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Overexpression of sorcin, a calcium-binding protein, induces a low level of paclitaxel resistance in human ovarian and breast cancer cells

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Abstract

Paclitaxel, an antimitotic, anticancer agent, induces cell cycle arrest in the mitotic phase by binding to the β-tubulin subunit and forming highly stable microtubule polymers that resist depolymerization. The overexpression of the P-glycoprotein (P-gp) and/or alteration in the cellular microtubules is associated with the development of paclitaxel resistance. However, we have established a paclitaxel-resistant human ovarian carcinoma subline (2008/13/4) wherein the degree of resistance could not be correlated with overexpression of P-gp, alterations in the α- and β-tubulin isotypes, or changes in the drug-binding affinity of the microtubules. mRNA differential display analysis revealed the overexpression of sorcin, a calcium-binding protein in the 2008/13/4 cells. However, no detectable changes in the intracellular calcium levels were detected in the parental and the paclitaxel-resistant variant. Furthermore, co-treatment with A23187, a calcium ionophore, did not alter the cytotoxicity of paclitaxel against the parental and the paclitaxel-resistant cells. Transfection of the parental 2008 cells with full-length sorcin cDNA induced a low level (3–5-fold) of paclitaxel resistance. In addition, transfection of human breast cancer cells with the full-length sorcin cDNA also led to the induction of a low level of paclitaxel resistance in the transfectants. Although the overexpression of sorcin did not produce high levels of paclitaxel resistance, the results obtained present compelling evidence of the involvement of sorcin in developing low-level paclitaxel resistance in a variety of tumor cells. The precise biochemical mechanism(s) by which sorcin overexpression induces low-level paclitaxel resistance is currently under investigation.

Keywords: Sorcin overexpression; Atypical paclitaxel resistance; Calcium independent; Human ovarian; Breast carcinoma cells

1. Introduction

Ovarian cancer is one of leading causes of mortality in the United States. Platinum-containing drugs initially are effective in the treatment of this disease. However, the development of resistance to these drugs often occurs. Paclitaxel, an antimitotic agent, has shown a greatly improved therapeutic index against primary ovarian cancers [1–3]. Isolated from the bark of the Western Pacific yew *Taxus brevifolia* [4], paclitaxel has been shown to possess significant preclinical and clinical antitumor activity against a variety of malignancies [1–3,5]. This anticancer drug has a unique mechanism of action: unlike other mitotic spindle poisons that induce disassembly of microtubules, paclitaxel binds to the N-terminal end of β-tubulin and promotes tubulin assembly [6–8]. Thus, drug binding promotes the formation of stable microtubule polymers that resist depolymerization and result in mitotic arrest [6–8].

For paclitaxel to be employed rationally in the clinic, it is important to understand the mechanisms by which a tumor cell can develop the ''paclitaxel resistance phenotype''. Resistance to paclitaxel has been associated with

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Abbreviations: P-gp, P-glycoprotein; MDR, multidrug resistant; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Fluo-3/AM, Fluo-3 acetomethoxyester; RT-PCR, reverse transcription-polymerase chain reaction; PVDF, polyvinyl difluoridine; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; G418, geneticin.

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either overexpression of the P-gp (a drug efflux pump that induces the multidrug resistant (MDR) phenotype [9,10]), alterations in the binding of the drug to microtubules, or a decrease in the levels of polymerized tubulin [11–22].

Earlier studies by us and others have shown that rodent tumor cells (rat, mouse, and hamster) display a markedly lower sensitivity to paclitaxel and other natural product drugs than human tumor cells [23–26]. This species-specific difference in paclitaxel sensitivity probably is due to a defect in the transport of paclitaxel in the rodent cells. Sarris *et al.* [27] reported a lack of clinical response in paclitaxel-resistant non-Hodgkin's lymphoma patients treated with a combination of cyclosporin A (a known inhibitor of P-gp function) and paclitaxel, suggesting that P-gp-mediated drug efflux was unlikely to be the sole cause of paclitaxel resistance. Some cisplatin-resistant (P-gp negative) human ovarian carcinoma cells also have been reported to be markedly more sensitive to paclitaxel than the parental cisplatin-sensitive human ovarian carcinoma cells [28,29]. Thus, it is apparent that sensitivity and/or resistance of tumor cells to the cytotoxic action of paclitaxel can be mediated by different mechanisms; some are known and others have yet to be identified.

Considering the potential utility of paclitaxel in the treatment of primary and cisplatin-resistant ovarian cancers (as well as in the treatment of other tumors), it is of great clinical importance to elucidate the different mechanisms by which a tumor cell develops resistance to paclitaxel. We have produced, by sequential exposure to increasing concentrations of paclitaxel, human ovarian carcinoma cells resistant to paclitaxel that do not overexpress any drug transport proteins [30]. Furthermore, these atypical paclitaxel-resistant ovarian carcinoma cells do not display an alteration in the expression of various α- and β-tubulin isotypes, nor do the microtubules have decreased binding affinity for paclitaxel [30]. Utilizing mRNA differential display analysis, we have determined which genes are differentially expressed in these atypical paclitaxel-resistant human ovarian carcinoma cells. Sorcin, a calcium-binding protein, was found to be up-regulated in the 2008/13/4 cells as compared with the parental 2008 cells. In this study, we present evidence that suggests that overexpression of sorcin is responsible, in part, for the observed paclitaxel resistance of the 2008/13/4 cells.

2. Materials and methods

2.1. Chemicals

Paclitaxel was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH. It was dissolved in DMSO at a final concentration of 20 mM, and the concentration of the solvent never exceeded 0.1% in the experimental protocol. Thapsigargin and A23187 were purchased from Calbiochem, and MTT was purchased from the Sigma Chemical Co. Fluo-3 acetomethoxyester (Fluo-3/AM) was purchased from Molecular Probes Inc. [α-^{33}P]dATP (2000 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) was purchased from Dupont-NEN Research Products. The RNAimage kits (1–10) and the MessageClean Kit were purchased from the GenHunter Corp. The AmpliTaq DNA polymerase (5 U/μL) was purchased from the Perkin-Elmer Corp. The multiprime DNA labeling system was obtained from the Amersham Corp. The sorcin polyclonal antibody was provided by M. Myers, Albert Einstein School of Medicine. The alkaline phosphatase conjugated anti-rabbit monoclonal antibody was obtained from Jackson Immunoresearch Laboratories, Inc. The eukaryotic expression vector pCR3.1 was purchased from Invitrogen.

