater apoptotic cell death in the survivin-positive mesothelioma cell line H28 (42.5%) as compared with the control oligonucleotides (16.2%; P < 0.001; Fig. 6). No treatment and treatment with Lipofectin alone did not have a significant apoptotic effect on these cells.

Time Course Inhibition of Survivin Expression by Antisurvivin Oligonucleotides. To investigate the duration of survivin expression inhibition by antisense oligonucleotides, we carried out a time course analysis by Western blot. H28 and MS-1 cells were transfected for 5 h with 500 nM antisurvivin oligonucleotides, and cells were collected at 12 and 24 h, respectively, after the start of transfection. Survivin expression was inhibited in samples harvested at 12 and 24 h after transfection in both cell lines (Fig. 7). Transfection using control oligonucleotides and Lipofectin alone did not affect survivin expression. These results indicate that treatment with antisurvivin oligonucleotides can specifically inhibit survivin protein synthesis and lower survivin protein levels within 12 h.

Figure 2. a, detection of survivin gene expression in mesothelioma cell lines. A, RT-PCR analysis. Survivin cDNA primers and L19 ribosomal protein cDNA primers used as internal control were described in “Materials and Methods.” PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide. Mesothelioma cell lines are indicated at the top. Relative sizes of RT-PCR products are as follows: survivin, 436 bp; and L19, 395 bp. Molecular size markers are the 1-kb ladders. B, Western blot analysis of survivin expression. For each cell line, 30 µg of soluble proteins were analyzed by Western blot and detected by antisurvivin monoclonal antibody, and β-actin antibody was used as control for equal sample loadings. b, detection of survivin expression in freshly resected human mesothelioma tissues. A, RT-PCR analyses. Total RNA was isolated from 12 mesothelioma tissues (179, 242, 2120, 9120T, 0390T, 10799T, 4WTT, 237, 10, 12, 32, and 246) and analyzed by RT-PCR using survivin- and L19-specific primers. Four cell lines (MCF7, A549, CF210, and U87) were used for comparison. A normal human lung total RNA was used as a negative control for PCR. B, Western blot analysis. Soluble proteins (30 µg) from each mesothelioma tissue were analyzed by Western blot and immunochemistry as described above. The mesothelioma sample case numbers are shown on the top. The H28 cell line was used as a positive control. β-Actin antibody was used as a control for equal sample loading (data not shown).
Discussion

Expression of the IAP family member survivin is up-regulated in a variety of human cancers, with high levels documented in breast and lung cancer (18). Overexpression of survivin in tumors correlates with resistance to chemotherapy and poor prognosis in patients with non-small cell lung cancer (19), colorectal cancer (20), and neuroblastoma (21). In this study, we demonstrate, for the first time, that survivin is up-regulated in both mesothelioma cell lines (7 of 8 cell lines; 87.5%) and freshly resected mesotheliomas (12 of 12 samples; 100%). These results confirmed our previous observation by cDNA microarray analysis that survivin was overexpressed in mesothelioma and may play an important role in tumor development, progression, and, importantly, resistance to chemotherapy.

Therapeutic strategies using antisense oligonucleotides have been found to be an effective way to down-regulate survivin and reduce the apoptotic threshold in tumor cells. A number of antisurvivin oligonucleotides have been tested for

![Anti-survivin Cells](image1.png)

Fig. 3. Effects of antisurvivin oligonucleotides on mesothelioma cell growth. Cells were treated with oligonucleotide (500 μM)-Lipofectin complex for 5 h, medium was removed, and cells were cultured in fresh medium for an additional 19 h. Cell viability was examined, and photographs were taken under an inverted phase-contrast microscope. A, MS-1 with antisurvivin oligonucleotide; B, MS-1 with control oligonucleotide; C, H28 with antisurvivin oligonucleotide; D, H28 with control oligonucleotide; E, LRK1A with antisurvivin oligonucleotide. The status of survivin expression for each cell line is shown in the inset. Magnification, ×400.

