Inhibition of *ABCB1* (*MDR1*) and *ABCB4* (*MDR3*) expression by small interfering RNA and reversal of paclitaxel resistance in human ovarian cancer cells

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Abstract

Ovarian cancer is currently the most lethal gynecologic malignancy in developed countries, and paclitaxel is a cornerstone in the treatment of this malignancy. Unfortunately, the efficacy of paclitaxel is limited by the development of drug resistance. Clinical paclitaxel resistance is often associated with *ABCB1* (*MDR1*) overexpression, and *in vitro* paclitaxel resistance typically demonstrates overexpression of the *ABCB1* gene. In this study, we demonstrate that paclitaxel-resistant cell lines overexpress both *ABCB1* and *ABCB4* (*MDR3*). To evaluate the role of these transporters in paclitaxel-resistant ovarian cancer cells, small interference RNAs (siRNAs) were used to target *ABCB1* and *ABCB4* RNA in the paclitaxel-resistant SKOV-3_{TR} and OVCAR8_{TR} ovarian cancer cell lines. Treatment of these lines with either chemically synthesized siRNAs or transfection with specific vectors that express targeted siRNAs demonstrated decreased mRNA and protein levels of *ABCB1* or *ABCB4*. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays of siRNA-treated cells demonstrated 7- to 12.4-fold reduction of paclitaxel resistance in the lines treated with the synthesized siRNA of *ABCB1* and 4.7- to 7.3-fold reduction of paclitaxel resistance in the cell lines transfected with siRNA of *ABCB1* expressing vectors. *ABCB4* siRNA-treated cell lines showed minor reduction in paclitaxel resistance. These results indicate that siRNA targeted to *ABCB1* can sensitize paclitaxel-resistant ovarian cancer cells *in vitro* and suggest that siRNA treatment may represent a new approach for the treatment of *ABCB1*-mediated drug resistance. [Mol Cancer Ther 2004;3(7):833–8]

Introduction

Ovarian cancer is currently the leading cause of death from gynecologic malignancies in the United States. Paclitaxel, originally isolated from *Taxus brevifolia* (the Pacific yew), is a microtubule stabilizing chemotherapeutic drug used in the treatment of ovarian, breast, and nonsmall cell lung cancers (1, 2). The primary limitation of efficacy for paclitaxel is the development of drug-resistant cancer cells that exhibit the multidrug resistance phenotype (3). Overexpression of the ATP binding cassette transporters such as *ABCB1* (*MDR1*) has been directly implicated in resistance to a broad spectrum of chemotherapeutic agents *in vitro* including paclitaxel, the anthracyclines, and the *Vinca* alkaloids (4-6). For various types of cancer, accumulated evidence from *in vitro* studies indicates that *ABCB1* overexpression may be the predominant factor in limiting the efficacy of chemotherapeutic agents. Introduction and overexpression of the *ABCB1* gene into drug-sensitive cells or into mice to produce transgenic animals confers resistance to the agents described above (7, 8). The *ABCB1* gene encodes a membrane-bound P-glycoprotein (P-gp), which has been shown to transport a variety of chemotherapeutic drugs out of the cell in an energy-dependent process. Further study showed that some of these resistant cell lines also overexpress another ATP binding cassette transporter gene, *ABCB4* (also known as mouse *MDR2* or human *MDR3*; refs. 8, 9). *ABCB4* expression has also been negatively correlated with clinical outcome (10). In brief, cell culture, animal, and clinical studies strongly suggest that overexpression of the *ABCB1* gene may be the single most important determinant of drug resistance in some cancers.

Current strategies to prevent or reverse multidrug resistance have focused primarily on the development of agents that are competitive inhibitors of P-gp. Development of these agents for clinical use has been hindered by toxicity and limited efficacy (11). In addition, some studies have indicated that these and related P-gp inhibitors stimulate *ABCB1* expression (12, 13). Alternative strategies involving the inhibition of transporter expression may offer superior mechanisms for reversing the multidrug resistance phenotype.