2.2. Cell culture conditions

The parental (2008) and paclitaxel-resistant (2008/13/4 and 2008/17/4) human ovarian carcinoma cells were maintained in RPMI medium as described previously [30]. The human ovarian carcinoma cell line (2780) was obtained from T. Hamilton, Fox Chase Cancer Center, and was maintained in RPMI medium as described previously [25]. The breast carcinoma cell lines (MCF-7 and MDA-MB435S) were obtained from ATCC and maintained as per the instructions of the supplier.

2.3. Differential display analysis

Total RNA from parental 2008 cells, ''atypical'' paclitaxel-resistant 2008/13/4 cells, and ''typical'' paclitaxel-resistant 2008/17/4 cells were used as templates for the differential display analysis [31–35]. RNA extracted from the 2008/17/4 cells was used in this analysis as a ''control'' for visualizing differentially expressed genes in a ''typical'', P-gp positive, paclitaxel-resistant cell. It is our contention that the differentially expressed genes that are common to both the 2008/17/4 cells (typical paclitaxel-resistant cells) and the 2008/13/4 cells (atypical paclitaxel-resistant cells) will be of minimal significance as a candidate gene(s) responsible for the atypical paclitaxel resistance observed in the 2008/13/4 cells. Furthermore, to assess the reproducibility of the differential display pattern, DNA-free total RNA was prepared from two different cultures of each cell line at two different time points. The reverse transcription-polymerase chain reaction (RT-PCR) reactions (using these two different RNA preparations from each cell line) in the presence of each primer pair combination were performed simultaneously. A gene was considered as being differentially expressed only if its presence/absence was altered markedly (>2–3-fold) in both RNA preparations from each cell line. These ''quality control'' features were adopted to reduce the number of false positives and allow us to identify genes that are

differentially expressed, exclusively in the 2008/13/4 cell line.

Total RNA from 2008 cells and from the paclitaxel-resistant 2008/13/4 and 2008/17/4 cells was extracted using the RNAzol B reagent (Tel-Test Inc.). To ensure removal of contaminating DNA, the RNA samples were treated with RNase-free DNase I (Gibco-Brl) in the presence of 20 mM Tris–HCl (pH 8.3), 50 mM KCl, and 2.5 mM $MgCl_2$ for 15 min at room temperature. DNase I was inactivated by the addition of EDTA (2.5 mM) and heating for 10 min at 65°. The RNA was re-extracted using standard techniques and resuspended at a final concentration of 0.1 mg/mL.

The entire mRNA differential display protocol was performed essentially as described by the RNAimage kit supplier (GenHunter Corp.) using [α-^{33}P]dATP (2000 Ci/mmol) as the radiolabeled nucleotide.

The absence/presence of the differentially displayed cDNA fragments observed in the denaturing polyacrylamide gels was verified by Northern blotting, using the differentially expressed cDNA as a probe that was radiolabeled using the random priming labeling method. Northern blotting was performed as described earlier [30].

After confirming that the unique cDNA fragment was differentially expressed, it was subcloned using the pCR-TRAP™ cloning system (GenHunter Corp.) and subjected to DNA sequencing using the dideoxy chain termination method. Then the nucleotide sequence of the insert was compared with known sequences in the GenBank database, using the GCG software.

2.4. Western blotting analysis of sorcin expression

The parental 2008 and the paclitaxel-resistant 2008/13/4 and 2008/17/4 cells were lysed in 1% Triton X-100 in a 50 mM Tris–HCl buffer (pH 7.2). The cytosolic supernatant was separated from the cellular debris by centrifugation at 14,000 *g* for 20 min at 4°. The protein content of the cytosol fraction was determined by the Coomassie Brilliant Blue dye-binding assay using the commercially available Bio-Rad Protein Assay reagent (Bio-Rad). Aliquots of the cytosol fraction containing 50 μg protein were subjected to electrophoresis on a 12% (w/v) SDS-polyacrylamide gel. After electrophoretic separation, the proteins from the gels were transferred onto polyvinyl difluoridine (PVDF) nylon membranes with a semidry blotter as described previously [30]. The nylon membranes were blocked with 5% (w/v) nonfat dried milk in 25 mM Tris–HCl (pH 7.4), 150 mM NaCl (TBS) for 1 hr and then washed with three changes of the TBS buffer containing 0.05% Tween 20 (TBST). Then the membranes were probed with the antibody raised against sorcin at a 1:5000 dilution in the TBST buffer for 1 hr at room temperature. After thoroughly washing the membranes with TBST, the blots were incubated with an alkaline phosphatase-conjugated anti-rabbit antibody in TBST for 1 hr at room temperature. At the end of the time period, the blots were washed thoroughly in TBST and then stained with an alkaline phosphatase detection kit (Bio-Rad).

2.5. Southern blotting analysis of the sorcin gene

High molecular weight genomic DNA was isolated from exponentially growing 2008, 2008/13/4, and 2008/17/4 cells using phenol–chloroform extraction and ethanol precipitation. The DNA (20 μg) was then digested overnight at 37° using 30 unit of *Eco*RI. The digested DNA was subjected to electrophoresis in a 0.8% (w/v) agarose/1× Tris–borate-EDTA gel at a constant voltage of 1 V/cm. After depurination and denaturation, the separated DNA was transferred onto Nytran membranes, and Southern blot analysis was performed as described [36] using the 300 bp sorcin cDNA obtained from the differential display analysis.

2.6. Isolation of full-length sorcin cDNA

The full-length sorcin cDNA was isolated by RT-PCR using the total RNA extracted from 2008/13/4 paclitaxel-resistant cells as a template and a sense primer (1–20) and a reverse primer (837–862) (the primer sequence information was obtained from GenBank Accession No. G13283). The RT reaction was performed using 100 ng of total RNA, 200 U of MMLV reverse transcriptase, 100 nM oligo-dT as primer, 1.0 mM dNTPs in a buffer containing 50 mM Tris–HCl (pH 8.3), 50 mM KCl, and 10 mM $MgCl_2$ (Perkin-Elmer) at 37° for 1 hr. A one-fifth volume of the RT reaction was then used for the PCR reaction containing the sense and reverse primers (250 ng per reaction) for sorcin, Amplitaq DNA polymerase (0.2 U per reaction), 200 μM dNTP, 50 mM Tris–HCl (pH 8.3), and 2.0 mM $MgCl_2$ (Perkin-Elmer). The PCR conditions were: an initial denaturation at 99° for 5 min followed by 25 cycles of 94° for 1 min, 55° for 1 min, and 72° for 1 min. The last cycle consisted of elongation at 72° for 5 min. The RT-PCR products were electrophoresed on a 1.5% (w/v) agarose gel, and the sorcin band (862 bp) was visualized by ethidium bromide (5 μg/mL) staining. The individual sorcin band was gel purified using the Qiagen kit and cloned into the pCR3.1 vector as per the instructions of the manufacturer. The full-length cDNA was sequenced to ascertain its identity with published human sequences and thereafter was used for transfection experiments as outlined below.

