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ORIGINAL ARTICLE

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Description of paclitaxel resistance-associated genes in ovarian and breast cancer cell lines

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Abstract *Purpose*: To identify genes involved in the paclitaxel resistance phenotype. *Methods*: High-density Affymetrix HG-U95Av2 microarrays were used to quantify gene expression in the resulting cell lines, SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR}, and their drug-sensitive parental lines, SKOV-3, OVCAR8 and MCF-7. *Results*: Three paclitaxel-resistant human ovarian and breast cancer cell lines were established. We identified 790 (SKOV-3_{TR}), 689 (OVCAR8_{TR}) and 964 (MCF-7_{TR}) transcripts that were more than twofold overexpressed relative to their expression in the corresponding parental cell line. A comparison of these transcripts identified eight genes that were significantly overexpressed in all three drug-resistant daughter cell lines. These genes included *MDR1*, a gene often implicated in both in vitro and in vivo resistance to multiple chemotherapeutics, including paclitaxel. The remaining seven genes have not been previously associated with resistance to paclitaxel in human cancer. Furthermore, we identified 815 (SKOV-3_{TR}), 430 (OVCAR8_{TR}) and 332 (MCF-7_{TR}) transcripts that were more than twofold decreased relative to their expression in the corresponding parental cell line. Comparison of these transcripts identified three genes that were significantly underexpressed in all three drug-resistant cell lines, none of which have been previously associated with paclitaxel resistance. *Conclusions*: Our results confirm that the paclitaxel resistance phenotype is associated with a large number of transcriptional changes. In addition, acquired paclitaxel resistance was associated with distinct transcriptional changes in each of the cell lines studied, suggesting that paclitaxel resistance is a complex phenotype that can arise through multiple mechanisms.

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Introduction

Drug resistance is a major obstacle to successful chemotherapy. Often a tumor exposed to one chemotherapeutic will develop resistance to several unrelated chemotherapeutics. The mechanisms of drug resistance are polygenetic and incompletely defined. At present, drug resistance has been associated with changes in gene expression or mutations in a number of individual genes, such as *MDR1*, *MRP*, *LRP* and *p53*. In addition, changes in genes that serve as the primary chemotherapeutic target or that regulate apoptosis have been implicated [1–4]. Previous transcriptional array studies have implicated a large number of genes in the development of drug resistance [5, 6]. Elucidating the molecular mechanisms of this complex process may lead to the identification of novel molecules that in turn may serve as targets for therapeutics, diagnostic tests or alternatively predictive markers. Therefore, understanding the basis of drug resistance is a principal goal of molecular oncology.

Paclitaxel, originally isolated from *Taxus brevifolia* (Pacific yew), is a microtubule-stabilizing chemotherapeutic used to treat many malignancies including ovarian, breast and non-small-cell lung cancers [7, 8]. Unfortunately, the efficacy of paclitaxel therapy is limited by the development of paclitaxel resistance. Specific mechanisms of acquired resistance in vitro have included overexpression of *MDR1*, differential expression of β-tubulin isotypes and mutations in β-tubulin [9–11].

This study was designed to characterize transcriptional changes associated with paclitaxel resistance. Specifically, 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. The individual expression profiles of the three parental cell lines and their corresponding resistant daughter cell lines were determined using microarrays. Transcriptional changes associated with acquired paclitaxel resistance were identified in each individual pair of cell lines. Subsequently, significant transcriptional changes were compared between pairs of cell lines. Using the resulting data sets it was possible to determine the diversity of the transcriptional changes associated with acquired paclitaxel resistance, and the consistency of these transcriptional changes in the development of paclitaxel resistance.

Materials and methods

Cell culture

The human ovarian cancer cell line SKOV-3 and the human breast cancer cell line MCF-7 were obtained from the American Type Tissue Collection (Rockville, Md.). Dr. Patricia Donahoe (Massachusetts General Hospital, Boston, Mass.) provided the human OVCAR8 ovarian cancer cell line. The paclitaxel-resistant SKOV-3_{TR}, OVCAR8_{TR}, and MCF-7_{TR} cell lines were established as previously reported [12, 13]. Briefly, the cell lines were selected to be paclitaxel resistant by continuous culture in medium containing step-wise increases in paclitaxel concentration over a period of 8 months. The cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin (all obtained from Life Technologies, Grand Island, N.Y.). Resistant cell lines were continuously cultured in paclitaxel. Previously, we have grown SKOV-3_{TR} without paclitaxel for 6 months and found no changes in either the paclitaxel-resistant phenotype or *MDR1* expression. Paclitaxel was purchased from a commercial source.