Small interfering RNAs (siRNAs) are double-stranded RNA molecules that induce sequence-specific degradation of homologous single-stranded RNA. In plants and insects, siRNA activity plays a role in host cell protection against viruses and transposons (14, 15). From a biological research perspective, siRNA is proving to be a very powerful technique to ‘‘knockdown’’ specific genes, thereby enabling the evaluation of their physiologic roles in *Caenorhabditis elegans*, *Drosophila melanogaster*, and human cells. siRNA

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technology has several major advantages over other post-transcriptional gene silencing techniques (such as antisense or gene knockout technology), in that it is easier to deliver, requires only small doses of siRNA to produce its silencing effect, and can inactivate a gene at almost any stage in development.

In this study, the utility of siRNA to reverse paclitaxel resistance in human ovarian cancer cells selected for resistance by treatment with paclitaxel is evaluated. These cells have been characterized previously and exhibit the classic multidrug resistance phenotype accompanied by increased expression of *ABCB1* (16). In this investigation, some of these resistant cell lines were also found to overexpress *ABCB4*. The purpose of this study was to characterize siRNA inhibition of *ABCB1* and *ABCB4* in paclitaxel-resistant ovarian cancer cells and to determine if siRNA inhibition might offer advantages over classic P-gp inhibitors.

Materials and Methods

Cell Culture

The human ovarian cancer cell line SKOV-3 and its paclitaxel-resistant subline SKOV-3_{TR} were established as reported previously (16, 17). The MCF-7_{TR} breast cancer cell line and OVCAR8_{TR} ovarian cancer cell line were established using a similar protocol. These cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. The SKOV-3_{TR}-, OVCAR8_{TR}-, and MCF-7_{TR}-resistant cell lines were grown in medium containing 0.3, 0.2, and 0.03 μmol/L concentrations of paclitaxel, respectively.

Synthesis of *ABCB1* and *ABCB4* siRNA

The siRNA sequence targeting *ABCB1* (Genbank accession no. NM_000927) or *ABCB4* (Genbank accession no. NM_018849) corresponded to coding regions of these genes. Two target sequences were selected for each gene: *ABCB1a*, *ABCB1b*, *ABCB4a*, and *ABCB4b* (Table 1). Each of the 19-nucleotide siRNAs contained a 3′-dTdT extension. Synthetic siRNA duplexes, as described in Table 1, were obtained from Qiagen (Chatsworth, CA). The siRNAs were dissolved by adding 1 mL of the buffer [100 mmol/L potassium acetate, 30 mmol/L HEPES-KOH, and 2 mmol/L magnesium acetate (pH 7.4)] to each tube, heated for 1 minute at 90°C and 60 minutes at 37°C, and kept at −20°C until the following transfection experiment.

Vector-Based *ABCB1* and *ABCB4* siRNA

Currently, siRNA can be introduced by transfection of short synthetic siRNA or via expression from an appropriate vector as a small RNA hairpin (siRNAs with a self-complementary stem loop). The plasmid pSuppressorNeo was purchased from Imgenex Corp. (San Diego, CA). These vectors employ the type III class RNA polymerase promoter U6 to drive the expression of siRNA molecules. The pSuppressorNeo vector DNA (provided in the kit) was digested with *Sal*I and *Xba*I to generate compatible ends for cloning. To add compatible restriction sites, the *Xho*I site was placed at the 5′ end and a *Xba*I site was added to the 3′ end of the primer sequences. The *Xho*I restriction site is compatible with the *Sal*I site and will allow cloning into a *Sal*I site (see manufacturer's protocol for details). Plasmids expressing *ABCB1* or *ABCB4* siRNA hairpins were constructed by cloning the *ABCB1* or *ABCB4* coding region into pSuppressorNeo according to the manufacturer's instructions. The primers used to generate the human *ABCB1* and *ABCB4* specific siRNAs were obtained from Qiagen. For example, to generate the *ABCB1* siRNA expression plasmid, a double-stranded oligonucleotide siRNA insert was created by annealing primers corresponding to nucleotides 889 to 908 of the *ABCB1* cDNA coding region with a six-nucleotide spacer (AGTACT). This insert was cloned to pSuppressorNeo plasmid. Two target sequences for each gene were selected for testing as described above. To compare the efficiency, we used the same sequence (Table 1) for vector-based siRNA and synthetic siRNA.