2.7. Effect of paclitaxel treatment on intracellular Ca^{2+} levels in parental and paclitaxel-resistant ovarian carcinoma cells

The cytosolic Ca^{2+} levels were measured with the fluorescent indicator dye Fluo-3/AM, excited by a 488-nm krypton:argon laser using a Nikon Eclipse TE300

inverted epifluorescence microscope connected to a photo multiplier detection system (Photon Technology International) before and after treatment with paclitaxel and thapsigargin. Thapsigargin is a specific and irreversible inhibitor of the ATP-dependent calcium pump (sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pumps) present in the endoplasmic reticulum [37]. Thapsigargin treatment leads to inhibition of the SERCA pump and release of the endoplasmic reticulum Ca^{2+} stores into the cytosol, causing a sustained elevation of the cytosolic [Ca^{2+}] [38]. The fluorescence signal was acquired and processed by photomultiplier tubes that were connected to a Digi-data interface, using Clampex 8.0 software (Axon Instruments Inc.). All measurements were performed at room temperature. Tumor cells were plated at a density of 0.5×10^3 in a 35-mm dish in growth medium and allowed to grow for 48–72 hr. At the end of this time period, several colonies consisting of approximately 8–12 cells were visible in each of the cell lines studied. The cells were loaded for 1 hr with 5 μM Fluo-3/AM and then washed extensively with dye-free growth medium. The background fluorescence (of the medium) was measured first, and then the fluorescence associated with an untreated colony of cells was ascertained. Thereafter, growth medium containing 500 nM paclitaxel, 2.5 μM paclitaxel, and 100 nM thapsigargin were added sequentially to the plate, and the changes (if any) in the fluorescence emission from the same colony of cells (as above) were measured for 2 min between each drug addition. The relative change in cytosolic calcium was calculated as a ratio ($\Delta F/F_0$) of the treatment-associated Fluo-3 emission subtracted from the growth medium-associated baseline Fluo-3 emission over the untreated sample fluorescence subtracted from the growth medium-associated fluorescence.

2.8. Transfection of the full-length sorcin cDNA in human ovarian and breast cancer cells

Subconfluent cells (2×10^5/60-mm dish) were transfected with pCR3.1 vector alone or the pCR3.1 vector with the full-length sorcin insert (2 μg/mL) using the Lipofectamine reagent (Gibco-Brl). Cells were propagated in a medium containing geneticin (700 μg/mL; geneticin (G418) sulfate) for 3 weeks. Individual G418-resistant colonies were picked, propagated, and screened for the constitutive expression of sorcin by the RT-PCR assay using the primers and conditions described for isolation of the full-length sorcin cDNA.

2.9. Cytotoxicity studies

The paclitaxel sensitivity of parental 2008 and 2780 human ovarian carcinoma cells and the full-length sorcin cDNA transfected 2008 and 2780 cells was assessed using the tetrazolium dye (MTT) method as described earlier [25,30]. The sensitivity of the parental and sorcin-transfected human breast carcinoma cells (MCF-7 and MDA-MB435S) was assessed by colony-forming assays. Briefly, cells (100–200/60-mm dish) were plated in fresh medium and allowed to attach for 24 hr at 37° in 5% CO_2. Then various concentrations of paclitaxel (0.5–100 nM) were added to individual dishes, and the plates were incubated as before for a further 72 hr. At the end of the time period, the medium containing paclitaxel was aspirated, and fresh medium was added. The cells were allowed to grow for a further 8–12 days (for a total of 12–16 days). Then the medium was aspirated, and each of the dishes was washed with phosphate-buffered saline. The cell colonies (at least 50 cells per clone) were stained with 0.2% (w/v) methylene blue and counted. The paclitaxel IC_{50} value (defined as the concentration of paclitaxel that inhibited cell growth by 50%) for each cell line was calculated by linear regression analysis. Each of the cell lines was exposed to various concentrations of paclitaxel in triplicate, and each experiment was repeated at least three times.

2.10. Statistical analysis

The linear regression analysis was performed using the SigmaStat Statistical Analysis System, Version 1.01.

3. Results

We have established two series of paclitaxel-resistant cell lines designated 2008/13/4 and 2008/17/4 from a human ovarian carcinoma cell line (2008) [30]. The 2008/17/4 cells display a classical MDR-phenotype with an increased expression of the drug-efflux protein P-gp [30]. In contrast, the 2008/13/4 cells express very low levels of P-gp; they have no obvious alterations in the expression of various tubulin isotypes, nor are their microtubules defective in paclitaxel binding [30]. To identify the genetic changes responsible for the atypical paclitaxel-resistant phenotype, we performed mRNA differential display analysis using total RNA extracted from the parental 2008 cells, and the paclitaxel-resistant 2008/13/4 as well as the 2008/17/4 cells. The RNA from 2008/17/4 cells was used in this analysis as a ''control'' for visualizing differentially expressed genes in a ''typical'', P-gp positive, paclitaxel-resistant cell line. It was our belief that the differentially expressed genes that were common to both the 2008/17/4 cells (typical paclitaxel-resistant cells) and the 2008/13/4 cells (atypical paclitaxel-resistant cells) would be of little interest as candidate genes for the atypical paclitaxel resistance observed in the latter.

One of the candidate genes up-regulated in the 2008/13/4 cells is shown in Fig. 1A, lane 2 (arrow). The expression of this gene was restricted to the 2008/13/4 cells, as negligible levels were observed in both the 2008 (Fig. 1, lane 1) and the 2008/17/4 cells (Fig. 1, lane 3). As presented below, upon DNA sequencing, this gene was

