Silencing Survivin Splice Variant 2B Leads to Antitumor Activity in Taxane-Resistant Ovarian Cancer

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Abstract

Purpose: To study the role of survivin and its splice variants in taxane-resistant ovarian cancer.

Experimental Design: We assessed the mRNA levels of survivin splice variants in ovarian cancer cell lines and ovarian tumor samples. siRNAs targeting survivin were designed to silence all survivin splice variants (T-siRNA) or survivin 2B (2B-siRNA) in vitro and orthotopic murine models of ovarian cancer. The mechanism of cell death was studied in taxane-resistant ovarian cancer cells and in tumor sections obtained from different mouse tumors.

Results: Taxane-resistant ovarian cancer cells express higher survivin mRNA levels than their taxane-sensitive counterparts. Survivin 2B expression was significantly higher in taxane-resistant compared with -sensitive cells. Silencing survivin 2B induced growth inhibitory effects similar to silencing total survivin in vitro. In addition, survivin 2B-siRNA incorporated into DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) nanoliposomes resulted in significant reduction in tumor growth ($P < 0.05$) in orthotopic murine models of ovarian cancer, and these effects were similar to T-siRNA-DOPC. The antitumor effects were further enhanced in combination with docetaxel chemotherapy ($P < 0.01$). Finally, we found a significant association between survivin 2B expression and progression-free survival in 117 epithelial ovarian cancers obtained at primary debulking surgery.

Conclusions: These data identify survivin 2B as an important target in ovarian cancer and provide a translational path forward for developing new therapies against this target. Clin Cancer Res; 17(11); 3716–26. ©2011 AACR.

Introduction

Survivin [baculoviral IAP repeat-containing protein (BIRC-5)] belongs to the inhibitor of apoptosis protein (IAP) family (1, 2) characterized by 1 to 3 BIR (baculoviral IAP repeats) domains, a COOH-terminal RING finger domain, and a caspase recruitment domain (2). However, in contrast with other IAPs, survivin lacks a RING finger domain (2). The mechanisms by which survivin, and other IAPs, exert their antiapoptotic effect have been well-characterized in vitro and in vivo (3–5). This mechanism involves formation of a complex between IAPs and effector caspases (2, 6) that result in inhibition of the apoptotic pathway (6). As a subunit of the chromosomal passenger complex (CPC), survivin also plays a critical role in the regulation of mitosis (7, 8).

Survivin has been amply considered an anticancer target because it is highly expressed in several types of cancer but is largely undetectable in most normal adult tissues (1, 9). Evidence also indicates that survivin plays a central role in cancer drug resistance (10–13). In ovarian cancer, progressive taxane resistance in vitro is associated with increasing survivin expression (11). Likewise, higher levels of survivin have been observed in advanced ovarian carcinomas associated with clinical resistance to a paclitaxel/platinum chemotherapy compared with drug-sensitive cases (11).

Five different isoforms of survivin, arising from alternative splicing of the survivin (BIRC-5) gene, have been identified (14–17). These include the wild-type (WT) survivin, survivin 2B, survivin 3B, survivin ΔEx3, and survivin...
2α (14, 15, 17, 18). Growing evidence indicates that the survivin splice variants could have different, even opposite, biological roles (16, 17, 19, 20). While some survivin splice variants could play an antipapoptotic role, others could be associated with mitosis (14–16, 21, 22). The lack of information regarding the biological role of survivin splice variants in taxane-resistant ovarian cancer prompted us to consider their specific roles. Our data indicate that compared with taxane-sensitive counterparts, taxane-resistant ovarian cancer cells express higher mRNA levels of specific survivin splice variants, particularly survivin 2B. Here, we examined the in vitro and in vivo effects of silencing of survivin splice variants in preclinical models of ovarian cancer and determined the clinical significance of survivin 2B mRNA expression levels in ovarian cancers.

Materials and Methods

Cells and culture conditions

The human ovarian cancer cell lines SKOV3ip1, SKOV3.TR, HeyA8, HEYA8.MDR, A2780PAR, and A2780CP20 cells have been described elsewhere (23–26). All tumor cell lines were screened for Mycoplasma using MycoAlert (Cambrex Bioscience) as described by the manufacturer. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS in 5% CO2/95% air at 37°C. In vitro assays were carried out at 70% to 85% cell density.