Cytotoxicity assay

In vitro cytotoxicity was assessed using the MTT assay as previously described [14]. MTT was purchased from Sigma (St Louis, Mo.). Briefly, 2×10^3 cells per well were plated in 96-well plates. Cells were plated in RPMI 1640 medium containing increasing concentrations of paclitaxel. After 7 days of culture with paclitaxel, 10 μl MTT (5 mg/ml in PBS) was 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.). The IC_{50} was defined as the paclitaxel concentration required to decrease the A_{490} to 50% that of the control (no paclitaxel) value. The absorbance values were normalized assigning the value of the parental line in medium without drug to 1.0 and the value of the no-cell control to 0. Experiments were performed in duplicate.

RNA extraction

Total RNA was collected from SKOV-3, SKOV-3_{TR}, OVCAR8, OVCAR8_{TR}, MCF-7 and MCF-7_{TR} using TRIzol Reagent (GIBCO, Grand Island, N.Y.) according to the manufacturer's instructions. RNA from the resistant cell lines was collected from cells grown with paclitaxel. To account for and eliminate biologic noise, RNA was isolated from three distinct flasks of each cell line. These biologic replicates were not pooled but rather arrayed as three replicates of the same cell line. RNA quality was determined via ethidium bromide staining following agarose/formaldehyde gel electrophoresis.

Transcriptional profiling and analysis

Total RNA was processed and hybridized to Affymetrix Genechip HG-U95Av2 arrays (Affymetrix, Santa Clara, Calif.) by the Gene Array Technology Center at Partners Healthcare (Brigham and Women's Hospital, Boston, Mass.). Each array contains 12,386 probes corresponding to approximately 9000 known human genes. Each probe consists of 20 separate 23-mer oligonucleotides. The expression level of each mRNA is quantified by measuring its hybridization to these 23-mers in comparison to its hybridization to a one-base mismatch oligonucleotide. Affymetrix Microarray Suite 5.0 and Affymetrix Data Mining Tool 3.0 were used to analyze the microarray data. Fold change in expression between sensitive and resistant cell lines was evaluated using the Mann-Whitney test. A twofold or greater change in intensity combined with a Mann-Whitney-associated *P* value less than 0.05 was used as the criterion for inclusion in our filtered data set. Intensity information was exported to Microsoft Excel as needed.

Real-time quantitative RT-PCR (QRT-PCR)

Real-time QRT-PCR was performed with the Mx300P Real-Time PCR system (Stratagene, La Jolla, Calif.). Primers and probes (Applied Biosystems, Foster City, Calif.) were designed with Primer Express software (Applied Biosystems). *MGC4175* primers were: sense 5′-AGTGGCTTGGGGAAAGTGAA-3′, and antisense 5′-AGACAGCAAAGAATATATTC-3′. The *MGC4175* probe (5′-AAACCCCAAAATGGAGGACTTTGC-3′) was 5′-labeled with 6-carboxyfluorescein (FAM) and 3′-labeled with 6-carboxytetramethylrhodamine (TAMRA). TaqMan *GAPDH* control reagents were obtained from Applied Biosystems (Foster City, Calif.). QRT-PCR was performed using a Brilliant Single-Step QRT-PCR

Master Mix kit (Stratagene, La Jolla, Calif.). Reaction conditions were: 30 min at 50°C, 10 min at 95°C, then 40 cycles of 15 s at 95°C, then 1 min at 55°C. *MGC4175* mRNA expression was quantified in comparison to *GAPDH* mRNA. All assays were performed in duplicate.

Results

SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR} are paclitaxel-resistant

Three paclitaxel-resistant cell lines were generated from paclitaxel-sensitive cell lines during 8 months of continuous culture in paclitaxel. The resistant phenotype was found to be stable after 12 months of continuous culture in medium containing paclitaxel. MTT cytotoxicity experiments demonstrated that SKOV-3_{TR}, OVCAR8_{TR}, and MCF-7_{TR} were 130-fold, 50-fold and 15-fold resistant to paclitaxel as compared to their sensitive parental cell lines, respectively (Fig. 1).