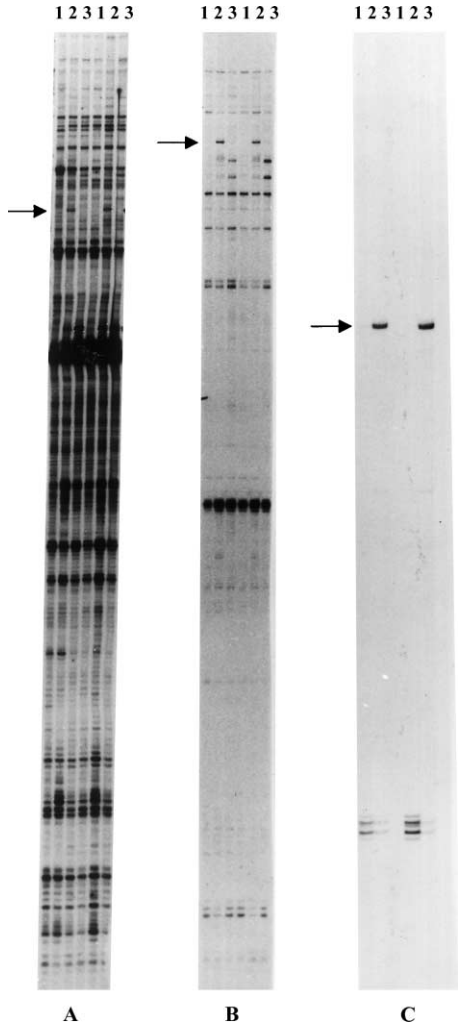

Fig. 1. Identification of differentially expressed genes in 2008/13/4 cells. Differential mRNA display (in duplicate) of parental and paclitaxel-resistant 2008 cells was performed using total RNA isolated from two different cultures of 2008 (lane 1), 2008/13/4 (lane 2), and 2008/17/4 (lane 3) cells, reverse transcribed, and followed by PCR in the presence of [α-^{33}P]dATP. The PCR fragments were displayed on 6% denaturing polyacrylamide gels and autoradiographed as detailed in Section 2. The primer pairs utilized for differential display were: (A) H-T11A (5′-AAGCTTTTTTTTTTA-3′) and AP77 (5′-AAGCTTTGAATTC-3′); (B) H-T11A (5′-AAGCTTTTTTTTTTA-3′) and AP58 (5′-AAGCTTAACTGAG-3′); and (C) H-T11A (5′-AAGCTTTTTTTTTTA-3′) and AP72 (5′-AAGCTTTCAAAGA-3′).

identified as being 100% homologous to sorcin, the calcium-binding protein. Similarly, the mRNA expression profile displayed using two other primer pair combinations also identified sorcin as being expressed exclusively in the 2008/13/4 cells (Fig. 1B and C, lane 2) as compared with its expression in the 2008 cells (Fig. 1B and C, lane 1) and in the 2008/17/4 cells (Fig. 1B and C, lane 3).

The cDNA from the differentially expressed band (Fig. 1A, lane 2, approximately 300 bp in size) was extracted, re-amplified, and subjected to electrophoresis to ascertain its presence after re-amplification. The cDNA was then labeled and used as a probe in Northern blotting procedures. It hybridized to a mRNA (of approximately

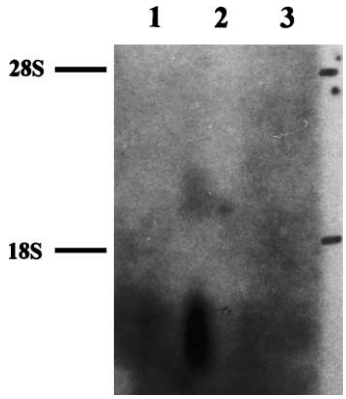

Fig. 2. Northern blot analysis to confirm the differential expression of cDNA observed in Fig. 1A–C. Total RNA (20 μg) was isolated from parental and paclitaxel-resistant cells and subjected to Northern blotting utilizing the randomly primed cDNA (from Fig. 1A) as probe. A positive signal (below the 18S band) was observed in lane 2 containing total RNA from the 2008/13/4 cells. Lane 1, parental 2008 cells; lane 3, 2008/17/4 cells.

800 bases) from the 2008/13/4 cells (Fig. 2, lane 2), while no hybridization was observed with mRNAs from the parental 2008 cells (Fig. 2, lane 1) and the 2008/17/4 cells (Fig. 2, lane 3). This confirmed the results obtained with the differential display analysis (Fig. 1).

This cDNA was then cloned and subjected to automated sequencing. The sequence of the cDNA exhibited 100% homology to the 3′-end of the human sorcin gene (from nucleotide 530–794 when compared with the sequence in GenBank Accession No. L12387). Similar results were obtained when the cDNAs extracted from gels shown in panels B and C of Fig. 1 were subjected to automated sequencing (nucleotides 518–788 and nucleotides 426–788, respectively, when compared with the sequence of sorcin in GenBank Accession No. L12387; data not shown).

Western blot analysis using a polyclonal antibody raised against hamster sorcin (provided by M. Meyers, Albert Einstein College of Medicine) also demonstrated its overexpression only in the 2008/13/4 cells (Fig. 3, lane 2; M_r 22 kDa). The identity of the lower band visible in all three lanes is not known at present and probably represents nonspecific binding by the sorcin polyclonal antibody.

Although karyotyping of the Geimsa-stained metaphase chromosome spreads from 2008/13/4 cells failed to reveal the presence of double minute chromosomes or homogenous staining regions, both hallmarks of gene amplification (unpublished observations), Southern blotting demonstrated that the sorcin gene was amplified only in the 2008/13/4 cells (Fig. 4, lane 2; cDNA extracted from gel shown in Fig. 1A was used as the probe). In contrast, the P-gp gene was not amplified in any of the cell lines (2008, 2008/13/4, and 2008/17/4) tested (data not shown).

The calcium-binding properties of sorcin have been amply demonstrated, both directly as well as indirectly, by analyzing its structural motifs. Thus, we investigated

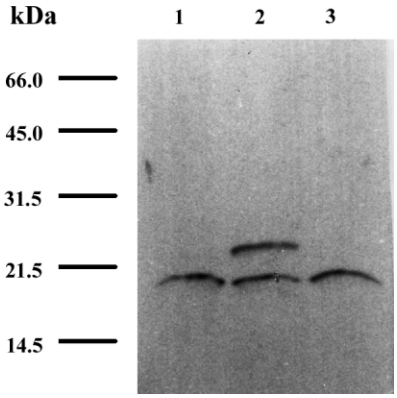

Fig. 3. Western blot analysis of sorcin expression. Cytosolic proteins (50 μg/lane) from parental and paclitaxel-resistant cells were subjected to western blotting using a polyclonal antibody against sorcin at a 1:2000 dilution. Lane 1, 2008 cells; lane 2, 2008/13/4 cells; lane 3, 2008/17/4 cells. Sorcin expression (band in lane 2 at approximately 22 kDa) was observed only in the 2008/13/4 cells. Migration of the molecular mass markers (in kDa) is indicated to the left.

