Cancer Chemother Pharmacol (2006) 57: 25–33
DOI 10.1007/s00280-005-0026-3

ORIGINAL ARTICLE

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GBP1 overexpression is associated with a paclitaxel resistance phenotype

Received: 3 December 2004 / Accepted: 12 March 2005 / Published online: 19 July 2005

Abstract In the search for novel genes involved in the paclitaxel resistance phenotype, prior studies of gene expression in paclitaxel-resistant cell lines and their paired drug-sensitive parental lines using high-density Affymetrix GeneChip arrays identified guanylate-binding protein 1 (*GBP1*) gene as an overexpressed transcript. The *GBP1* gene encodes a large GTPase that is induced by interferon gamma (IFN-γ) in a variety of eukaryotic cells. In this report we characterize *GBP1* and demonstrate that *GBP1* expression is consistently up-regulated in 7 of 8 paclitaxel or doxorubicin-resistant human cancer cell lines as compared to its expression in the relevant drug-sensitive parental lines. Analysis of *GBP1* expression using the Cancer Profiling Array showed that *GBP1* is ubiquitously expressed with no significant difference in expression levels between normal and tumor tissue. Parallel analysis of the Cancer Cell Line Profiling Array determined that *GBP1* expression in a majority of cell lines derived from human tumors of different tissue origin was induced to variable levels following exposure to multiple stress agents including paclitaxel and doxorubicin. Importantly, stable expression of a *GBP1* transgene in the paclitaxel-sensitive ovarian cancer cell line OVCAR8 was sufficient to confer moderate paclitaxel resistance. Our data suggest that increased expression of the *GBP1* gene may play an important role in the development of multi-drug resistance (MDR).

Keywords Gene array · *GBP1* · Paclitaxel · Chemotherapy · Multi-drug resistance

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Introduction

The treatment of cancer patients with chemotherapy is still seriously hindered by the occurrence of multi-drug resistance (MDR) and understanding the basis of MDR is a principal goal of molecular oncology. The mechanisms of drug resistance are polygenic and at present are incompletely defined. Specific changes in gene expression or mutations linked to drug resistance have been described for a number of individual genes including *MDR1, MRP, LRP*, and *p53* [1–3]. Clinical studies, however, have shown that inhibition of the MDR1- or MRP-related drug efflux proteins does not significantly improve the effectiveness of chemotherapy in patients [4, 5]. These observations suggest that MDR1 or MRP-related P-glycoproteins are not the primary mediators of clinical drug resistance and multiple mechanisms involving other cellular processes are likely to exist. Indeed, other genes implicated in the MDR phenotype encode either regulators of apoptosis or individual proteins that serve as the primary targets of specific antineoplastic agents [2, 6–8].

Paclitaxel (Taxol), originally isolated from *Taxus brevifolia* (Pacific yew), is a microtubule-stabilizing chemotherapeutic commonly utilized in the treatment of ovarian, breast, and non-small-cell lung cancers [9, 10]. Although paclitaxel is a clinically effective chemotherapy agent, the efficacy of paclitaxel therapy is limited by the development of recurrent disease that is frequently drug resistant. Specific mechanisms of acquired paclitaxel resistance have been defined in vitro and include overexpression of *MDR1*, differential expression of β-tubulin isotypes, and accumulation of β-tubulin mutations [3, 11, 12]. Analyses of clinical samples, however, clearly show that these in vitro mechanisms are rarely involved in the development of clinical paclitaxel resistance [4, 5, 13–17], suggesting that cellular processes distinct from P-glycoprotein activity or microtubule function are involved in the paclitaxel resistance phenotype *in vivo*.

Oligonucleotide or cDNA-based gene array analysis is an efficient technology that allows for a comprehensive analysis of gene expression. In an attempt to identify novel genes that are differentially expressed between paclitaxel-resistant and paclitxel-sensitive cells, the paclitaxel-sensitive ovarian cancer cell lines SKOV-3 and OVCAR8 and the paclitaxel-sensitive breast cancer cell line MCF-7 were exposed to incrementally increasing concentrations of paclitaxel. This procedure resulted in the establishment of three paclitaxel-resistant daughter cell lines, SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR}, respectively. By using high-density Affymetrix GeneChip arrays, we have identified *GBP1* as a paclitaxel-resistance associated gene in a previously published study [18]. *GBP1* is one of only eight genes that showed overexpression in SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR} as compared with their sensitive parental cell lines [19]. The *GBP1* gene encodes an interferon-inducible GTPase that belongs to the dynamin family of guanine nucleotide binding proteins [20, 21]. The function of GBP1 is unclear although the protein has been implicated in the interferon-mediated viral response and in the regulation of endothelial cell proliferation and angiogenic activity [22–24]. The *GBP1* gene has not previously been linked to paclitaxel resistance and was selected for further study. The studies outlined here demonstrate that *GBP1* is a paclitaxel-resistance associated gene expressed in a broad spectrum of human cancer cell lines and may play an important role in the development of MDR.