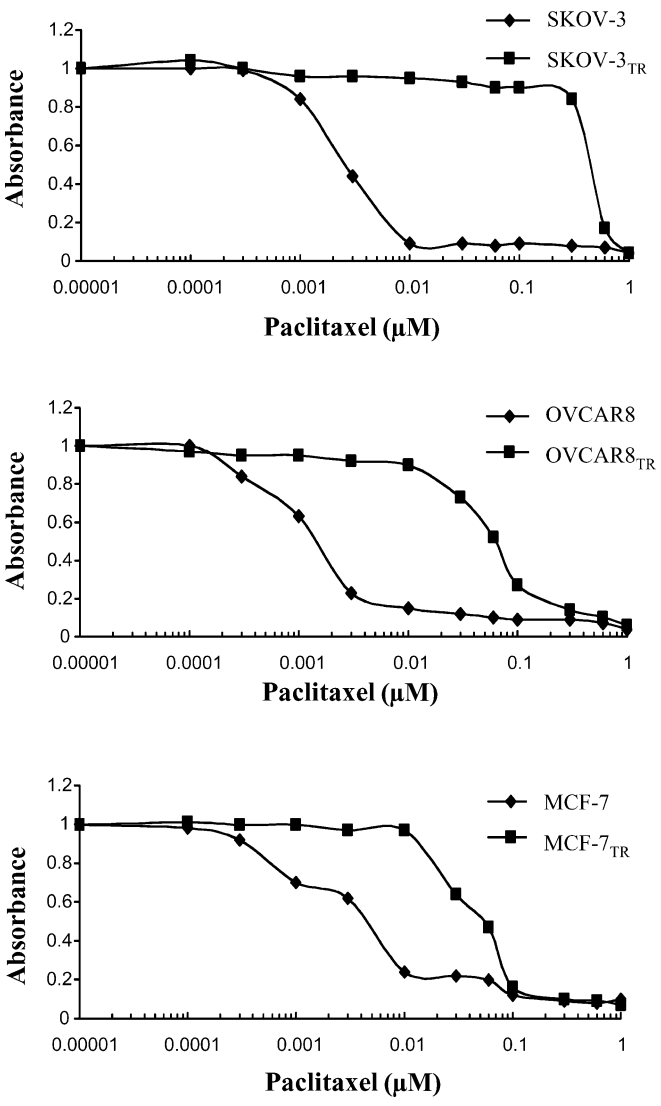

Fig. 1 Paclitaxel resistance of SKOV-3 versus SKOV-3_{TR}, OVCAR8 versus OVCAR8_{TR} and MCF-7 versus MCF-7_{TR}. Data are the means of three replicates at each concentration

A large number of genes are misexpressed in SKOV-3_{TR}, OVCAR8_{TR} and MCF-7_{TR} as compared to their respective parental cell lines

Affymetrix array technology was used to evaluate the transcriptional changes within three pairs of paclitaxel-sensitive and paclitaxel-resistant cell lines. The expression profiles of four ovarian cancer cell lines (SKOV-3, SKOV-3_{TR}, OVCAR8, OVCAR8_{TR}) and two breast cancer cell lines (MCF-7, MCF-7_{TR}) were evaluated by microarray analysis. Each cell line was arrayed in triplicate using RNA isolated as biologic replicates. Individual arrays were normalized and analyzed using Affymetrix Microarray Suite 5.0.

To determine the effect of using biologic replicates in our experiments, individual transcript expression values were compared between biologic replicates. The coefficient of determination (R^2) was calculated for each of six comparisons per cell line pair (Table 1). The resulting R^2 values for the SKOV-3 comparisons ranged from 0.97 to 0.98.

To determine the variance that could be attributed to the paclitaxel resistance phenotype, additional comparisons were performed between sensitive and resistant cell lines (SKOV-3 replicate 1 vs SKOV-3_{TR} replicate 1). As shown in Table 1, these R^2 values were lower, 0.91–0.93, between the sensitive parental and resistant daughter cell lines. Similar calculations demonstrated that R^2 values were 0.93–0.96 for OVCAR8/OVCAR8_{TR} and 0.94–0.97 for MCF-7/MCF-7_{TR} cell line pairs. Interestingly, SKOV-3_{TR} demonstrated the highest degree of paclitaxel resistance, followed by OVCAR-8_{TR} and then MCF-7_{TR}.