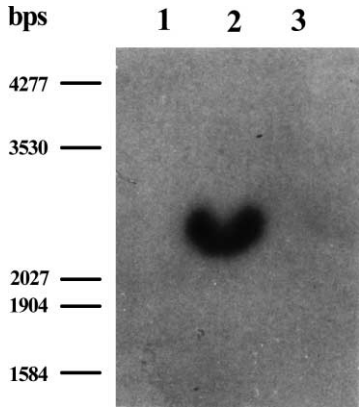

Fig. 4. Southern blotting to assess sorcin gene amplification. Genomic DNA (20 μg per lane) from parental and paclitaxel-resistant cells was digested with 30 U of *Eco*RI overnight and then subjected to Southern blotting using randomly primed cDNA (purified from the gel shown in Fig. 1A) as probe. A positive signal was observed in the lane containing DNA from 2008/13/4 cells (lane 2), whereas no signal was observed in the lanes containing DNA from 2008 (lane 1) and 2008/17/4 (lane 3) cells, after a 4-hr exposure. Longer exposures (>24 hr) displayed a positive signal in the lanes containing DNA from the 2008 and 2008/17/4 cells (unpublished observation).

whether the treatment with paclitaxel would produce a differential alteration in the level of cytosolic-free calcium in the atypical paclitaxel-resistant ovarian carcinoma cells compared with the parental cells. Thapsigargin, an irreversible inhibitor of SERCA-type Ca^{2+} pumps [37], was utilized in these studies to demonstrate that an increase in the cytosolic Ca^{2+} (due to release from intracellular stores) does occur following thapsigargin treatment. Treatment with either 500 nM or 2.5 μM paclitaxel did not alter the levels of cytosolic-free calcium in the parental and the paclitaxel-resistant human ovarian carcinoma cells (data not shown). In contrast, treatment with thapsigargin (100 nM) caused a rapid and sustained elevation (3-fold) in the cytosolic-free calcium in the 2008 as well as the 2008/13/4 cells, indicating that drug-induced perturbation can result in the release of Ca^{2+} from intracellular stores causing an elevation of cytosolic calcium. These results suggest that, although sorcin is overexpressed in the paclitaxel-resistant cells, an altered level of cytosolic-free calcium (due to release from intracellular stores) in response to paclitaxel treatment is not associated with the drug-resistant phenotype of the 2008/13/4 cells.

We also investigated the effects of increasing the intracellular Ca^{2+} concentration (via treatment with a calcium ionophore, A23187) on paclitaxel cytotoxicity. Pretreatment with A23187 (10 nM) did not alter the paclitaxel sensitivity of the parental (2008) or that of the paclitaxel-resistant (2008/13/4) cells (Table 1). Similar results were obtained with simultaneous exposure of the 2008 and the 2008/13/4 cells to A23187 and paclitaxel (data not shown).

The role of sorcin in the MDR phenomenon is debatable [39–43]. Thus, an investigation of whether sorcin overexpression can produce paclitaxel resistance was initiated. Transfection of a full-length sorcin cDNA into the parental 2008 cells was performed. The transfectants (19 individual clones) were subjected to a growth inhibition assay in the presence of various concentrations of paclitaxel. As shown in Table 2, 9 of the 19 clones were found to be between 3- and 5-fold resistant to paclitaxel, and this correlated well with the level of sorcin expression (as determined by RT-PCR analysis, Fig. 5) in these clones. The RT-PCR results for sorcin expression in the transfected cells should be viewed with caution, because using the polyclonal antibody we were unable to detect the expression of sorcin in these cells. Additionally, it is also likely that the low levels

Table 1
Effect of a 24-hr pretreatment of the 2008 and 2008/13/4 cells with the calcium ionophore A23187 on paclitaxel cytotoxicity

Cells	IC_{50} (nM)		Fold-sensitization
	Paclitaxel alone	A23187 + paclitaxel	
2008	13.6 ± 1.7	11.9 ± 3.6	1.1
2008/13/4	8100.01 ± 1100.0	7700.0 ± 2300.0	1.0

Paclitaxel sensitivity of the 2008 and the 2008/13/4 cells in the presence and absence of A23187 (10 nM) was determined by the MTT assay [30]. The concentration of A23187 (10 nM) utilized did not inhibit cell growth by itself. Data presented are means of three separate experiments, each performed in triplicate. The IC_{50} values (means ± SD; defined as the concentration of paclitaxel required to kill 50% of the cells) were calculated using linear regression analysis.

Table 2
Paclitaxel sensitivity of 2008 cells transfected with full-length sorcin cDNA

Cells	Paclitaxel, IC_{50} (nM)	Fold-resistance
2008	11.1 ± 2.4	–
2008/13/4	7600.0 ± 1900	–
2008/pCR3.1	10.6 ± 1.6	0.95
2008/sor6	51.7 ± 4.6*	4.7
2008/sor7	44.6 ± 2.3*	4.0
2008/sor8	39.0 ± 4.3*	3.5
2008/sor15	30.0 ± 1.5*	2.7
2008/sor16	32.4 ± 2.6*	2.9
2008/sor17	59.1 ± 4.1*	5.3
2008/sor18	56.0 ± 2.8*	5.0
2008/sor19	29.1 ± 3.9*	2.6
2008/sor20	31.6 ± 1.8*	2.8

Paclitaxel sensitivity of individual sorcin-transfected 2008 clones was assessed by the MTT assay as described previously [30]. Each clone was analyzed in two separate experiments, each performed in triplicate. The IC_{50} values (means ± SD; defined as the concentration of paclitaxel required to kill 50% of the cells) were calculated using linear regression analysis. Fold-resistance was defined as the IC_{50} value of the sorcin-transfected clone/IC_{50} value of parental 2008 cells.

* $P < 0.001$ compared with the paclitaxel IC_{50} against 2008 cells.

of paclitaxel resistance observed in the sorcin-transfected cells could be a direct result of low levels of sorcin expression in these clones, and an increase in the sorcin levels could presumably increase the degree of resistance in the transfected cells. The remaining 10 clones that expressed either none or low levels of sorcin were all sensitive to paclitaxel.

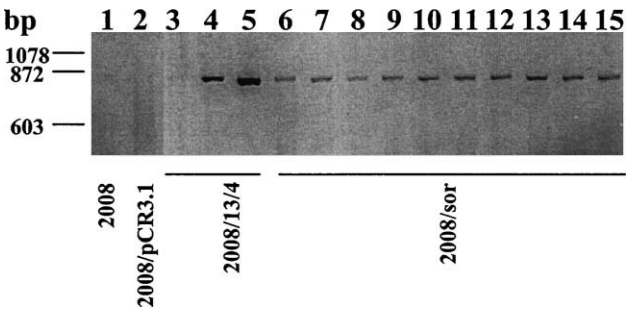

Fig. 5. Semiquantitative RT-PCR analysis of the levels of sorcin expression in the paclitaxel-resistant 2008 cells transfected with a full-length sorcin cDNA. Total RNA (1 μg) from parental 2008 cells, paclitaxel-resistant 2008/13/4 cells, 2008 cells transfected with vector alone, and 2008 cells transfected with full-length sorcin cDNA was subjected to reverse transcription using oligo dT primers, and a one-fifth volume (equivalent to 200 ng of RNA; except where indicated) of the RT reaction was amplified by PCR using primers described in Section 2. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The level of sorcin expression (as determined by PCR product intensity) in the transfected cells was compared to the expression of sorcin observed in the 2008/13/4 cells. Lane 1, 2008; lane 2, 2008/pCR3.1; lane 3, 2008/13/4 (20 ng); lane 4, 2008/13/4 (100 ng); lane 5, 2008/13/4 (200 ng); lane 6, 2008/sor6; lane 7, 2008/sor7; lane 8, 2008/sor8; lane 9, 2008/sor12; lane 10, 2008/sor15; lane 11, 2008/sor16; lane 12, 2008/sor17; lane 13, 2008/sor18; lane 14, 2008/sor19; and lane 15, 2008/sor20.