Chemicals, reagents, and antibodies

For in vitro testing, docetaxel and paclitaxel were reconstituted in 100% dimethyl sulfoxide (DMSO). When added to the cells, the final concentration of DMSO on the culture medium was 0.1% or less. Monoclonal antibodies against caspases-8, -3, -9, and PARP-1, cyclin-dependent kinase (cdk) 2, p27, and cyclin E were purchased from Cell Signaling. Monoclonal anti-β-actin was purchased from Sigma-Aldrich. Mouse and rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Sigma-Aldrich.

Western blot analysis

Cells were washed with cold phosphate-buffered saline (PBS), lysed with ice-cold lysis buffer, and incubated on ice for 30 minutes. Lysates were centrifuged, supernatants were collected, and protein concentration was determined using Bio-Rad Protein Reagents (Bio-Rad). Protein lysates (30 μg) were separated by SDS-PAGE, blotted onto membranes, and probed with the appropriate dilution of each primary antibody. Membranes were rinsed and incubated with the appropriate HRP-conjugated secondary antibody, rinsed again, and the bound antibodies were detected using enhanced chemiluminescence (GE Healthcare) following autoradiography in a FluorChem 8900 (Alpha Innotech Corporation).

RNA isolation and SYBR-1–based real-time PCR

Total RNA was extracted from cells using the Rneasy kit from Qiagen. Total RNA (1.0 μg) was subjected to reverse transcription in a reaction mix containing 500 μg/mL oligo (dT), 1 × First Strand Buffer, 0.01 mol/L DTT, 0.5 mmol/L dNTP mix, and 200 units of MMLV RT (Invitrogen) to a final volume of 20 μL, and incubated at 40°C for 2 hours. The resulting first-strand complementary DNA (cDNA) was used as a template for SYBR-1–based real-time PCR analysis (27). Primers (Invitrogen) were as follows: total survivin: forward: AGCCCTTTCTCAAGGACCAC, reverse: CACGT-CTGGAAAGCGAAGAAG; common forward primer for WT, 2B, and ΔEX3 isoforms: GACACCCGGCATCCTCTATTC; reverse: WT: TGCTTTTTATGTTCCTTCTATGG, 2B: AAGTG-CTGATTATACAGGCGT, ΔEX3: ATTTTGTTCTTCTTG-CATG; survivin 2B: forward: GCCTTGTGTTAGAAGTGTGCAA, reverse: GCAATGAGGGTGGAAAGCA (21); 3B: forward: GAGGCTGTGCTTACAGGGT, ΔEX3: GTCTCTCAATT- TTGTCITGT (18) AGCATGCCTCCGGTGCCT; and β-actin: forward: ATAGCAACGCTCGATGACAGCTAC, reverse: CACCTTCTACAATGAGCTGCTGT. Two micro- liters of cDNA was added to QuantiTect SYBR Green PCR Master Mix (Applied Biosystems) containing 200 nmol/L of each primer. PCR was carried out in a StepOne plus (Applied Biosystems) real-time PCR using the following thermal settings: 1 cycle of 15 minutes at 95°C and 40 cycles of 15 seconds at 94°C, 30 seconds at X°C (X = 58°C for WT, 2B, and ΔEX3; 60°C for β-actin; 50°C for 2α; and 54°C for 3B) and 30 seconds at 72°C. Relative mRNA expression was calculated with the ΔΔCt method (28, 29).

siRNA and in vitro siRNA transfection

To silence total human survivin (NM_001168), siRNA (5’-CAGACTTGGCCACGTTT-3’) was designed in exon 2, which is common to all survivin splice variants. siRNA duplex targeting the 5’-AAATCCAGACCTTTGGAG-3’ sequence in the open reading frame of the human survivin 2B (NM_001012271) and a nonsilencing negative control siRNA (C-siRNA) was used. siRNAs were purchased from Sigma. Briefly, 3 × 10^4 cells/mL were plated in 6-well plates. Twenty-four hours later, 3 μg of siRNA were mixed with HiPerFect transfection reagent (Qiagen) at 1:3 ratio (siRNA:transfection reagent). The mix was incubated for 10 minutes at room temperature (RT) and then added to the cells. Cells were collected 24 hours later to assess the downregulation of each target.
**In vitro cell viability assay**
Cells (2 × 10^4 cells/mL) were plated in a 96-well plate. Twenty-four hours later, docetaxel or siRNAs were added to the cells and incubated for 72 hours. The medium was then removed and 100 µL of Alamar blue dye (Invitrogen) was added following the manufacturer’s instructions. Optical density (OD) values were obtained spectrophotometrically in a plate reader (Kinetic Microplate Reader; Molecular Devices Corp.) after 3 hours of dye incubation. To assess the effect of combining siRNA and docetaxel, cells were plated, and 24 hours later, siRNA was added to cells and incubated for another 24 hours. Docetaxel was then added and cells were incubated for other 72 hours. In all cases, percentages of cell growth inhibition were obtained after blank OD subtraction, taking the untreated cells values as 0% of inhibition.