Having established that our experiment could potentially identify significant transcript expression changes between paclitaxel-sensitive and paclitaxel-resistant cell lines, individual expression values were averaged across replicates to get a single expression value per transcript per cell line. The resulting 12,386 average transcript expression values were compared between sensitive and resistant cell lines (SKOV-3 vs SKOV-3_{TR}, OVCAR8 vs OVCAR8_{TR} and MCF-7 vs MCF-7_{TR}) to give a fold change in expression. A large number of transcripts were identified as differentially expressed between sensitive and resistant cell lines (Fig. 2). To focus on statistically significant changes, we defined altered expression as a twofold or greater change in expression with a Mann-Whitney P value less than 0.05. Using these criteria, 790 (SKOV-3_{TR}), 689 (OVCAR8_{TR}) and 964 (MCF-7_{TR}) gene transcripts demonstrated more than twofold overexpression in the paclitaxel-resistant lines relative to their expression in the sensitive parental lines. In addition, 815 (SKOV-3_{TR}), 430 (OVCAR8_{TR}) and 332 (MCF-7_{TR}) transcripts

Table 1 R^2 values

			Parental			Resistant		
			Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
SKOV-3	Parental	Replicate 1	1.00	–	–	–	–	–
		Replicate 2	0.97	1.00	–	–	–	–
		Replicate 3	0.98	0.97	1.00	–	–	–
	Resistant	Replicate 1	0.93	0.93	0.93	1.00	–	–
		Replicate 2	0.92	0.92	0.93	0.98	1.00	–
		Replicate 3	0.92	0.91	0.93	0.96	0.97	1.00
OVCAR8	Parental	Replicate 1	1.00	–	–	–	–	–
		Replicate 2	0.98	1.00	–	–	–	–
		Replicate 3	0.98	0.99	1.00	–	–	–
	Resistant	Replicate 1	0.95	0.95	0.96	1.00	–	–
		Replicate 2	0.94	0.93	0.95	0.98	1.00	–
		Replicate 3	0.95	0.95	0.95	0.98	0.99	1.00
MCF-7	Parental	Replicate 1	1.00	–	–	–	–	–
		Replicate 2	0.99	1.00	–	–	–	–
		Replicate 3	0.98	0.98	1.00	–	–	–
	Resistant	Replicate 1	0.96	0.96	0.96	1.00	–	–
		Replicate 2	0.96	0.97	0.96	0.99	1.00	–
		Replicate 3	0.95	0.95	0.94	0.98	0.98	1.00