Table 3
Etoposide and vincristine sensitivity of 2008 cells transfected with full-length sorcin cDNA

Cells	Etoposide, IC_{50} (μM)	Fold-resistance	Vincristine, IC_{50} (nM)	Fold-resistance
2008	1.6 ± 0.3	–	31.9 ± 4.9	–
2008/13/4	59.0 ± 8.0	37	7500.0 ± 900.0	235
2008/sor6	1.5 ± 0.6	1.0	10.9 ± 3.3*	0.3
2008/sor7	2.1 ± 0.3	1.3	68 ± 0.4*	0.2
2008/sor8	1.7 ± 0.3	1.0	99 ± 0.7*	0.3

Etoposide and vincristine sensitivity of individual sorcin-transfected 2008 clones was assessed by the MTT assay as described previously [30]. Each clone was analyzed in two separate experiments, each performed in triplicate. The IC_{50} values (means ± SD; defined as the concentration of drug required to kill 50% of the cells) were calculated using linear regression analysis. Fold-resistance was defined as the IC_{50} value of the sorcin-transfected clone/IC_{50} value of parental 2008 cells.

* $P < 0.005$ compared with the vincristine, IC_{50} against the parental 2008 cells.

Our preliminary studies had demonstrated that the atypical paclitaxel-resistant 2008/13/4 cells were cross-resistant to etoposide and vincristine. Thus, we investigated the effect of constitutive overexpression of sorcin further, in three of the paclitaxel-resistant transfectants, on their etoposide and vincristine sensitivity. As shown in Table 3, sorcin overexpression did not lead to resistance to etoposide and vincristine. In fact, the sorcin-transfected paclitaxel-resistant clones were found to have a significantly increased sensitivity (3–5-fold) to the cytotoxic effects of vincristine. The observation that the sorcin-transfected cells were sensitized to vincristine is intriguing. However, it should be noted that the fold-resistance to vincristine was reduced in the 2008/13/4 cells from the 420-fold resistance observed in the 2008/13/2 cells [30]. This decrease in vincristine resistance was observed concomitantly with increased expression of sorcin in the 2008/13/4 cells.

To determine whether the association of sorcin expression with low level paclitaxel resistance was cell line and/or tissue specific, an additional human ovarian tumor cell line (2780) and human breast cancer cell lines (MCF-7 and MDA-MB435S) were transfected with the full-length sorcin cDNA. Individual clones from each of the transfected cell lines were grown separately, and their sensitivity to paclitaxel was determined (data not shown). Paclitaxel-resistant clones were generated upon transfection of the full-length sorcin cDNA into the breast cancer cells (MDA-MB435S); four of the seven transfected clones were found to be between 3- and 6-fold resistant to paclitaxel. This correlated with the level of sorcin expression (as assessed by RT-PCR analysis) in these clones. However, sorcin overexpression in the human ovarian carcinoma cells (2780) and the estrogen receptor positive breast carcinoma cell line (MCF-7) did not produce any paclitaxel-resistant clones. The reason(s) for the difference in the paclitaxel sensitivity of the two breast cancer cell lines transfected with sorcin (and demonstrating increased protein expression) is

unclear at the present time, but a difference in the estrogen receptor status of these cells could presumably be an important factor in the response of tumor cells (overexpressing sorcin) to paclitaxel.

4. Discussion

In this study, we have demonstrated that sorcin, a calcium-binding protein is overexpressed (as a result of gene amplification) in a human ovarian carcinoma cell line (2008/13/4) in the absence of overexpression and/or amplification of the P-gp. Furthermore, we have also shown that constitutive overexpression of sorcin (via transfection of a full-length sorcin cDNA) in a variety of tumor cell lines can lead to the development of low levels (2.5–6-fold) of paclitaxel resistance.

Paclitaxel has been shown to bind to microtubules, resulting in the formation of highly stable polymers that resist depolymerization [6–8]. This stability causes mitotic arrest and ultimately cell death. Historically, overexpression of the drug efflux protein P-gp and/or alterations/mutations in the tubulins have been identified as the major mechanisms by which tumor cells become resistant to paclitaxel [9]. More recently, alterations in several signal transduction effector molecules and apoptosis-associated proteins that act either in a p53-dependent or -independent manner have been observed in a variety of *in vitro* tumor cells resistant to paclitaxel [44].

In the case of the atypical paclitaxel-resistant 2008/13/4 cells, we have demonstrated previously that expression of P-gp is negligible (further confirmed by the levels of intracellular radiolabeled paclitaxel in the 2008/13/4 cells that were similar to those observed in the parental 2008 cells [30]). In addition, the paclitaxel-binding affinity of the microtubules from the resistant (2008/13/4) cells was similar to that observed in those derived from the parental cells [30]. Furthermore, no alterations in the levels of Bcl-2, Bax and $Bclx_{(s/l)}$ (when compared with the parental 2008 cells) were observed (unpublished observations). Yet the 2008/13/4 cells were resistant to paclitaxel and displayed a multidrug-resistant phenotype that was distinct from the ''classical'' MDR phenotype. Thus, the 2008/13/4 cells were only marginally resistant to adriamycin (2.7-fold), although resistance to etoposide (37-fold) and vincristine (260-fold) was observed [30].