Materials and methods

Cell culture

The human ovarian cancer cell lines SKOV-3 and NIH:OVCAR3, the breast cancer cell lines MCF-7 and MDA435, and the colon cancer cell line SW480 were obtained from the American Type Culture Collection (Rockville, MD, USA). Dr. Patricia Donahoe (Massachusetts General Hospital, Boston, MA, USA) provided the human ovarian cancer cell line OVCAR5,OVCAR8 and IGROV1. The human multiple myeloma cell line 8226/S and its doxorubicin-resistant subclones 8226/Dox40 and 8226/MDR_{10}V [25] were provided by Dr. William S. Dalton (University of South Florida, H. Lee Moffitt Cancer Center, Tampa, FL, USA). The paclitaxel-resistant subclones SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR} were established by continuous culture in media containing step-wise increases in paclitaxel concentration over a period of eight months [26, 27]. The gemcitabine-resistant SKOV-3_{GR}, OVCAR5_{GR} and IGROV1_{GR} subclones were established as previously reported [26]. All cell lines were cultured in either RPMI 1640 or DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (all obtained from Invitrogen Life Technologies, Inc., Grand Island, NY, USA). Resistant subclones were continuously cultured in paclitaxel. Paclitaxel and doxorubicin were purchased from commercial sources.

RNA extraction

RNA was collected from all cell lines using TRIzol Reagent (GIBCO, Grand Island, NY, USA) according to the manufacturer's instructions. RNA quality was determined via ethidium bromide staining following agarose/formaldehyde gel electrophoresis.

RT-PCR

RT-PCR of *GBP1* was performed using the sense and anti-sense primer pairs, 5′-CTG CAC AGG CTT CAG CAA AA-3′ and 5′-AAG GCT CTG GTC TTT AGC TT-3′, respectively. TRIzol-extracted total RNA from the OVCAR8TR cell line was DNAase-treated to remove contaminating genomic DNA. RT-PCRs were performed using the Titan One Tube RT-PCR system (Roche, Indianapolis, IN, USA) following the manufacturer's protocol.

Northern analysis

RT-PCR products of *GBP1* from OVCAR8_{TR} were cloned into the pCR®2.1 vector using a TA Cloning kit (Invitrogen, Carlsbad, CA, USA) and the integrity of the cloned fragment was confirmed by sequencing. The cDNA inserts were released from the vector by Eco RI (Promega, Madison, WI, USA) digestion and purified using the QIAEX II Gel Extraction Kit (Qiagen Inc, Chatsworth, CA, USA). The purified fragments were labeled with ^{32}P-dCTP using the Megaprime DNA Labeling System (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for use as probes in Northern blot analyses. Total RNAs from matched drug-resistant cell lines and their corresponding drug-sensitive parental cell lines were extracted using TRIzol, separated by electrophoresis in 1.2% agarose/formaldehyde gels (approximately 5 μg total RNA per lane), transferred to Hybond N-plus nylon membranes (Amersham Biosciences, Piscataway, NJ, USA), and cross-linked to the membrane by UV irradiation. A one-hour pre-hybridization step was performed in Rapid-hyb buffer (Amersham Biosciences, Piscataway, NJ, USA) followed by a 2-h hybridization of the labeled probe in the same buffer. The blots were washed twice at room temperature with 2X SSC, 0.1%SDS for 15 min and twice at 65°C with 0.2X SSC, 0.1% SDS for 15 min. Blots were exposed to autoradiography X-ray film with an intensifying screen. For sequential hybridization of the blots, bound ^{32}P-labeled probe was stripped from the nylon membrane by incubating the blot for 5 min at room temperature in 0.1X SSC, 0.5% SDS heated to

100°C. The blots were exposed to X-ray film to confirm that the initial labeled probe was effectively stripped from the blot stripped prior to re-probing. Subsequent pre-hybridization, hybridization, washing, and autoradiography of the blot were carried out as described above.

Cancer profiling array and cancer cell line profiling array

The Cancer Profiling Array (BD Biosciences, Clontech) and the Cancer Cell Line Profiling Array (Clontech) allow evaluation of specific gene expression in a large number of human tumors. The Cancer Profiling Array consists of 241 pairs of cDNAs generated from matched human tumors and normal tissues. Each pair was independently normalized based on the expression of three housekeeping genes (http://www.bdbioscience.com/clontech/techinfo/manuals/PDF/7841-1.pdf). The Cancer Cell Line Profiling Array includes normalized BD SMART amplified cDNA from 26 different human cancer cell lines each treated with 26 different chemotherapeutic agents, oxidative stress inducers, or radiation. The array also includes an untreated cDNA control for each cell line (http://www.bdbiosciences.com/clontech/techinfo/manuals/PDF/7848-1.pdf). The hybridization of both arrays with a ^{32}P-labeled *GBP1* cDNA probe was carried out following the manufacturer's specifications. Quantitative analysis of the primary array data was performed using Scion Image Beta 4.02 Win software to obtain the signal intensities for individual spots (Scion Corporation, Frederick, MD, USA).