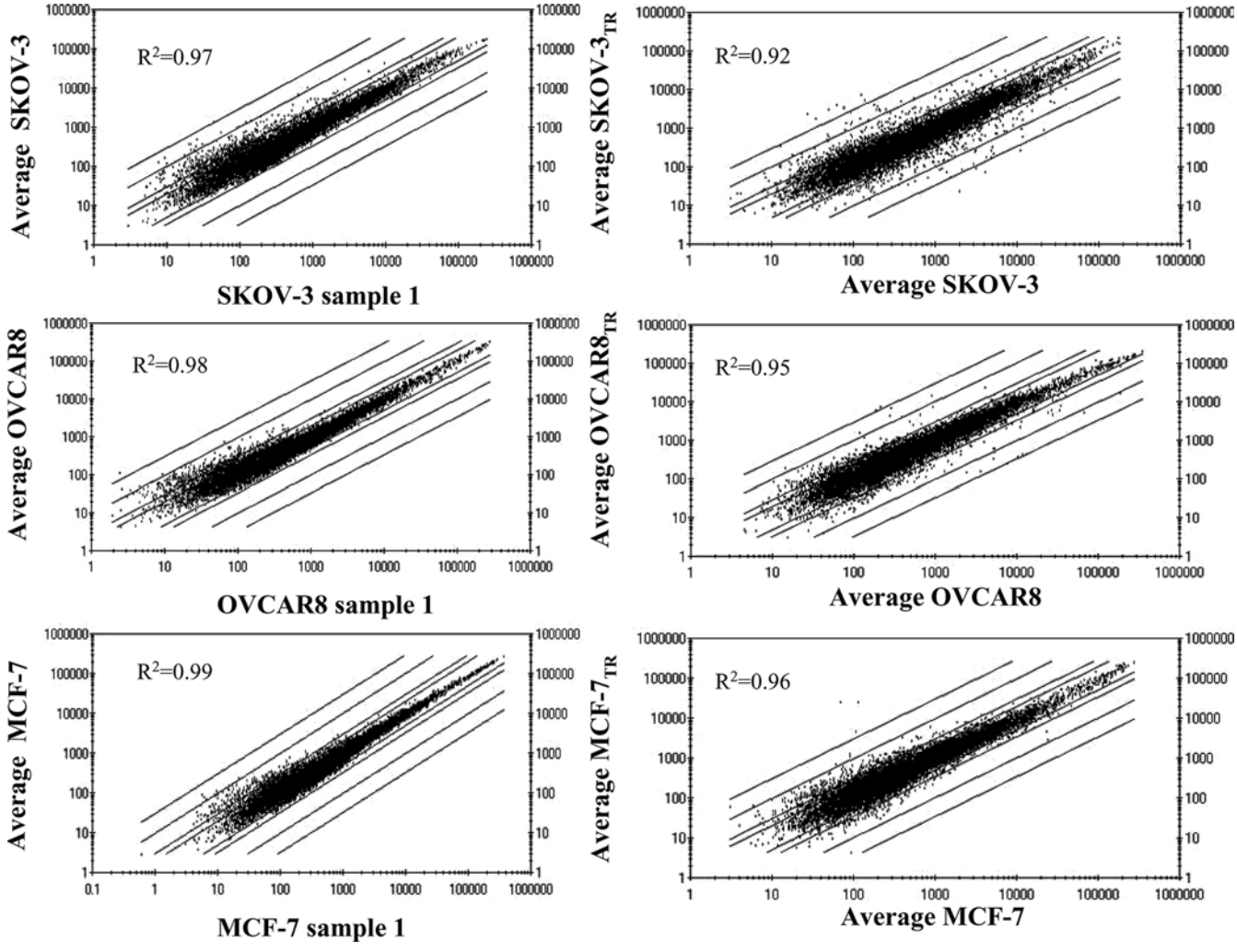

Fig. 2 Comparison of average transcript expression values. Lines parallel to the line of identity indicate the relative fold change (2, 3, 10, and 30-fold, respectively)

were more than twofold decreased in the resistant cell lines as compared to the sensitive cell lines. Transcript expression changes ranged from 2-fold to 240-fold. The top 20 most highly overexpressed and underexpressed

Table 2 A list of the 20 most differentially expressed genes in each cell line pair. The number in parentheses following the gene name is the fold overexpression/underexpression compared to the respective sensitive cell line. Genes in bold type are differentially expressed in at least two of the three resistant cell lines