Differential display analysis revealed that sorcin, a calcium-binding protein, was overexpressed in the atypical paclitaxel-resistant 2008/13/4 cells as compared with its expression in the parental 2008 cells. As demonstrated by Southern blotting, sorcin gene amplification was the most likely cause of this overexpression. Increased expression of sorcin, a 22-kDa calcium-binding protein (a result of sorcin gene amplification), has been demonstrated in several MDR cell lines [39–42]. The amplification of the gene that encodes sorcin is thought to be tightly associated with the amplification of the P-gp gene (*mdr1*). However, to date the contribution of sorcin to the MDR phenomenon has been questionable. DNA amplification analysis indicates that the development of the MDR phenotype observed upon selection of tumor cells with natural product anticancer drugs occurs due to P-gp overexpression and that overexpression of the other genes (like sorcin) occurs only because they are located in close proximity to the MDR gene [39]. However, overproduction of sorcin is not always observed in conjunction with *mdr1* gene amplification. In fact, studies using the vincristine-resistant HOB1 lymphoma cell lines revealed that the sorcin gene was amplified upon exposure of parental HOB1 cells to a high concentration of vincristine, but this phenomenon was not related to *mdr1* gene amplification in the drug-resistant cells [40–42]. In addition Beyer-Sehlmeyer *et al.* [43], have recently demonstrated increased expression of sorcin in drug-resistant tumor cell lines of hematopoietic origin without a concomitant increase in the expression of P-gp. These conflicting results suggest that sorcin might be independently involved in mediating resistance to vincristine. These observations correlate well with our studies, wherein overexpression of sorcin was observed only in the paclitaxel-resistant cells (2008/13/4) that have very low levels of P-gp expression, have no defect in paclitaxel transport, and yet are resistant to paclitaxel. These results present compelling evidence that sorcin may play a role in the development of paclitaxel resistance in the 2008/13/4 cells.

Indeed, forced expression of sorcin in a variety of tumor cell lines (human ovarian and breast cancer) led to the induction of 2.5–6-fold paclitaxel resistance in these cells. Interestingly, some of the sorcin-transfected human ovarian carcinoma clones (Table 3) were found to be more sensitive (3–5-fold) to the cytotoxic effects of vincristine. Clinically, these observations raise some important questions regarding the role of sorcin in the development of paclitaxel resistance and possibly to other natural product drugs. Experimental studies aimed at elucidating the mechanisms by which resistance is produced compare parental cells with a highly drug-resistant cell population. Tumor cells with a relatively low degree of resistance that emerge rapidly and may possess different resistance mechanisms have been largely ignored. In addition, the development of low levels of paclitaxel resistance upon overexpression of sorcin has important clinical ramifications considering that patients are treated with the maximally tolerated dose of paclitaxel and generation of even a 2–7-fold resistant tumor subpopulation will lead to treatment failure.

Our observations, thus, far indicate that sorcin is a factor involved in the development of paclitaxel resistance in the 2008/13/4 cells, although other mechanisms that maintain the resistance phenotype of the 2008/13/4 cells are also operative. It is important to identify these other mechanisms, but an important question for the current study is:

how does up-regulation of sorcin lead to the development of paclitaxel resistance? From a functional aspect, it is well known that sorcin is a calcium-binding protein, and our earlier observation that verapamil (a calcium channel blocker) sensitizes these cells to the cytotoxic effects of paclitaxel without increasing its intracellular concentration [30] suggested that calcium may play a role in the cytotoxicity of paclitaxel. However, in the present study, we demonstrate that paclitaxel treatment does not alter the cytosolic-free calcium levels. Our observations indicate that treatment with paclitaxel does not affect Ca^{2+} influx (from the growth medium) in either the parental or the paclitaxel-resistant human ovarian tumor cells. In addition, although treatment with thapsigargin caused a sustained elevation in the cytosolic calcium (indicating a release from intracellular stores) in both the 2008 and the 2008/13/4 cells, treatment with paclitaxel did not demonstrate any changes in the levels of cytosolic calcium, suggesting that paclitaxel did not induce release of calcium from the intracellular stores.

These observations, although preliminary, suggest a role for sorcin in the development of low-level paclitaxel resistance that is distinct from its calcium-binding property. Indeed, sorcin has been shown to interact with several different cellular proteins including the ryanodine receptor, presenilin-2 and annexin VII, albeit in a calcium-dependent manner [45–47]. These interactions of sorcin with the cellular proteins have been speculated to serve some signaling functions on the surface of the cell membrane. In this regard, Han *et al.* [48] recently reported that increased expression of annexin IV (another calcium binding protein) also leads to the development of low levels of paclitaxel drug resistance. In the case of the present study, what is not known at present is how the interactions of sorcin influence the cytotoxic action of paclitaxel, leading to the development of a drug-resistant phenotype.

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References

[1] Rowinsky EK, Onetto N, Canetta RM, Arbuck SG. Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 1992;19:646–62.

[2] Rowinsky EK, Donehower RC. The clinical pharmacology of paclitaxel (taxol). Semin Oncol 1993;20:16–25.

[3] Trimble EL, Arbuck SG, McGuire WP. Options for primary chemotherapy of epithelial ovarian cancer: taxanes. Gynecol Oncol 1994;55:S114–21.

[4] Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. Part VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 1971;93:2325–7.

[5] Liebmann JE, Cook JA, Lipshultz C, Teague D, Fisher J, Mitchell JB. Cytotoxic studies of paclitaxel (taxol) in human tumor cell lines. Br J Cancer 1993;68:1104–9.

[6] Horwitz SB. Mechanism of action of taxol. Trends Pharmacol Sci 1992;13:134–6.

[7] Parekh HK, Simpkins H. The transport and binding of taxol. Gen Pharmacol 1997;29:167–72.

[8] Rao S, Krauss NE, Heerding JM, Swindell CS, Ringel I, Orr GA, Horwitz SB. 3′-(*p*-Azidobenzamido)taxol photolabels the N-terminal 31 amino acids of β-tubulin. J Biol Chem 1994;269:3132–4.

[9] Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 1993;62:385–427.

[10] Bradley G, Ling V. P-Glycoprotein, multidrug resistance and tumor progression. Cancer Metastasis Rev 1994;13:223–33.

[11] Horwitz SB, Cohen D, Rao S, Ringel I, Shen HJ, Yang CP. Taxol: mechanisms of action and resistance. J Natl Cancer Inst Monogr 1993;15:55–61.

[12] Keates RAB, Sarangi F, Ling V. Structural and functional alterations in microtubule protein from Chinese hamster ovary cell mutants. Proc Natl Acad Sci USA 1981;78:5638–42.

[13] Houghton JA, Houghton PJ, Hazelton BJ, Douglass EC. *In situ* selection of a human rhabdomyosarcoma resistant to vincristine with altered β-tubulin. Cancer Res 1985;45:2706–12.

[14] Cabral F, Barlow SB. Mechanisms by which mammalian cells acquire resistance to drugs that affect microtubule assembly. FASEB J 1989;3:1593–9.

[15] Minotti AM, Barlow SB, Cabral F. Resistance to antimitotic drugs in Chinese hamster ovary tumor cells correlates with changes in the levels of polymerized tubulin. J Biol Chem 1991;266:3987–94.

[16] Ohta S, Nishio K, Kubota N, Ohmori T, Funayama Y, Ohira T, Nakajima H, Adachi M, Saijo N. Characterization of a taxol-resistant human small-cell lung cancer cell line. Jpn J Cancer Res 1994;85:290–7.