pIRES$_{GBP1}$ expression vector construction

The mammalian expression vector pIRESneo (Clontech) contains the internal ribosomal entry site (IRES) of the encephalomyocarditis virus (ECMV) that permits translation of two open reading frames (ORF) from a single mRNA. The expression cassette of pIRESneo contains the human cytomegalovirus, which is a major and an immediate early promoter/enhancer (pCMV) followed by a multiple cloning site (MCS) and a synthetic intron known to enhance mRNA stability. A 1837 base pair cDNA fragment containing the full ORF of human *GBP1* was amplified by RT-PCR from the RNA of OVCAR8, a paclitaxel-resistant cell line that highly overexpresses *GBP1*. RT-PCR was performed using the sense and anti-sense primer pair 5′-ATAAGAATGCGGCCGCcaagggaacagctggacat-3′(sense) and 5′-GGTGGATCCaaggctctggtctttagctt-3′(anti-sense). The underlined sequences represent the NotI (sense) and BamHI (anti-sense) sites required for cloning of the RT-PCR product into pIRES. The lower-case letters represent the sequence of the *GBP1* cDNA. The integrity of the *GBP1* construct was verified by restriction enzyme digestion and sequencing.

Transfection and production of stable cell lines

Transfections were performed using LipofectAmine Plus reagents (Life Technologies, Inc). Approximately 5×10^5 OVCAR8 cells were plated into 90-mm tissue culture dishes and cultured overnight. Prior to transfection, the growth medium was replaced with serum-free RPMI 1640 and the cells were cultured for 3 h. LipofectAmine reagent containing 5 μg of pIRES or pIRES$_{GBP1}$ was combined with Plus reagent and applied to the cells. After 4 h in culture, the medium was replaced with RPMI 1640 containing 10% fetal bovine serum. Selection for stable G418 sulfate-resistant clones was initiated 24 h post-transfection. Selection medium containing G418 (Invitrogen) at a concentration of 300 μg/ml was added to the cells and the medium was changed every 2 days. Following selection, G418-resistant colonies were ring cloned and expanded as individual clonal cell lines for subsequent analysis.

Cytotoxicity assay

Paclitaxel cytotoxicity was assessed in vitro using the MTT assay as previously described [28]. Briefly, 2×10^3 cells per well were plated in 96-well plates in culture medium (RPMI 1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin) containing increasing concentrations of paclitaxel. After 7 days of culture, 10 μl MTT (5 mg/ml in PBS, obtained from Sigma) were added to each well and the plates were incubated for 4 h. The resulting formazan product was dissolved with acid-isopropanol and the absorbance at a wavelength of 490 nm (A_{490}) was read on a BT 2000 Microkinetics Reader (Bio-Tek Instrument, Winooski, VT, USA). The absorbance values were normalized by assigning the value of the parental line in medium without drug to 1.0 and the value of the no cell control to 0. The IC50 is defined as the drug concentration required to decrease the A_{490} to 50% of the control (no paclitaxel) value. Experiments were performed in triplicate.

Western blot analysis

Cells were lysed by scraping in 50 mm Tris, pH 8.0, 150 mM NaCl, 1.0% igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. The lysates were clarified by centrifugation at 13,000*g* and the concentration of protein in each was determined using the DC protein assay (Biorad). Thirty micrograms of total cellular protein from each lysate were electrophoresed in duplicate sample sets through a 10% SDS-polyacrylamide gel. The resolved proteins were transferred to nitrocellulose in a buffer of 25 mM Tris, 192 mM glycine and 20% methanol at 60 V for 14 h. The filter was blocked for 1 h with 5% non-fat

dry milk (NFDM) in Tris-buffered saline containing 0.05% Tween-20 (TBST) and cut into two, with each half containing a complete sample set. One filter was incubated for 1 h with a 1:5,000 dilution of a rabbit polyclonal antibody directed against *GBP1* (kindly provided by Dr. Michael Sturzl, Neuherberg, Germany). The second filter was incubated for 1 h with a 1:1,000 dilution of a rabbit polyclonal antibody directed against human actin (H-300, Santa Cruz Biotechnology, Inc.). The filters were then washed with TBST and incubated for 1 h with a 1:5000 dilution of a horseradish peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham), washed with TBST, and developed with SuperSignal West Pico chemiluminescent substrate (Pierce Biotechnology, Inc.). All primary and secondary antibodies were diluted in 0.5% NFDM in TBST.