![Anti-survivin Dose Response](image2.png)

Fig. 4. Dose-response curve of the effect of antisurvivin oligonucleotides on the growth and viability of H28 cells. Cells were treated for 5 h with an increasing concentration of antisurvivin oligonucleotide 4003 (10). Untreated cells, cells treated with Lipofectin alone, and cells treated with the control oligonucleotide were used for comparison. Cell viability was determined in triplicate cultures 24 h after the start of transfection using the trypan blue staining method. Each value represents the means ± SD of three independent experiments.
their ability to block survivin expression in tumor cell lines primarily for the elucidation of the biological function of survivin during cell division and apoptosis (22). Olie et al. (10) tested six antisurvivin oligonucleotides, of which antisense oligonucleotide 4003 was found to be the most effective in inducing growth inhibition and apoptosis in a lung carcinoma cell line A549. This same antisurvivin oligonucleotide used in the present study effectively down-regulated survivin expression in two survivin-positive cell lines, H28 and MS-1. Cell viability was significantly decreased in the cells after antisurvivin oligonucleotide treatment as compared with untreated controls. Results from caspase-3 activity assay and flow cytometry analysis further confirmed that the reduced cell viability in mesothelioma cells was due to apoptotic cell death induced by antisurvivin oligonucleotides. These results are consistent with previous studies using the A549 cell line that showed elevated caspase-3-like protein activity after antisense treatment (10). Previous studies have shown that survivin can inhibit a number of effector caspases, including caspase-3, -7, and -9 (4, 18), blocking the apoptotic cell death pathways. Immunoprecipitation study and protein structure indicate that the antiapoptotic function of survivin results from an indirect inhibitory role on caspase-3, possibly by promoting a pro-caspase-3-p21 complex (23, 24).

In the present study, we also found that survivin expression could be suppressed within 12 h after antisense oligonucleotide transfection (Fig. 7). We have detected a significant reduction of survivin expression by Western blot analysis as early as 12 h after transfection that correlates with the induction of apoptosis in both H28 and MS-1 cell lines. Olie et al. (10) observed reduction of survivin mRNA 20 h after transfection and apoptosis 64–72 h after transfection with antisense oligonucleotides. Our results suggested that transfection of antisurvivin oligonucleotides was efficient in inhibiting survivin expression in mesothelioma cells.

Mesothelioma is highly resistant to chemotherapy and radiotherapy (12, 13) and is resistant to apoptosis (17). Resistance to apoptosis may contribute to the overall insensitivity of mesothelioma to standard therapies. Because mesothelioma typically harbors wild-type p53 (25, 26), a major modulator of apoptosis, its resistance to apoptosis would be expected to arise downstream from p53, such as the Bcl-2 family of proteins that regulate cell death (27). Overexpression of Bcl-2, a protein that suppresses apoptosis in response to a variety of treatments, has been found to correlate with poor prognosis in several solid tumors including breast, prostate, and lung cancer (28–30). However, expression of Bcl-2 varied in different tumors and was limited to a fraction of cases. For examples, Bcl-2 expression was detected in 3 of 14 cases of mesothelioma (17) and in only 7.5% of 174 cases of gastric carcinomas (31). Thus, overexpression of Bcl-2 alone cannot account for the resistance to apoptosis in mesothelioma and other tumors.
In contrast to the level of Bcl-2 expression, we have determined that survivin is expressed overwhelmingly in both mesothelioma cell lines and freshly resected tumors in the present study (87.5% and 100%, respectively). Furthermore, we have shown that targeted down-regulation of survivin expression by antisense oligonucleotides results in increased cell death and enhanced apoptosis in mesothelioma cells. These findings are consistent with others in different cell systems (5, 10). Our studies suggest that the overexpression of survivin plays a more important role than Bcl-2 in developing resistance to apoptosis and contributes to the poor response of mesothelioma cells to chemotherapy and radiation therapy.

These results suggest that targeting survivin expression using antisense oligonucleotides may hold promise as an effective therapy for mesothelioma. Previous studies have shown synergic effects of antisurvivin oligonucleotides in sensitizing cancer cells to chemotherapeutic drugs (10, 22, 32). Experiments are now under way in our laboratory to test the efficacy of survivin antisense oligonucleotides in combination with cytotoxic chemotherapy in vitro and in vivo. It is hoped that translation of this strategy to the clinic may improve the efficacy of treatment of patients with mesothelioma.

In conclusion, we have shown that survivin is overexpressed in both mesothelioma cell lines and fresh tumor samples. Survivin antisense oligonucleotides efficiently down-regulate the expression level of survivin and cause apoptotic cell death in vitro. Survivin overexpression in mesothelioma may play an important role in the development of resistance to apoptosis and thus to insensitivity to standard chemotherapy and radiation therapy. Targeting survivin expression using an antisense strategy in combination with chemotherapy may increase clinical effectiveness in mesothelioma treatment.

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References
18. Togin, S., Villa, A., Marchisio, P. C., and Altieri, D. C. Pleiotropic cell division defects and apoptosis induced by Fas (CD95), Bax, caspases, and anti-
19. Nonzo, M., Rosell, R., Felip, E., Astudillo, J., Sanchez, J. J., Maestre, T., and Reed, J. C. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anti-


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