**Assessment of cell apoptosis and cell-cycle progression**
Apoptosis was measured with the FITC-Apoptosis Detection Kit (BD Biosciences), which uses Annexin-V and propidium iodide (PI) as the apoptotic and necrotic markers, respectively. Briefly, HEYA8.MDR cells (1 × 10^5) were collected, washed, and resuspended in 1 × binding buffer. Cells were incubated for 10 minutes with Annexin-V and/or PI according to the manufacturer’s instructions. After the addition of 500 µL of 1 × binding buffer, apoptotic cells were analyzed in a FACScalibur flow cytometer (BD Biosciences). CellQuest Pro software (BD Biosciences) was used to determine the number of apoptotic and necrotic cells. To assess cell cycle, cells were transfected with siRNAs and 48 hours later, cells were collected and fixed with 70% cold ethanol and stored at −20°C. Twenty-four hours later, cells were collected and resuspended in PBS containing 50 µg/mL (final concentration) PI and 100 units/mL (final concentration) RNase A. The mixture was incubated at 37°C for 30 minutes in the dark and then analyzed by flow cytometry in a FACScalibur flow cytometer (BD Biosciences). CellQuest Pro software (BD Biosciences) was used to determine the number of cells in each phase of the cell cycle.

**SiRNA incorporation into DOPC nanoliposomes**
For in vitro delivery, siRNAs were incorporated into 1,2-dioleoyl-sn-glycero-3-phosphocholine (siRNA-DOPC; Avanti Polar Lipids, Inc.). siRNA and DOPC were mixed in the presence of excess t-butyl alcohol at a ratio of 1:10 (w/w) as described previously (23, 24, 30–32). After Tween 20 was added, the mixture was frozen in an acetone/dry ice bath and lyophilized. Before in vivo administration, the lyophilized powder was hydrated with Ca2+ and Mg2+-free PBS at a concentration of 25 µg/mL to achieve the desired dose in 200 µL per injection.

**Orthotopic tumor implantation and drug treatment**
Female athymic nude mice (NCr-nu, 8–12 weeks old) were purchased from Taconic (Hudson). To generate tumors, HEYA8.MDR (5 × 10^5 cells/0.2 mL HBSS) and SKOV3.TR (1 × 10^6 cells/0.2 mL HBSS) were injected into the peritoneal cavity of nude mice. To assess the efficacy of each siRNA for silencing the target in vivo, nude mice bearing HEYA8.MDR tumors were randomly divided into 3 groups (N = 3 per group): (i) nonsilencing siRNA (C-siRNA), (ii) total survivin siRNA (T-siRNA), and (iii) 2B-siRNA. One dose (5 µg siRNA per mouse) of each siRNA was administered intraperitoneally 3 weeks after tumor implantation. Two days after siRNA injection, animals were sacrificed, tumors were removed, and stored at −80°C for total RNA isolation.

To evaluate the therapeutic activity of survivin siRNAs alone or in combination with docetaxel HEYA8.MDR were injected intraperitoneally. Seven days later, the mice (N = 10 per group) were randomly assigned to 7 treatment groups (all treatments were administered intraperitoneally): (i) C-siRNA, (ii) docetaxel (DTX) alone, (iii) c-siRNA plus DTX, (iv) T-siRNA, (v) T-siRNA plus DTX, (vi) 2B-siRNA, and (vii) 2B-siRNA plus DTX. Liposomal siRNAs (5 µg siRNA per injection) were injected twice a week and docetaxel (50 µg per injection) once a week (23, 24). The therapeutic experiment was repeated with SKOV3.TR cells.