SKOV3$_{TR}$ vs SKOV-3	OVCAR8$_{TR}$ vs OVCAR8	MCF-7$_{TR}$ vs MCF-7
Overexpressed genes		
cDNA clone IMAGE-782636 (84)	cDNA clone IMAGE-1953089 (36)	***MDR1*** (240)
MDR1 (67)	***MDR1*** (33)	*IGFBP5* (14)
GAGE7 (64)	*Prostaglandin D2 synthase* (16)	*5′* nucleotidase (12)
MAGE2 (54)	***MDR3*** (16)	***MDR3*** (11)
GAGE4 (49)	*Activator of S phase kinase* (11)	*KIAA 0013* (9)
GAGE2 (45)	cDNA clone 24511 (10)	*CENP-E* (9)
Wnt-5a (39)	cDNA clone RG060N22 (9)	*B-HLH DNA binding protein* (9)
cDNA clone DKFZp566k 192 (30)	*Phosphatidylinositol 3-kinase* (8)	*HTF10* (8)
cDNA clone IMAGE-2429487 (22)	*CHK1* (8)	*TRAIL* (8)
MAGE6 (19)	*p53TG1-A* (8)	***RecQL*** (8)
Inositol polyphosphate 4-phosphatase type II (18)	*CTSE* (8)	*ASH1* (7)
MAGE12 (18)	*Sorcin CP-22* (7)	cDNA DKFZp434G173 (7)
MAGE3 (16)	PAC 262D12 (7)	*PTP-PEST* (7)
GAGE6 (15)	*Striatin* (7)	*Rip-1* (7)
cDNA clone IMAGE-567001 (15)	*Phospholipase C-gamma* (7)	cDNA clone IMAGE-2489058 (7)
ABCB6 (15)	*MKK7* (7)	*HIS2* (7)
cDNA clone DKFZp564P116 (14)	***RecQL*** (6)	*LD5-1* (6)
Interferon gamma treatment-inducible mRNA (13)	*Transcriptional intermediary factor 2* (6)	*ICErel-II* (6)
GAGE5 (13)	*KIAA 1033* (5)	*DLC-1* (6)
ABCC2 (12)	*Stress-activated protein kinase 4* (5)	*znfp 192* (6)
Underexpressed genes		
Carboxypeptidase E (116)	*Heme oxygenase 1* (146)	***Heat shock protein 70B*** (21)
GOS2 (90)	*c-fos* (46)	*Angiopoietin-2* (17)
Claudin-10 (67)	*Fibronectin* (31)	*Calpastain* (13)
Fibronectin (50)	***Heat shock 70-kDa protein 1B*** (27)	*Granule membrane protein-140* (12)
VEGF (42)	*Activating transcription factor 3* (23)	*Cyclic nucleotide phosphodiesterase* (11)
Fibroblast tropomysin (39)	***Heat shock protein 70B*** (23)	*Nel-related protein* (11)
E-cadherin (36)	*Helix-loop-helix basic phosphoprotein* (20)	*KIAA 0995* (11)
Calmegin (33)	*Gem GTPase* (15)	*CCG1 protein* (11)
KIAA 0711 (29)	*TNF-α* converting enzyme (15)	*Neural cell adhesion protein* (11)
Retinol-binding protein (28)	cDNA clone 198754 (15)	*Cleavage signal 1 protein* (10)
cDNA DKFZp564J102 (26)	*KIAA 0930* (15)	*KIAA 0001* (10)
Map kinase phosphatase, MKP-2 (25)	*Pirin* (14)	*p54/58 N* (10)
Bene (24)	*CREBL2* (14)	*Sulfate transporter* (10)
Cytidine deaminase, CDA (22)	*KIAA 0199* (4)	*HRFX2* (9)
Serine protease with IGF-binding motif (20)	*Angelman syndrome gene* (12)	*ORCTL3* (9)
DEC-205 (20)	*eps8 binding protein e3B1* (12)	*SIM1* (9)
ZNFpT17 (20)	*Prostacyclin synthase* (12)	*Hep27 protein* (9)
Beta III spectrin (19)	*RET finger protein-like 1* (12)	*HYPE* (9)
GA733-2 (19)	*KIAA 0471* (11)	cDNA DKFZp564C152 (8)
Heat shock protein 40 (18)	*Sec23B isoform* (10)	*Lumican* (8)

genes in each of the three resistant cell lines are summarized in Table 2.

The paclitaxel-resistant phenotypes of SKOV-3$_{TR}$, OVCAR8$_{TR}$ and MCF-7$_{TR}$ are largely non-overlapping

Although SKOV-3$_{TR}$, OVCAR8$_{TR}$ and MCF-7$_{TR}$ all demonstrate a paclitaxel-resistant phenotype, the transcripts identified with altered expression in each cell line pair were largely non-overlapping and encode proteins with a wide variety of biochemical functions. Indeed, the genes identified encode transcription factors, protein kinases, protein phosphatases, cell cycle regulators, cancer testis antigens, fibronectins (FN), heat shock proteins, as well as a large collection of genes that have limited characterization.

Genes differentially expressed in all three paclitaxel-resistant cell lines

Eight genes were overexpressed in the three paclitaxel-resistant cell lines in comparison to their respective parental line (Table 3). One of these eight genes was *MDR1*, which encodes an ATP requiring drug efflux pump. In our experiments, *MDR1* was upregulated 33- to 240-fold. The remaining seven genes have not been previously associated with drug resistance. Two of the seven genes, *MGC4175* and *MGC14772*, were identified in the NIH Mammalian Gene Collection Project and encode hypothetical proteins. *MDR1*, *MGC4175* and *MGC14772* are all located at chromosome position 7q21, raising the question as to whether the paclitaxel-associated overexpression of these three genes is interrelated. In addition, three genes were