Synthetic siRNA Transfection

SKOV-3_{TR} and OVCAR8_{TR} were cultured as described above. For transfection, cells were either plated on 96-well plates for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays or plated on dishes for Northern blot RNA isolation or Western blot protein isolation. Transfections were performed with TransMessenger transfection reagent (Qiagen) as directed by the manufacturer. For *ABCB1* and *ABCB4*, each 96-well plate received 0.1 μg siRNA per well in a volume of 200 μL in triplicate, and each 60 mm dish received 5 μg siRNA per dish in a volume of 10 mL. Medium was replaced with RPMI 1640 supplemented with 10% fetal bovine serum 24 hours after transfection. Total RNA was isolated after 48 hours of siRNA transfection.

Table 1. Synthetic siRNAs used in this study

Target Gene	siRNA	siRNA (Sense Strand Only) 5′ to 3′	Target Location (bp)
ABCB1	*ABCB1a*	r(UGCGACAGGAGAUAGGCUG)d(TT)	889
ABCB1	*ABCB1b*	r(GCGAAGCAGUGGUUCAGGU)d(TT)	2,113
ABCB4	*ABCB4a*	r(CUCAAUACGCGGCUAACAG)d(TT)	2,387
ABCB4	*ABCB4b*	r(GAGGCCAACGCCUAUGAGU)d(TT)	13,392

Transfection of Plasmids Expressing siRNA into Resistant Cells

SKOV-3_{TR} and $OVCAR8_{TR}$ were grown to 40% to 70% confluence in complete medium. Using Lipofectamine Plus transfection reagent (Invitrogen, Carlsbad, CA) and the suggested method, the cells were transfected with pSupressorNeo plasmids expressing siRNA *ABCB1*, *ABCB4*, or just pSupressorNeo empty vector without insert as a control. Medium containing 250 μg/mL G418 (Invitrogen) was added to the cells 24 hours after transfection. Stable G418-resistant clones were selected after 8 to 10 days of transfection for MTT cytotoxicity assay or Northern blot analysis.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted using Trizol reagent (Invitrogen) and quantified by a spectrophotometer. Total RNA (6 μg) was treated with formaldehyde and subjected to electrophoresis in a 1.2% agarose gel using standard techniques. The gels were transferred to Hybond nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, England) and probed with ^{32}P-labeled *ABCB1* or *ABCB4* specific gene probes that had been labeled using the Megaprime DNA Labeling System (Amersham Pharmacia Biotech). The isolation and characterization of *ABCB1* and *ABCB4* cDNA specific probes were used as described previously (18-20). The sequence of cDNA fragments encoding part of the transmembrane segment is sufficiently divergent to discriminate between these two genes (18). The full-length cDNA of the *ABCB1* plasmid (pGEM3ZF (−)XBA-MDR1.1) and *ABCB4* plasmid (pJ3omega-MDR3) were purchased from American Type Culture Collection (Manassas, VA). The *ABCB1* specific gene probe was prepared by purifying a 600-bp *Stu*I-*Kpn*I fragment from the *ABCB1* plasmid, and the *ABCB4* specific gene probe was prepared by purification of a 450-bp *Hin*dII-*Pst*I fragment from the *ABCB4* plasmid as described previously (18).

SDS-PAGE and Western Blot Analysis

Western blots were performed using an Enhanced Chemiluminescence Plus Western Blotting Detection Kit (Amersham Pharmacia Biotech). Eighty-percent confluent cells were washed with PBS and detached with trypsin-EDTA. Equal numbers of paclitaxel-sensitive or paclitaxel-resistant cells transfected with *ABCB1* siRNA were lysed in M-PER Mammalian Protein Extraction Reagent buffer (Pierce Chemical Co., Rockford, IL) and centrifuged at 1,200 × *g* for 15 minutes at 4°C to remove insoluble debris. The detergent soluble fraction was loaded in equal aliquots of protein and resolved using 6% SDS-PAGE. The transferred nitrocellulose blot was blocked with 5% fat-free milk powder in TBS-T (pH 7.6; 20 mmol/L Tris-HCl, 100 mmol/L NaCl, and 0.01% Tween 20) at room temperature for 2 hours. The membrane was immunoblotted with monoclonal antibody C219 (product ID SIG-8710, Signet, Dedham, MA) for human ABCB1 and polyclonal antibody H-300 (Santa Cruz Biotechnology, Santa Cruz, CA) for human β-actin in TBS-T for 2 hours at room temperature. The molecular weight of *ABCB1* encoded P-gp is 170 kDa, while *ABCB4* encoded P-gp is 140 kDa. Antibody C219 recognized both of the ABCB1 and ABCB4 proteins (21, 22). Detection was performed using the reagents provided in the Enhanced Chemiluminescence Plus kit.