**Immunohistochemistry**
Expression of Ki67 (cell proliferation marker) and immunohistochemistry for CD31 (microvessel density, MVD) were done on paraffin-embedded tumors as described before (23, 24, 30). Ki67 primary antibody (BioCare Medical) and secondary goat HRP anti-rabbit (Jackson Laboratories) were used. The primary antibody used for CD31 was an anti-CD31 (platelet/endothelial cell adhesion molecule-1, rat IgG; BD Pharmingen). Secondary antibody was goat HRP anti-rat (Jackson Laboratories). Assessment of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)-positive cells (apoptosis) were determined by immunohistochemical analysis using freshly cut frozen tissue as described previously (23, 24, 30, 31).

To quantify MVD, 10 random fields at 100× final magnification were examined for each tumor (1 slide per mouse, 5 slides per group) and the number of microvessels per field was counted (23, 24, 26). To quantify Ki67 expression, the number of positive and negative cells (3,3′-diaminobenzidine staining) was counted in 10 random fields at 100× magnification and the percentage of Ki67-positive cells was calculated for each group (23, 24, 26). To quantify TUNEL-positive cells, the number of positive cells was counted in 10 random fields at 100× magnification (23, 24, 26).

**RNA extraction from human epithelial ovarian cancer specimens**
After obtaining Institutional Review Board approval, we obtained 117 human epithelial ovarian carcinoma specimens that were archived and freshly frozen at the time of primary debulking at the University of Texas MD Anderson Cancer. These samples (35 mg each) were frozen in liquid
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Figure 1. Expression of survivin splice variants in ovarian cancer cells. Total RNA was extracted from cells to doing SYBR-I–based real-time PCR as described in the "Materials and Methods" section. β-Actin was used as the "endogenous control." Messenger RNA expression levels were calculated with the ΔΔCt method. The relative mRNA expression level of each survivin splice variant was expressed relative to the total survivin of the SKOV3ip1 cell line, which was taken as 1.0. *, P < 0.05; **, P < 0.01. Columns represent the means of triplicates ± SD.

Statistical analysis

For in vivo experiments, differences in continuous variables (mean body weight, tumor weight, MVD, Ki67, and TUNEL staining) were analyzed using the Student’s t test for comparing 2 groups and by ANOVA for multiple group comparisons with P < 0.05 considered statistically significant. To examine the interaction index between T-siRNA, 2B-siRNA, and docetaxel, an isobolanalysis was used as described by Feleszko and colleagues (33). Briefly, equieffective concentrations (concentrations of either drug, alone, or in combination that gave equivalent inhibition of cell growth as compared with untreated control cells) were calculated. The interaction index for combination of the 2 drugs was calculated according to the following equation:

$$\text{Interaction index} = \frac{\text{siRNA}_c}{\text{siRNA}_a} + \frac{\text{DTX}_c}{\text{DTX}_a}$$

where siRNAa and DTXa are concentrations of T-siRNA, 2B-siRNA, and docetaxel, respectively, that produce some specified effect when used alone, and siRNAc and DTXc are concentrations of T-siRNA, 2B-siRNA, and docetaxel, respectively, that produce the same effect when used in combination. The interaction is considered synergistic when the interaction index is less than 1.0.

Results

Expression of survivin splice variants in ovarian cancer cells

We first carried out growth inhibition experiments to confirm the responsiveness of the ovarian parental cancer cells lines HEYA8 and SKOV3ip1, and their taxane-resistant counterparts, HEYA8.MDR and SKOV3i.TR, respectively (refs. 31–33; Supplementary Fig. S1). As expected, the resistant cells had no response, whereas the sensitive cells had a dose-dependent response.