Table 3 Genes differentially expressed in all three resistant cell lines

Gene name	Genbank accession no.	Chromosome location	Function
Overexpressed genes			
GBP1[a]	M55542	1p22	Guanine nucleotide binding (GTP, GDP, GMP)
Toll-like receptor 6	AB020807	4p15	Activation of NF-κB mediating cytokine secretion and the inflammatory response
CATP-III	M54995	4q12	Stimulation of DNA synthesis, mitosis, glycolysis
Human testis specific basic protein	U60665	6p21	Unknown
MDR1[a]	M14758	7q21	Plasma membrane efflux pump, expression decreases accumulation of drug
MGC 4175[a]	NM_024315	7q21	Unknown
MGC 14772[a]	BC008478	7q21	Unknown
NF-κB-2[a]	U09609	10q24	Involved in the immune response and acute phase reaction
Underexpressed genes			
Cyclic nucleotide gated channel alpha 3	AF065314	2p11	Visual signal transduction
Fibronectin	A14133	2q34	Cell adhesion, cell motility and maintenance of cell shape
Opioid receptor	U12569	6q24	Inhibition of neurotransmitter release

[a]Genes confirmed by QRT-PCR and/or Northern analysis.

identified as underexpressed in all three drug-resistant cell lines (Table 3). These three genes have not been previously associated with drug resistance.

Figure 3 is a Venn diagram demonstrating the number of transcripts overexpressed (Fig. 3a) and underexpressed (Fig. 3b) in each pair of cell lines and the relationship of these transcriptional changes to the other cell line pairs. In addition to the eight genes overexpressed in all three resistant cell lines, there were 79 genes that were overexpressed and shared between SKOV-3_{TR} and MCF-7_{TR}, 52 genes between SKOV-3_{TR} and $OVCAR_{TR}$ and 152 genes between MCF-7_{TR} and $OVCAR8_{TR}$. In addition to the three genes underexpressed in all three resistant cell lines, there were 40 transcripts with decreased expression in both SKOV-3_{TR} and MCF-7_{TR}, 46 genes decreased in both SKOV-3_{TR} and $OVCAR_{TR}$ and 23 genes decreased in both MCF-7_{TR} and $OVCAR8_{TR}$.

Real-time QRT-PCR and/or Northern analysis confirmed the array findings of selected genes

To validate the array data, we performed QRT-PCR and/or Northern analysis of the 11 genes that were differentially expressed in the three resistant cell lines. We confirmed the expression patterns for 5 genes (Table 3). As an example, *MGC4175* transcript expression is shown in Fig. 4. Overall, 5 of the 11 differentially expressed genes were confirmed by Northern blot/RT-PCR. The other genes showed either no difference in expression level or inconsistent RT-PCR and/or Northern data. These failures in validation could be attributed to various experimental factors including the sequence context and optimal Northern/RT-PCR conditions for these specific genes, and other factors that we do not understand at present.

Discussion

The behavior of a cancer cell is governed, in part, by its expressed genetic repertoire. One previously unanswered question is whether paclitaxel resistance activates the same, overlapping, or distinct sets of target genes in different resistant cell lines. Given the diverse genetic backgrounds of SKOV-3, OVCAR8 and MCF-7, the three resulting resistant cell lines could have acquired paclitaxel resistance through unique expression profiles with little or no similarity to one another. If there was a preferred mechanism of paclitaxel resistance, then the three resistant cell lines might have converged on this mechanism, demonstrating overlap in the transcriptional changes associated with paclitaxel resistance. Third, if only one mechanism of paclitaxel resistance was available, then the three resistant cell lines would have demonstrated one very similar expression profile. Distinguishing these three possibilities is best facilitated by large-scale transcriptional array analysis.

While, our results do not conclusively answer the question as to whether one mechanism versus a preferred mechanism versus multiple mechanisms of paclitaxel resistance exists, important observations can be made. First, there are a large number of transcriptional changes associated with paclitaxel resistance acquired in vitro. Indeed, between 9% and 13% of transcripts are overexpressed or underexpressed in the resistant cell lines as compared to the drug-sensitive parental lines, confirming our initial experiments using smaller scale arrays [12]. Second, gene overexpression is more common than gene underexpression in acquired paclitaxel resistance (Fig. 3). Third, it is also notable that the fold overexpression or underexpression was most dramatic in the SKOV-3_{TR} cell line, which has the greatest relative paclitaxel resistance as compared to its parental cell line.