Cytotoxicity Assay

In vitro cytotoxicity assays were performed by MTT assays as described previously (23). MTT was obtained from Sigma Chemical Co. (St. Louis, MO). Briefly, for synthetic siRNA transfection, 2×10^3 cells per well were plated in 96-well plates with RPMI 1640 containing *ABCB1* or *ABCB4* siRNA (0.1 μg per well in volume of 200 μL) with increasing concentrations of paclitaxel from 0.0001 to 1 μmol/L. For vector-based siRNA transfection, 2×10^3 cells of stable *ABCB1* or *ABCB4* transfected clones were plated in 96-well plates with RPMI 1640 containing the same increasing concentrations of paclitaxel. Untransfected SKOV-3 and OVCAR8 were used as controls. After culturing for 6 days, 10 μL of MTT (5 mg/mL in PBS) were added to each well and incubated for 4 hours. After dissolving the resulting formazan product with acid isopropanol, the absorbance was read on a BT 2000 Microkinetics Reader (Bio-Tek Instruments, Inc., Winooski, VT) at a wavelength of 490 nm. The IC_{50} is defined as the drug concentration required to reduce the $A_{490\ nm}$ to 50% of the control value. Fold reversal is the IC_{50} for paclitaxel-resistant cells divided by the IC_{50} for the siRNA-treated paclitaxel-resistant cells. The percentage of cell growth was calculated by defining the absorbance of parental cells not treated with paclitaxel as 1.0 and the value of the no-cell control to 0. Experiments were performed in triplicate. MTT cytotoxicity assays were also performed with 5 μmol/L verapamil (Sigma Chemical) using the same protocol as above. Presented results reflect at least three independent experiments performed in triplicate.

Results

ABCB1 and *ABCB4* Overexpression in Paclitaxel-Resistant Cells

The paclitaxel-resistant SKOV-3_{TR}, $OVCAR8_{TR}$, and MCF-7_{TR} ovarian cancer cell lines were evaluated for expression of *ABCB1* and *ABCB4* by Northern analysis. *ABCB1* was found to be overexpressed in all three resistant cell lines, while *ABCB4* showed modest overexpression in SKOV-3_{TR} and more significant overexpression in $OVCAR8_{TR}$ and MCF-7_{TR}. The expression of *ABCB1* and *ABCB4* in SKOV-3_{TR} and $OVCAR8_{TR}$ is shown in Figs. 1 and 3, with MCF-7_{TR} data not shown.

siRNA Inhibition of *ABCB1* and *ABCB4* mRNA Expression in Paclitaxel-Resistant Cells

To determine whether siRNA specific to the *ABCB1* or *ABCB4* gene sequences could inhibit gene expression, we first tested the synthetic siRNA and vector-based siRNA. Two different siRNAs were synthesized per gene: *ABCB1a*, *ABCB1b*, *ABCB4a*, and *ABCB4b* (see Materials and Methods for details). In addition, with the pSuppressorNeo system, two *ABCB1* and two *ABCB4* siRNA expression plasmids were created using the same sequences as defined for the

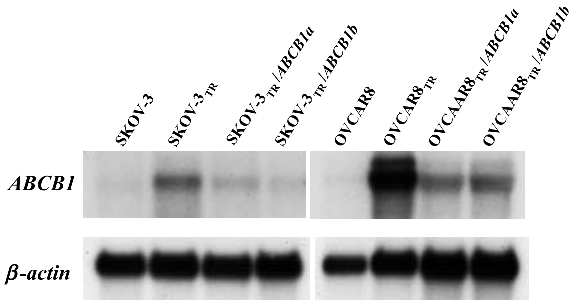

Figure 1. Northern blot analysis of *ABCB1* mRNA expression in paclitaxel-sensitive parental cell lines and resistant SKOV-3$_{\mathrm{TR}}$ and OVCAR8$_{\mathrm{TR}}$ cell lines treated with *ABCB1* synthetic siRNA. Total RNA was isolated after 48 hours of transfection. Blots were hybridized with an *ABCB1* specific cDNA probe (see Materials and Methods). β-actin hybridization was used as a loading control.