We next used SYBR-I–based real-time PCR to compare the mRNA expression levels of the 5 survivin splice variants in taxane-sensitive ovarian cancer cells and their taxane-resistant counterparts (Fig. 1). Although the most abundant isoform in all cell lines was WT, survivin 2B was approximately 3-fold more abundant in the taxane-resistant compared with the taxane-sensitive ovarian cancer cells. This pattern was not observed in the cisplatin-sensitive A2780PAR ovarian cancer cell and its cisplatin-resistant counterpart AP2780CP20. The mRNA expression levels of survivin 2α and survivin 3B were negligible as compared with other isoforms. Therefore, we focused on the survivin 2B isoform for all subsequent work.
Effects of survivin 2B silencing on cell growth

siRNAs were designed around exon 2 to inhibit all survivin splice variants at the same time, and 2 additional siRNAs were designed to inhibit only survivin 2B (Supplementary Fig. S2A). Real-time PCR experiments showed that T-siRNA significantly reduced the levels of total survivin and the survivin splice variants WT, 2B, and ΔEX3 in the HEYA8.MDR cell line. 2B-siRNA inhibited the expression of this isoform, but not survivin WT or ΔEX3, showing that this siRNA was specific for survivin 2B. Maximal silencing of both survivin 2B and total survivin was observed at 24 hours (Supplementary Fig. S2B).

Dose-dependent growth inhibition was observed after 24 hours of incubation of HEYA8.MDR cells with the T-survivin-siRNA or 2B-siRNA (Fig. 2B). We also studied the effects of combining nonactive doses of docetaxel (Fig. 2C). Isobologram analysis demonstrated synergism for growth inhibition at all siRNA-Docetaxel combinations tested (Supplementary Fig. S3A and B). Combination of an inactive dose of siRNA (25 nmol/L), with different docetaxel concentrations induced additive growth inhibitory effects, even at 10 nmol/L docetaxel (Supplementary Fig. S3C). Cell growth inhibition was also observed after transfection of HEYA8.MDR cells with other different siRNAs against total survivin and survivin 2B (Supplementary Fig. S4). These results indicate that the growth inhibitory effects observed were indeed due to survivin silencing.

Effects of survivin 2B silencing on apoptosis and cell-cycle progression

We next investigated whether the growth inhibition induced by T-siRNA or 2B-siRNA was due to apoptosis (Fig. 3A). Transfection of HEYA8.MDR cells with T-siRNA induced up to 35% apoptosis, whereas survivin silencing with nonactive doses of docetaxel (Fig. 2C)
Survivin 2B silencing induced up to 15% apoptosis (*, \( P < 0.01 \)). These results were confirmed by Western blot analysis, which showed that silencing survivin 2B induced lower levels of PARP-1 cleavage and lower activation of caspase-9 and caspase-3 (Fig. 3B) when compared with cells incubated with T-siRNA. No difference was observed in the cleavage product of caspase-8, between T-siRNA and 2B-siRNA. These latter effects were expected as caspase-8 is upstream of survivin in the apoptotic pathway (1, 2).

Survivin and its splice variants are known to play a central role during mitosis (1, 15, 34); thus, we also assessed whether cell-cycle arrest is induced by survivin 2B silencing (Fig. 3C). More than 80% of the HEYA8.MDR cells were arrested in G1-phase after 48 hours of transfection with 2B-siRNA (Fig. 3C). Western blot analysis confirmed that key proteins required for transition from G1- to S-phase (35–37) were downregulated [cyclin E (40%) and cdk2 (80%)], whereas p27 which blocks cyclin E and cdk2 function (35) was upregulated (50%), in 2B-siRNA transfected cells (Fig. 3D and E).

**Therapeutic effect of 2B-siRNA-DOPC**

DOPC-based nanoliposomes were used for the animal models as previously described (23, 24, 26, 30, 31). We first tested whether the siRNAs will silence the respective survivin isoforms in an orthotopic model of ovarian cancer (Supplementary Fig. S5, see Materials and Methods). Mice were injected intraperitoneally with 5 \( \mu \)g siRNA per mouse of T-siRNA-DOPC or 2B-siRNA-DOPC. Mice were sacrificed 72 hours postinjection and the
tumors were dissected. T-siRNA-DOPC, as expected, significantly reduced the expression of all isoforms, whereas 2B-siRNA-DOPC led to significant silencing of only survivin 2B.