Results

Identification of *GBP1* overexpression by cDNA array

We examined the expression profiles of the cell lines SKOV-3 vs. SKOV-3_{TR}, OVCAR8 vs. $OVCAR8_{TR}$ and MCF-7 vs. MCF-7_{TR} in triplicate, using independent RNA preparations. In addition to *MDR1*, one of the genes, guanylate-binding protein 1 (*GBP1*) gene also demonstrated overexpression in the SKOV-3_{TR}, $OVCAR8_{TR}$ and MCF-7_{TR} resistant cell lines [19].

Verification of *GBP1* overexpression by northern blot analysis

To confirm the altered gene expression patterns detected in the microarray experiments, we measured the expression of *GBP1* and *MDR1* in the $SKOV3_{TR}$, $OVCAR8_{TR}$, and MCF-7_{TR} paclitaxel-resistant cell lines and their drug-sensitive parents by Northern analysis (Fig. 1). Northern analysis demonstrated an increased expression in the range predicted by the microarray and RT-PCR results [19]. To validate the expression of *GBP1* in drug-resistant lines, we analyzed additional paclitaxel- and doxorubicin-resistant cancer cell lines (NIH:OVCAR3, SW480, MDA435, 8226/Dox40, and $8226/MDR_{10}V$) that were not included in the initial microarray experiments. Northern analysis demonstrated that *GBP1* was overexpressed in seven of the eight (SKOV-3_{TR}, MCF-7_{TR}, $OVCAR8_{TR}$, NIH:OVCAR3_{TR} $SW480_{TR}$, $MDA435_{TR}$ and $8226/MDR_{10}V$) paclitaxel- or doxorubicin-resistant cell lines (Fig. 1) all of which also overexpressed *MDR1*. *GBP1* expression was not invariably linked to the *MDR1* phenotype. Evaluation of gemcitabine-resistant ovarian cancer cell lines demonstrated *GBP1* overexpression in $OVCAR5_{GR}$ but not in SKOV-3_{GR}, or $IGROV1_{GR}$ (Fig. 2). As expected, these gemcitabine-resistant cell lines did not overexpress *MDR1*.

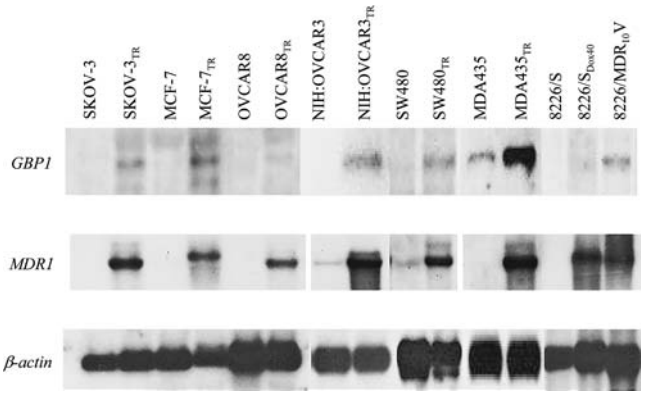

Fig. 1 *GBP1* is overexpressed in a subset of drug-resistant cancer cell lines. Total cellular RNA was isolated from the indicated drug-sensitive and drug-resistant cancer cell lines and subjected to Northern analysis as described in Materials and methods. The blot was sequentially hybridized to a ^{32}P-dCTP labeled cDNA probe specific to *GBP1* (*upper panel*), *MDR1* (*middle panel*), and β-actin (*lower panel*)

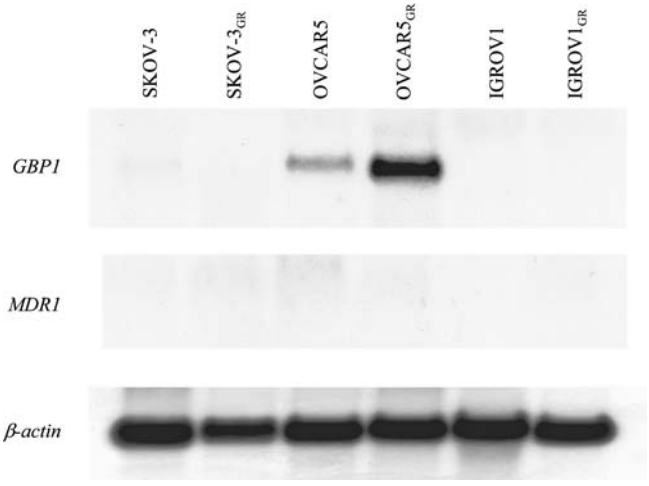

Fig. 2 *GBP1* expression is not invariably linked to the *MDR1* expression phenotype. Total cellular RNA isolated from the indicated gemcitabine-resistant ovarian cancer cell lines and their drug-sensitive parental lines was subjected to Northern analysis as described in Materials and methods. The blot was sequentially hybridized to a ^{32}P-dCTP labeled cDNA probe specific to *GBP1* (*upper panel*), *MDR1* (*middle panel*), and β-actin (*lower panel*)