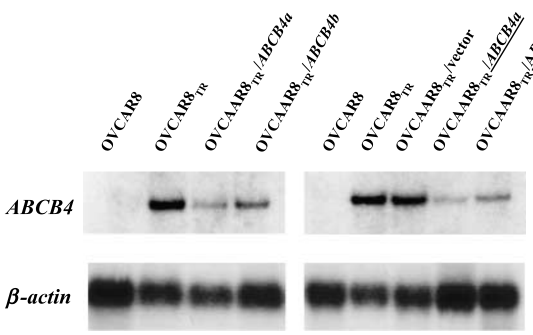

Figure 3. Northern blot analysis of *ABCB4* mRNA expression in OVCAR8$_{\mathrm{TR}}$ cells after transfection with synthetic or vector-derived siRNA (*underlined*). Total RNA was isolated after 48 hours of transfection. Blots were hybridized with an *ABCB4* specific cDNA probe. β-actin hybridization was used as a loading control.

synthetic siRNA. mRNA expression of *ABCB1* and *ABCB4* was evaluated by Northern blot analysis. The results showed that the expression of *ABCB1* was significantly decreased by addition of either the synthetic or the vector-based siRNA, although the vector-based method was less effective than the synthetic siRNA (Figs. 1 and 2). Both the SKOV-3$_{\mathrm{TR}}$ and the OVCAR8$_{\mathrm{TR}}$ cell lines revealed similar results. Targeting *ABCB4* with specific synthetic or vector-based siRNAs also decreased *ABCB4* mRNA to a similar magnitude (Fig. 3).

siRNA Inhibition of ABCB1 Protein Expression in Paclitaxel-Resistant Cells

The ABCB1 protein expression in siRNA transfected SKOV-3$_{\mathrm{TR}}$ and OVCAR8$_{\mathrm{TR}}$ cells was evaluated by Western blot analysis. As shown in Fig. 4, levels of ABCB1 protein were decreased 48 hours after transfection with the synthetic siRNA. The levels of ABCB1 proteins were also decreased after transfection with vector-based siRNA but less effectively than with the synthetic siRNA (data not shown).

Paclitaxel Resistance in *ABCB1* or *ABCB4* Transfected Cells

The *ABCB1* or *ABCB4* siRNA-mediated reversal of the paclitaxel-resistant phenotype was evaluated by comparison of the IC$_{50}$ values determined by MTT in siRNA-treated resistant and control cell lines. Cytotoxicity was measured 6 days after treatment with siRNA. As measured by MTT assay, the IC$_{50}$ values of the siRNA treated SKOV-3$_{\mathrm{TR}}$ and OVCAR8$_{\mathrm{TR}}$ cells were lower as compared with the untreated resistant lines, suggesting that *ABCB1* siRNA inhibits *ABCB1* expression and partially restores the sensitivity of these resistant cell lines to paclitaxel (Table 2). For *ABCB1*, the inhibition by synthetic siRNA was more effective than that by the vector-based siRNA. Cell lines treated with the synthesized siRNA of *ABCB1* demonstrated 7- to 12.4-fold reduction in paclitaxel resistance as compared with 4.7- to 7.3-fold reduction of resistance by transfection with vectors containing the *ABCB1* sequence. In contrast, the *ABCB4* siRNA duplex with resultant decrease in *ABCB4* RNA expression resulted in only modest reduction of paclitaxel resistance (Table 2).