The antitumor effects of T-siRNA and 2B-siRNA as compared with C-siRNA were tested in HEYA8.MDR and in SKOV3.TR tumor-bearing mice (Fig. 4). Tumor weight and number of nodules were assessed in all mice. Seven treatment groups (10 mice each) were studied: (i) C-siRNA-DOPC, (ii) DTX alone, (iii) C-siRNA-DOPC plus DTX, (iv) T-siRNA-DOPC, (v) T-siRNA-DOPC plus DTX, (vi) 2B-siRNA-DOPC, and (vii) 2B-siRNA-DOPC plus DTX. siRNA-DOPC (5 μg siRNA per injection) was injected twice a week and docetaxel (50 μg per injection) once a week (23, 24). All siRNA-DOPC treatments were given twice a week for 6 weeks. DTX by itself did not induce a significant effect on tumor growth (Fig. 4A). Decreased tumor weight was observed in both T-siRNA-DOPC and 2B-siRNA-DOPC groups (*, P < 0.05 for both, T-siRNA-DOPC and 2B-siRNA-DOPC). Tumor weight was further reduced when DTX was added to T-siRNA-DOPC and 2B-siRNA-DOPC (**, P < 0.01 for both, T-siRNA-DOPC plus DTX and 2B-siRNA-DOPC plus DTX). Similar results were obtained with treatment of SKOV3.TR-bearing mice with T-siRNA-DOPC and 2B-siRNA-DOPC alone or in combination with DTX (Fig. 4C). T-siRNA-DOPC or 2B-siRNA-DOPC induced a significant decrease in the number of tumor nodules in both, HEYA8.MDR tumor–bearing mice (**, P < 0.01; Fig. 4B), and SKOV3.TR (**, P < 0.01; Fig. 4D). No further reduction in the number of tumor nodules was observed when DTX was combined with either T-siRNA-DOPC or 2B-siRNA-DOPC (Fig. 4B and D).

**Effect of survivin silencing on angiogenesis, cell proliferation, and apoptosis**

Because silencing of T-survivin–induced cell growth inhibition and apoptosis in vitro and silencing of survivin 2B resulted in cell-cycle arrest with few apoptotic events, we...
examined the effects of the siRNA-DOPC therapy on cell proliferation, apoptosis, and MVD. T-siRNA-DOPC and 2B-siRNA-DOPC treatment resulted in decreased MVD when compared with controls (Fig. 5A), which was further decreased by combination treatment with docetaxel. Cell proliferation was assessed by staining for the nuclear marker Ki67. T-siRNA-DOPC and 2B-siRNA-DOPC induced a significant reduction in the Ki67 index. TUNEL data analysis (Fig. 5C) showed a significant induction of apoptosis in mice treated with T-siRNA-DOPC alone (*, P < 0.05) or in combination with DTX [T-siRNA-DOPC (***, P < 0.001) and 2B-siRNA-DOPC (**, P < 0.01)]. However, higher apoptotic events were observed in T-siRNA-DOPC plus docetaxel group compared with 2B-siRNA-DOPC plus docetaxel group.

Survivin 2B expression in human carcinoma

Survivin and its splice variants are overexpressed in most cancers (14, 38) including ovarian cancer (11, 39). However, most of these studies were carried out by real-time PCR or immunohistochemistry (with an antibody against all survivin splice variants) and do not represent truly quantitative measures. The expression of survivin 2B in ovarian cancer has not been studied. Thus, we examined the mRNA expression levels of survivin 2B in 117 epithelial ovarian cancers obtained at primary debulking surgery by real-time PCR. Two-fold difference between the mRNA abundance for each sample and the mRNA control (normal ovarian epithelial cells) was considered as survivin 2B overexpression (27, 28, 40). Of the 117 specimens, 24% (n = 28) showed high levels of survivin 2B mRNA (Supplementary Table S1). We found that survivin 2B overexpression was significantly (P < 0.005) associated with progression-free survival (Fig. 6A), and with overall survival (P < 0.009; Fig. 6B). The relation between clinicopathologic variables (stage, histology, grade, ascites, and cytoreduction) and survivin 2B mRNA expression was also examined (Supplementary Table S1). Survivin 2B mRNA expression was significantly (P < 0.07) higher in high stage tumors versus low stage disease. We also examined the effect of survivin 2B expression on patient survival in multivariable analysis. This analysis revealed that high survivin 2B expression (P = 0.02) remained statistically significantly associated with...
patient survival after controlling for primary cytoreductive surgery, tumor stage and grade, histology, and patient age.