GBP1 is ubiquitously expressed in human tissues and cancers

Our Northern analyses demonstrated that *GBP1* is overexpressed in a number of drug-resistant ovarian and non-ovarian cancer cell lines. We were interested in determining whether *GBP1* expression was also elevated in either primary human tumors or in human tumor-derived cell lines following exposure to multiple toxins. We utilized the commercially available Cancer Profiling Array and t Cancer Cell Line Profiling Array (Clontech) in these studies. These arrays provide a high-throughput format for analyzing differential gene expression and

studying multiple cellular functions. Analysis of the Cancer Profiling Array demonstrated that *GBP1* is constitutively expressed in various tissues and organs. Examination of relative expression levels in normal tissues indicated that *GBP1* is expressed at high levels in breast and lung with lower expression in stomach and kidney (Fig. 3). In general, there appeared to be no significant difference in *GBP1* expression between normal and tumor tissues. This was confirmed by quantification of the array data using Scion Image software (Table 1). In contrast, parallel analysis of the Cancer Cell Line Profiling Array showed an increased expression of *GBP1* in many cancer cell lines following a 48-h exposure to numerous individual toxins or stress agents (Fig. 4), indicating that increased expression of *GBP1* is a common event in a broad spectrum of tumor types. We specifically focused on *GBP1* expression levels in the individual tumor-derived cell lines following exposure to paclitaxel or doxorubicin. *GBP1* expression was elevated from 0.5- to 22.2-fold (mean 6.0; median 2.9) after 48 h of paclitaxel exposure and from 0.5- to 7.9-fold (mean 3.6; median 2.3) after 48 h of doxorubicin exposure (Fig. 5). These results support and extend our initial Northern analysis data and demonstrate that *GBP1* overexpression is a common consequence of not only long-term chemotherapy exposure but also relatively brief drug exposure time to paclitaxel or doxorubicin treatment.

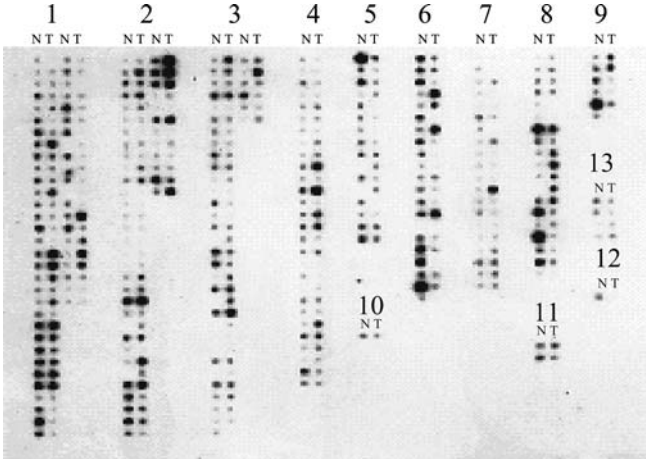

Fig. 3 *GBP1* is widely expressed in human cancer tissues. *GBP1* expression in matched normal and tumor tissues was analyzed by hybridization of a ^{32}P-labeled *GBP1* cDNA probe to the Cancer Profiling Array (Clontech) following the manufacturer's specifications. The numbers indicate the tissue source of the cDNA samples arrayed in each panel: *1* breast; *2* uterus; *3* colon; *4* stomach; *5* ovary; *6* lung; *7* kidney; *8* rectum; *9* thyroid gland; *10* cervix; *11* small intestine; *12* pancreas; *13* prostate. *N* Normal tissue samples, *T* tumor tissue samples

GBP1 induces paclitaxel resistance in OVCAR8

Our analyses of endogenous *GBP1* expression in multiple drug-resistant cancer cell lines have demonstrated that *GBP1* is frequently upregulated and suggest that the GBP1 protein may be critically involved in the development of MDR. It remains possible, however, that *GBP1* overexpression is an indirect consequence of acquired drug-resistance. To determine whether GBP1 directly participates in the establishment of the paclitaxel-resistant phenotype, we transfected the paclitaxel-sensitive ovarian cancer cell line OVCAR8 with a *GBP1* expression vector and generated the two independent stable and cloned cell lines OVCAR8$_{GBP1a}$ and OVCAR8$_{GBP1b}$. We then asked if exogenous overexpression of GBP1 was sufficient to confer increased paclitaxel-resistance. The results from the MTT assay measuring in vitro paclitaxel cytotoxicity in the OVCAR8$_{GBP1a}$ and OVCAR8$_{GBP1b}$ cell lines are shown in Fig. 6a. GBP1-transfected OVCAR8 cells are relatively resistant to paclitaxel, whereas no significant change in resistance was observed in OVCAR8 cells transfected with the empty vector. The paclitaxel IC50 values of both OVCAR8$_{GBP1}$ clones were four-fold higher than those of the parental (OVCAR8) and empty vector (OVCAR8$_{pIRES}$) control lines, suggesting that*GBP1* is directly involved in the development of paclitaxel-