Comparison of Paclitaxel Resistance Reversal by Verapamil and *ABCB1* siRNA

The paclitaxel-resistant phenotype can be reversed by the calcium channel blocker verapamil, a reagent that has been used to evaluate cells for a functioning *ABCB1*

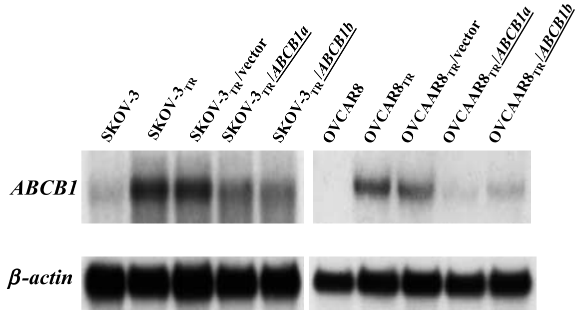

Figure 2. Northern blot analysis of *ABCB1* mRNA expression in SKOV-3$_{\mathrm{TR}}$ and OVCAR8$_{\mathrm{TR}}$ cell lines after transfection with vector-derived siRNA (*underlined*). Total RNA was isolated from stably transfected clones. Blots were hybridized with an *ABCB1* specific cDNA probe. β-actin hybridization was used as a loading control.

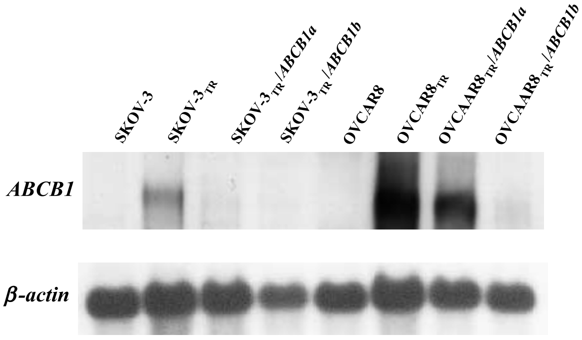

Figure 4. P-gp expression after *ABCB1* synthetic siRNA treatment of SKOV-3$_{\mathrm{TR}}$ and OVCAR8$_{\mathrm{TR}}$. Western blot analyses using a C219 monoclonal antibody for ABCB1 P-gp and a H-300 polyclonal antibody for β-actin were performed.

Table 2. Effect of synthetic and vector-based *ABCB1* or *ABCB4* siRNA on paclitaxel sensitivity

	Synthetic siRNA IC$_{50}$	Vector-Based siRNA IC$_{50}$	Verapamil IC$_{50}$
SKOV-3	0.006 ± 0.004		
SKOV-3$_{TR}$	0.410 ± 0.014		
SKOV-3$_{TR}$/vector		0.400 ± 0.028	0.022 ± 0.008 (18.6)
SKOV-3$_{TR}$/*ABCB1a*	0.033 ± 0.002 (12.4)	0.056 ± 0.016 (7.3)	
SKOV-3$_{TR}$/*ABCB1b*	0.045 ± 0.011 (9.1)	0.072 ± 0.004 (6.0)	
SKOV-3$_{TR}$/*ABCB4a*	0.322 ± 0.009 (1.3)	0.281 ± 0.021 (1.5)	
SKOV-3$_{TR}$/*ABCB4b*	0.380 ± 0.014 (1.1)	0.400 ± 0.006 (1.0)	
OVCAR8	0.008 ± 0.001		
OVCAR8$_{TR}$	0.422 ± 0.085		0.019 ± 0.012 (22.2)
OVCAR8$_{TR}$/vector		0.391 ± 0.029	
OVCAR8$_{TR}$/*ABCB1a*	0.054 ± 0.021 (7.8)	0.085 ± 0.007 (5.0)	
OVCAR8$_{TR}$/*ABCB1b*	0.064 ± 0.017 (7.0)	0.090 ± 0.012 (4.7)	
OVCAR8$_{TR}$/*ABCB4a*	0.267 ± 0.011 (1.6)	0.196 ± 0.009 (2.2)	
OVCAR8$_{TR}$/*ABCB4b*	0.235 ± 0.026 (1.7)	0.122 ± 0.025 (3.5)	

NOTE: *In vitro* cytotoxicity assays were performed by MTT assays as described in Materials and Methods. IC$_{50}$ is the concentration of paclitaxel (μmol/L) that produced 50% inhibition of cell growth. Results represent the mean ± SE of one experiment done in triplicate. Numbers in parentheses represent fold reversal of paclitaxel resistance. Results were reproduced in two additional experiments.

efflux pump. To determine whether the activity of *ABCB1* siRNA is as effective as verapamil, we compared SKOV-3$_{TR}$ exposed to synthetic *ABCB1* siRNA or to verapamil. *ABCB1* siRNA-induced reversal of paclitaxel resistance was not as strong as that seen with verapamil (Table 2).