Discussion

The key finding from our study is that siRNA-based silencing of the survivin splice variant 2B inhibits cell growth in vitro and reduces tumor growth in orthotopic models of taxane-resistant ovarian cancer. Furthermore, survivin 2B mRNA was detected in a substantial proportion of ovarian cancers and was associated with poor of progression-free and overall survival in ovarian cancer patients.

Several reports have shown the role of survivin in drug resistance of ovarian cancer cells (11, 39, 41). However, the role of survivin 2B in taxane-resistant ovarian cancer cells had not been addressed. Our finding that survivin 2B is more abundant in taxane-resistant than in taxane-sensitive ovarian cancer cells, confirm previous results that each survivin splice variant could have specific intracellular roles as antiapoptotic or cell-cycle regulator proteins (14, 15, 17, 42).

siRNA-based silencing of survivin 2B induced similar growth inhibitory effects than silencing of all survivin splice variants at the same time, showing that this survivin 2B could have a central role in cell growth, viability, and drug resistance of ovarian cancer cells. The cell-cycle arrest in G1 on survivin 2B silencing suggests that the main intracellular role of this isoform may be related to the regulation of cell cycle rather than apoptosis. In addition, our in vivo data confirmed that survivin 2B silencing induced lower apoptosis than silencing all survivin splice variants at the same time. This hypothesis is supported by the fact that survivin 2B is the only isoform that retains the C-terminal coiled coil domain necessary for the interaction with INCEP for the CPC formation during mitosis (14).

Earlier reports indicated that survivin 2B could have lost its antiapoptotic function (15, 16, 19, 21) or even have a proapoptotic role (22). More recent survivin overexpression studies in human epithelial carcinoma A431 cell line conducted by Knauer and colleagues showed that survivin 3B was the only survivin isoform able to protect clones against cisplatin-induced apoptosis (18). Our data showed that silencing of survivin 2B in taxane-resistant ovarian cancer cells induced cell growth arrest in vitro and in vivo. In fact, the additional siRNA against survivin 2B that we used here was the one used by Ling and colleagues (22) in which they observed an increase in cell resistance to paclitaxel-mediated growth inhibition of MCF-7 cells. Although our experiments were carried out in different cell lines than those reported by others (16, 21, 22), our observation that siRNA-based silencing survivin 2B induced cell-cycle arrest with minor apoptotic events (in vitro and in vivo) are more in agreement with a role of survivin 2B in the control of cell-cycle progression (14). Survivin 2B silencing could lead to a mitotic catastrophe as previously observed after survivin deletion in vitro and in vivo, with survivin dominant-negative mutants (5, 8, 43, 44). The increasing levels of p27 and the decreased levels of cyclin E and cdk2 with the concomitant arrest in G1 of survivin 2B–depleted HEYA8. MDR cells observed in our study correlates with the role of survivin 2B in the mitotic progression.

There is evidence suggesting that survivin induces drug resistance by stabilizing tubulin polymers (41, 45). The higher expression of mRNA in taxane-resistant compared with their taxane-sensitive counterparts ovarian cancer cells also showed the central role of the survivin 2B splice variant in taxane resistance. Survivin 2B could promote cell survival by interacting with cell-cycle–related kinases, including


The mRNA expression of survivin 2B has been assessed in different cancers (14, 46–49). However contrasting results were obtained regarding the correlation between survivin 2B expression levels and clinicopathologic markers (14, 46–49). These contrasting results are mainly due to the use of nonspecific primers to amplify specifically survivin 2B or the use of semiquantitative analysis of gel images obtained by RT-PCR (14, 49). This is the first report showing mRNA survivin 2B expression levels in ovarian cancer patients. The strong difference in the overall survival between ovarian cancer patients with high versus low survivin 2B suggests that this splice variant could be used as a prognostic markers for ovarian cancer.

Although survivin is highly abundant in most cancers, growing evidence indicate that survivin could regulate the proliferation and survival of normal adult cells (50), especially those with continual renewal. On the basis of our results that silencing survivin 2B induces similar growth inhibition in vitro and in vivo than inhibiting all survivin splice variants at the same time, we propose the use of survivin 2B-siRNA-DOPC as a novel and specific antisurvivin therapy without compromising all intracellular pools of survivin in normal adult tissues.

Disclosure of Potential Conflicts of Interest

G. Lopez-Berestein and A. K. Sood have ownership interest in BioPath, Inc. (including patents).

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