Table 1 Summary of *GBP1* expression levels in profiling arrays

Type of matched normal/tumor sample (number of cases)	Average expression level in normal tissue (range)	Average expression level in tumor tissue (range)	Tumor:normal expression level
Breast (53)	69 (24–142)	61 (21–172)	0.9
Uterus (44)	50 (12–137)	64 (12–204)	1.3
Colon (38)	44 (7–12)	45 (5–142)	1
Stomach (28)	28 (2–986)	44 (3–157)	1.6
Ovary (16)	62 (15–198)	25 (1–96)	0.4
Lung (21)	75 (3–204)	43 (2–154)	0.6
Kidney (20)	22 (0–61)	29 (0–119)	1.3
Rectum (19)	59 (5–182)	48 (0.5–130)	0.8
Thyroid gland (6)	71 (24–191)	46 (9–48)	0.7
Cervix (1)	51	5	0.1
Small intestine (2)	68 (56–79)	52 (44–61)	0.8
Pancreas (1)	48	29	0.6
Prostate (4)	29 (7–48)	20 (12–29)	0.7

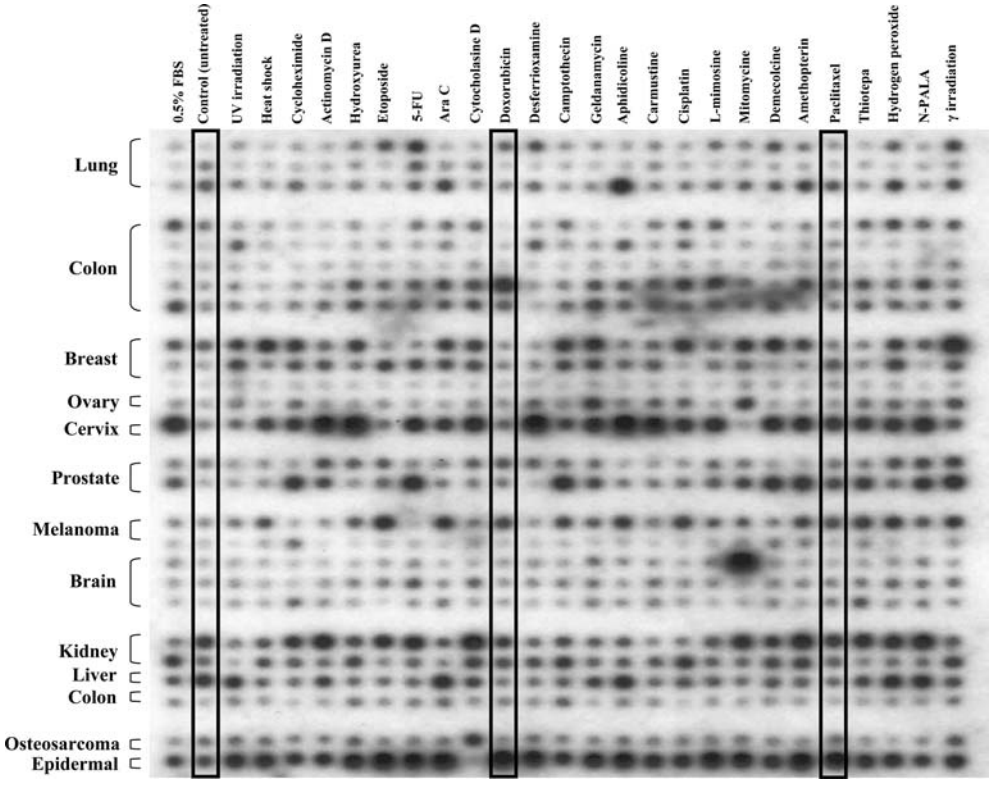

Fig. 4 *GBP1* expression in human cancer cell lines is induced by multiple toxins. *GBP1* expression in multiple human tumor-derived cell lines exposed to various stress agents was analyzed by hybridization of a ^{32}P-labeled *GBP1* probe to the Cancer Cell Line Profiling Array (Clontech) following the manufacturer's specifications. Each row contains cDNA generated from a single cell line derived from the indicated tumor type following a 48-h exposure to each of the toxins listed in the column headings. The control, doxorubicin and paclitaxel treatment are *boxed*