Discussion

In this study, it is demonstrated that both synthetic and vector-based expression of siRNA can specifically reduce the expression of the *ABCB1* and *ABCB4* genes, as well as P-gp expression for *ABCB1*, in paclitaxel-resistant cells. The effectiveness of siRNA in reducing expression of *ABCB1* and *ABCB4* was observed not only in paclitaxel-resistant SKOV-3$_{TR}$ cells but also in OVCAR8$_{TR}$ cells. Two approaches were taken to test whether siRNA was able to inhibit the expression of *ABCB1* and *ABCB4*. Both synthetic and vector-based siRNAs significantly decreased the expression of *ABCB1*, although inhibition of *ABCB1* by synthetic siRNA in paclitaxel-resistant cells was more effective than inhibition by the hairpin siRNA expressed from a vector containing the U6 promoter. Several reasons may explain this discrepancy, including transfection efficiency or the level of siRNA expression. We speculate that the amount of siRNA expressed from the vector is lower than the synthetic siRNA successfully transfected. Although, theoretically, siRNA is constantly expressed from the vector, the amount of siRNA expressed from these plasmids is not easily controlled, and current technology does not accurately monitor the siRNA level inside the cells. Further study using an inducible vector system to express siRNA may improve the efficiency of vector-based siRNA gene silencing.

By comparison, *ABCB1* siRNA demonstrates a more potent reversal of paclitaxel resistance in both SKOV-3$_{TR}$ and OVCAR8$_{TR}$ as compared with *ABCB4* siRNA. The *ABCB4* gene is located on chromosome 7q21.1, 34 kb downstream of the *ABCB1* gene (24). The *ABCB1* and *ABCB4* genes are very similar. They both contain 27 introns and are inserted at identical positions in the coding sequence (24). The proteins encoded by these genes have virtually identical hydropathy plots; they are 77% identical and 82% similar in amino acid sequence (18). The *ABCB4* P-gp is normally involved in the transport of phospholipids from liver hepatocytes into bile but is also involved in the transport of paclitaxel and vinblastine, albeit inefficiently unless it is mutated (25). *ABCB4* is also inhibited by verapamil, a classic inhibitor of the ABCB1 protein, P-gp. Transfection of *ABCB4* cDNA constructs resulted in low-level resistance to the antifungal agent aureobasidin, also a substrate of *ABCB1* (26). *ABCB4* expression also correlated negatively with clinical outcome (10). We demonstrated that *ABCB4* siRNA-treated cell lines showed minor reductions in paclitaxel resistance. This result is consistent with previous data that reported lower rates of transport of paclitaxel by *ABCB4* as compared with *ABCB1* (25). The reduction in paclitaxel resistance was unlikely to be caused by changes in *ABCB1* expression because we selected *ABCB4* specific sequences for the siRNA. In addition, inhibition of *ABCB1* will lead to more robust reduction of paclitaxel resistance. The previous studies are consistent with this study, which suggests that *ABCB4* does not play as significant a role in paclitaxel resistance as *ABCB1* at least *in vitro*.

Recently, two studies of synthetic siRNA-based suppression of *ABCB1* gene expression in multidrug resistance cancer cell lines have been reported (27, 28). These studies targeted *ABCB1* and showed that *ABCB1* siRNA markedly inhibited the expression of *ABCB1* mRNA and P-gp. This study confirms those findings with different target sequences and provides new data comparing synthetic and vector-based siRNA strategies as well as additional studies evaluating *ABCB4*.

In conclusion, our data confirm the effectiveness of siRNA in inhibiting *ABCB1* expression and the subsequent reversal of resistance to paclitaxel. Moreover, *ABCB4* siRNA also demonstrates very modest inhibition of the paclitaxel-resistant phenotype. These experiments lend credence to the hypothesis that siRNA treatment may represent a new approach for the treatment of *ABCB1*-mediated drug resistance.

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