[17] Debal V, Allam N, Morjani H, Millot JM, Braguer D, Breillout F, Manfait M. Characterisation of a navelbine-resistant bladder carcinoma cell line cross-resistant to taxoids. Br J Cancer 1994;70:1118–25.

[18] Giannakakou P, Sackett DL, Kang Y-K, Zhan Z, Buters JTM, Fojo T, Poruchynsky MS. Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J Biol Chem 1997;272:17118–25.

[19] Kavallaris M, Kuo DY-S, Burkhart CA, Regl DN, Norris MD, Haber M, Horwitz SB. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific β-tubulin isotypes. J Clin Invest 1997;100:1282–93.

[20] Ranganathan S, Benetatos CA, Colarusso PJ, Dexter DW, Hudes GR. Altered β-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. Br J Cancer 1998;77:562–6.

[21] Dumontet C, Duran GE, Steger KA, Beketic-Oreskovic L, Sikic BI. Resistance mechanisms in human sarcoma mutants derived by single-step exposure to paclitaxel (taxol). Cancer Res 1996;56:1091–7.

[22] Haber M, Burkhart CA, Regl DL, Madafiglio J, Norris MD, Horwitz SB. Altered expression of Mβ2, the class II β-tubulin isotype, in a murine J774.2 cell line with a high level of taxol resistance. J Biol Chem 1995;52:31269–75.

[23] Gupta RS. Species-specific differences in toxicity of antimitotic agents towards cultured mammalian cells. J Natl Cancer Inst 1985;74:159–64.

[24] Gupta RS. Intrinsic multidrug resistance phenotype of Chinese hamster (rodent) cells in comparison with human cells. Biochem Biophys Res Commun 1988;153:598–605.

[25] Parekh HK, Simpkins H. Species-specific differences in taxol transport and cytotoxicity against human and rodent tumor cells. Biochem Pharmacol 1996;51:301–11.
[26] Lopes NM, Adams EG, Pitts TW, Bhuyan BK. Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell lines. Cancer Chemother Pharmacol 1993;32:235–42.
[27] Sarris AH, Younes A, McLaughlin P, Moore D, Hagemeister F, Swan F, Rodriguez MA, Romaguera J, North L, Mansfield P, Callendar D, Mesina O, Cabanillas F. Cyclosporin A does not reverse clinical resistance to paclitaxel in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol 1996;14:233–9.
[28] Christen RD, Jekunen AP, Jones JA, Thiebaut F, Shalinsky DR, Howell SR. *In vitro* modulation of cisplatin accumulation in human ovarian carcinoma cells by pharmacologic alteration of microtubules. J Clin Invest 1993;92:431–40.
[29] Parekh HK, Simpkins H. Cross-resistance and collateral sensitivity to natural product drugs in cisplatin-sensitive and -resistant rat lymphoma and human ovarian carcinoma cells. Cancer Chemother Pharmacol 1996;37:457–62.
[30] Parekh H, Wiesen K, Simpkins H. Acquisition of taxol resistance via P-glycoprotein- and non-P-glycoprotein-mediated mechanisms in human ovarian carcinoma cells. Biochem Pharmacol 1997;53:461–70.
[31] Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 1992;257:967–71.
[32] Liang P, Averboukh L, Keyomarsi K, Sager R, Pardee AB. Differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells. Cancer Res 1992;52:6966–88.
[33] Wang X, Ruffolo RR, Feuerstein GZ. mRNA differential display: application in the discovery of novel pharmacological targets. Trends Pharmacol Sci 1996;17:276–9.
[34] Bauer D, Muller H, Reich J, Riedel H, Ahrenkiel V, Warthoe P, Strauss M. Identification of differentially expressed mRNA species by an improved display technique. Nucl Acids Res 1993;21:4272–80.
[35] Liang P, Bauer D, Averboukh L, Warthoe P, Rohrwild M, Muller H, Strauss M, Pardee AB. Analysis of altered gene expression by differential display. Methods Enzymol 1995;254:304–21.
[36] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, Vol. 1–3. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
[37] Treiman M, Caspersen C, Christensen SB. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends Pharmacol Sci 1998;19:131–5.
[38] Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. J Biol Chem 1991;266:17067–71.
[39] van der Bliek AM, Meyers MB, Biedler JL, Hes E, Borst P. A 22-kDa protein (sorcin/V19) encoded by an amplified gene in multidrug-resistant cells, is homologous to the calcium-binding light chain of calpain. EMBO J 1986;5:3201–8.
[40] Meyers MB, Schneider KA, Spengler BA, Chang T-D, Biedler JL. Sorcin (V19), a soluble acidic calcium-binding protein overproduced in multidrug resistant cells. Identification of the protein by anti-sorcin antibody. Biochem Pharmacol 1987;36:2373–80.
[41] Wang S-L, Tam M-F, Ho Y-S, Pai S-H, Kao M-C. Isolation and molecular cloning of human sorcin a calcium-binding protein in vincristine-resistant HOB1 lymphoma cells. Biochim Biophys Acta 1995;1260:285–93.
[42] Lee W-P. Purification, cDNA cloning, and expression of human sorcin in vincristine-resistant HOB1 lymphoma cell lines. Arch Biochem Biophys 1996;325:217–26.
[43] Beyer-Sehlmeyer G, Hiddemann W, Wörmann B, Bertram J. Suppressive subtractive hybridisation reveals differential expression of serglycin, sorcin, bone marrow proteoglycan and prostate-tumour-inducing gene I (PTI-1) in drug-resistant and sensitive tumour cell lines of haematopoetic origin. Eur J Cancer 1999;35:1735–42.
[44] Blagosklonny MV, Fojo T. Molecular effects of paclitaxel: myths and reality (a critical review). Int J Cancer 1999;83:151–6.
[45] Meyers MB, Pickel VM, Sheu SS, Sharma VK, Scotto KW, Fishman GI. Association of sorcin with the cardiac ryanodine receptor. J Biol Chem 1995;270:26411–8.
[46] Pack-Chung E, Meyers MB, Pettingell WP, Moir RD, Brownawell AM, Cheng I, Tanzi RE, Kim TW. Presenilin 2 interacts with sorcin, a modulator of the ryanodine receptor. J Biol Chem 2000;275:14440–5.
[47] Verzili D, Zamparelli C, Mattei B, Noegel AA, Chiancone E. The sorcin-annexin VII calcium-dependent interaction requires the sorcin N-terminal domain. FEBS Lett 2000;471:197–200.
[48] Han EK, Tahir SK, Cherian SP, Collins N, Ng SC. Modulation of paclitaxel resistance by annexin IV in human cancer cell lines. Br J Cancer 2000;83:83–8.