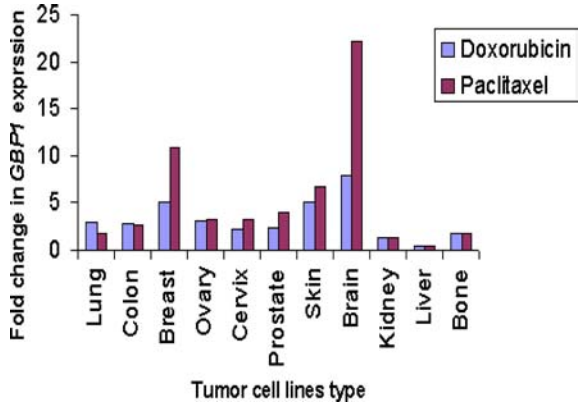

Fig. 5 *GBP1* expression in human cancer cell lines is induced to varying degrees by paclitaxel and doxorubicin. The data from the Cancer Cell Line Array were quantitated using Scion Image software. The values obtained following individual toxin treatment of multiple cell lines of shared tumor origin were averaged. The paclitaxel- and doxorubicin-induced *GBP1* expression levels in each collection of tumor-derived cell lines were determined by comparing the average expression level in the paclitaxel- or doxorubicin-treated samples to the average expression level in the control (untreated) samples for each tumor type

resistance. These results are in contrast to those obtained from similar functional studies previously carried out with *MM-TRAG* (*MDR1* and mitochondrial taxol resistance associated gene), another of the eight genes overexpressed in all three of the analyzed paclitaxel-resistant cell lines [19]. In those experiments, exogenous expression of MM-TRAG did not alter the paclitaxel sensitivity of OVCAR8, indicating that the moderate paclitaxel resistance we observed following transfection of OVCAR8 with GBP1 was not simply a non-specific consequence of exogenous gene expression. Elevated GBP1 expression in the OVCAR8$_{GBP1a}$ and OVCAR8$_{GBP1b}$ cell lines was confirmed by Western blotting (Fig. 6b). The significantly high level of GBP1 expression in the transfected cells resulted in only a moderate level of paclitaxel resistance. In addition, there was no significant changes in relative resistance to doxorubicin, cisplatin or topotecan in the *GBP1*-transfected cell lines (data not shown).

Discussion

In this study we have extended cDNA microarray analysis of differential gene expression in paclitaxel-resistant and paclitaxel-sensitive human cancer cell lines. This analysis had identified *MDR1* and 7 additional genes including *GBP1* that were associated with the paclitaxel-resistance phenotype. Early studies demonstrated that *GBP1* overexpression is broadly associated with the MDR phenotype with increased *GBP1* transcript levels detected in paclitaxel and doxorubicin-resistant cell lines and in a non-*MDR1*-expressing gemcitabine-resistant cancer cell line. Specifically, elevated expression of *GBP1* was observed in 7 of 8 paclitaxel or doxorubicin-resistant cell lines and one of three gemcitabine-resistant cell lines and was largely, but not completely, correlated with *MDR1*

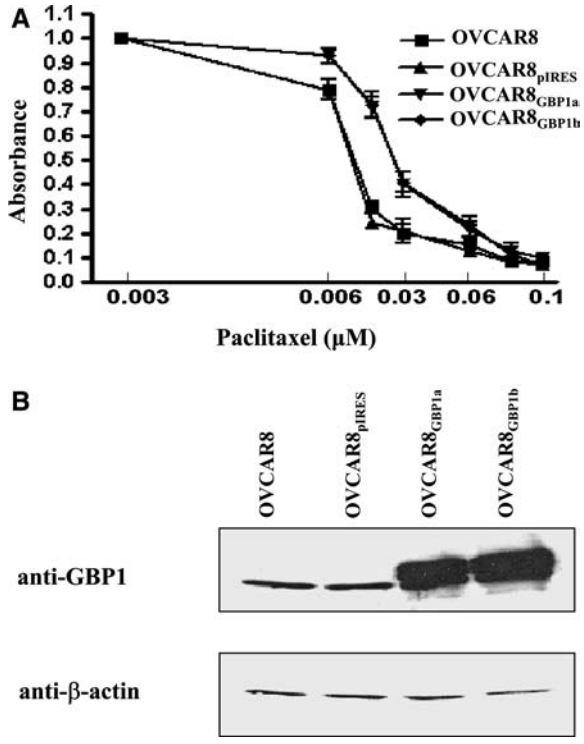

Fig. 6 Exogenous expression of *GBP1* confers paclitaxel resistance to the ovarian cancer cell line OVCAR8. **a** The relative cytotoxicity of paclitaxel in two independent OVCAR8-derived cell lines ($OVCAR8_{GBP1a}$ and $OVCAR8_{GBP1b}$) stably transfected with a *GBP1* expression vector and in the parental (OVCAR8) and empty vector (OVCAR8pIRES) controls were assessed using the MTT assay. All samples were analyzed in triplicate. **b** Duplicate sample sets of total cellular protein isolated from the OVCAR8, OVCAR8 pIRES, $OVCAR8_{GBP1a}$, and $OVCAR8_{GBP1b}$ cell lines analyzed in the MTT assay were subjected to Western blot analysis using antibodies directed against GBP1 (*upper panel*) and β-actin (*lower panel*)

overexpression. Further characterization of *GBP1* expression in cancer using both the Cancer Profiling Array and Cancer Cell Line Profiling Array revealed that *GBP1* is constitutively expressed in all evaluated tissues suggesting that the *GBP1* protein has an important role in normal tissue biology. While there was no significant difference in *GBP1* expression between normal and tumor tissues, many chemical agents induced *GBP1* expression within 48 h in various cancer cell lines (Fig. 4) with considerable overexpression induced by paclitaxel and doxorubicin (Fig. 5). Importantly, overexpression of the GBP1 protein in a paclitaxel sensitive–ovarian cancer cell line led to a modest level of paclitaxel resistance strongly implying an important role for GBP1 in the development of the paclitaxel-resistant phenotype. Several investigators have reported that non-MDR1 transporter proteins cause an MDR phenotype in human cancer cell lines [29, 30]. Additionally, transfection of the *HER2/neu*, *c-H-ras*, *Bcl-2*, *IL-6* or *MAGE* gene into drug-sensitive cell lines has conferred drug-resistance in vitro [31–35] consistent with previous observations that the perturbation of pathways regulating cell proliferation and apoptosis is a key event in the acquisition of MDR.

The GBP1 protein belongs to the large family of guanine nucleotide-binding proteins, which regulate fundamental cellular processes such as signal transduction, protein synthesis, and intracellular protein transport [36]. GBP1 is a large (67 kDa) interferon-induced GTPase which undergoes nucleotide-dependent oligomerization [20, 21]. GBP1 has an unusual ability to hydrolyze GTP to both GDP and GMP in a concentration-dependent manner, although the precise effect of the complete conversion of GTP to GMP on GBP1 activity and regulation is unknown [20, 37]. Structural analysis indicates that GBP1 is most closely related to the dynamin and Mx guanine nucleotide binding protein family which functions in many processes including cytokinesis, transport vesicle budding, organelle division, and pathogen resistance [38]. Like Mx, *GBP1* has been shown to play a role in the interferon-mediated anti-viral response [24].

The precise cellular function of *GBP1* remains unclear although recent studies of *GBP1* expression and activity in endothelial cells have provided some insight. *GBP1* expression in endothelial cells is selectively induced by the inflammatory cytokines (ICs) interferon alpha and gamma (IFN-α/IFN-γ), interleukin-1 alpha and beta (IL-1α/IL-1β), and tumor necrosis factor alpha (TNF-α) [23] via cooperative activation of a nuclear factor κB (NF-κB) motif and an IFN-α-stimulated response element (ISRE) in the*GBP1* promoter [39]. *GBP1* mediates the anti-proliferative effects of ICs in endothelial cells [40]. *GBP1* also functions as a major regulator of the IC-mediated anti-angiogenic response in endothelial cells and its expression is downregulated by angiogenic growth factors [40]. *GBP1* exerts its effect on angiogenesis by inhibiting MMP-1 expression and can block endothelial cell invasiveness and tube formation in three-dimensional collagen matrices [22]. The anti-proliferative and anti-angiogenic activities of *GBP1* in endothelial cells are not obviously consistent with its apparent role in the development of the MDR phenotype. However, the*GBP1* gene is expressed in a wide spectrum of both normal and neoplastic tissues and determination of *GBP1* function in non-endothelial cell types may clarify its association with acquired drug-resistance.

Other investigators have also identified genes whose expression is upregulated in paclitaxel-resistant MCF-7 breast cancer cells. One such gene is *IRF9*, which encodes IFN regulatory factor 9, a mediator of signaling by type 1 interferons. The observed interferon-independent transcriptional activation of *IRF9* and other IFN responsive genes correlated with paclitaxel-resistance. Significantly, the transient overexpression of IRF9 in drug-sensitive cells reproduced the drug-resistance phenotype [41]. These results are consistent with our own data on *GBP1* and suggest that independent activation of interferon pathways may be a common means of establishing paclitaxel resistance.

In summary, we have demonstrated that *GBP1* expression is associated with the multi-drug-resistance phenotype and is selectively upregulated by various chemical reagents in human cancer cell lines and is an early response to toxin exposure. Owing to the ubiquitous expression of this protein, understanding the mechanism of *GBP1* induction and the downstream effects of its overexpression may reveal potential drug targets in the future. Significantly, overexpression of the GBP1 protein in a drug-sensitive cell line was sufficient to moderately reduce paclitaxel sensitivity, suggesting that *GBP1* may directly participate in the development of clinical drug resistance. The precise definition of its role awaits further investigation.

Acknowledgements This work was supported by NIH Grant CA 89150 (to M.V. Seiden)

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