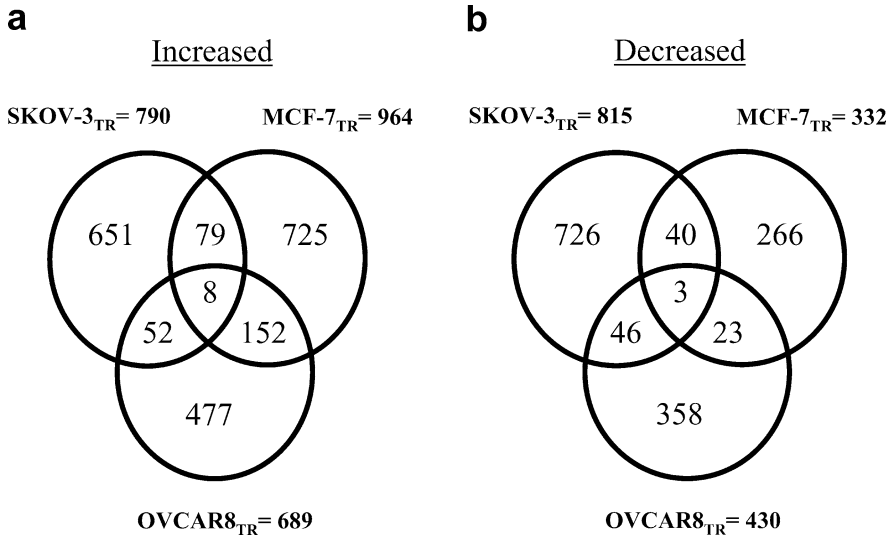

Fig. 3 Genes overexpressed (**a**) and underexpressed (**b**) in the three pairs of cell lines. Genes overexpressed/underexpressed in more than one cell line are indicated in the overlapping regions of the circles

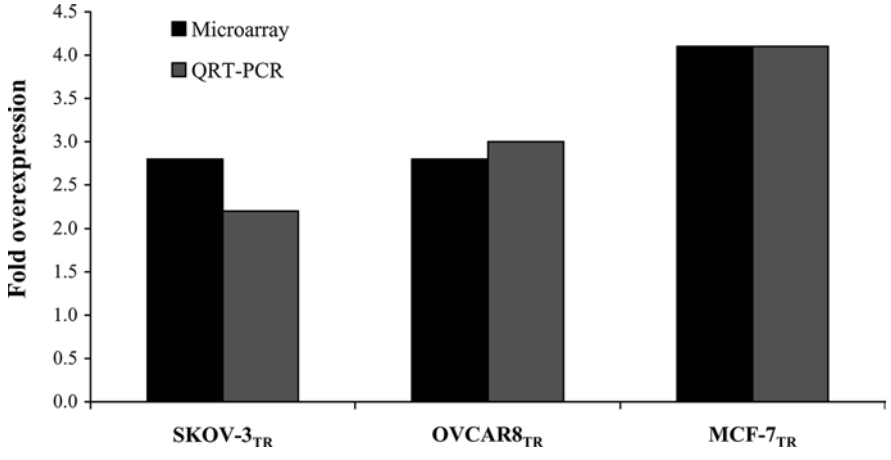

Fig. 4 Expression of *MGC4175* by microarray and QRT-PCR in all three pairs of cell lines. All QRT-PCR data have been normalized to *GAPDH*

While it is possible that some of the transcriptional changes identified in the array analysis do not represent true changes in RNA expression, the analysis of cell lines in triplicate should reduce sporadic changes (i.e., false positives). Northern blotting or QRT-PCR confirmed the altered expression of 5 of 11 transcripts identified in all three resistant cell lines. Extrapolating to the entire genome, these results suggest that hundreds of genes have altered expression in drug-resistant daughter lines as compared to their parental lines.

MDR1, which encodes P-glycoprotein, is frequently correlated with in vitro multidrug resistance. In the present study, *MDR1* was overexpressed in all three resistant cell lines. The undisputed role of *MDR1* in in vitro drug resistance raises the possibility that the majority of genes identified by transcriptional analysis are an epiphenomenon of *MDR1* overexpression and are not functionally relevant. While this remains a formal possibility, at least four lines of evidence suggest this is not the case. First, if the majority of the transcriptional changes identified were associated with upstream or downstream regulation of *MDR1*, one would hypothesize that the transcriptional changes of the three drug-resistant cell lines would overlap more broadly than is evident. Indeed, only 11–31% of the transcriptional changes seen in one cell line were duplicated in a second resistant cell line. Second, in earlier studies we identified the overexpression of *IL-6*, *PGK1* and *MAGE* genes in multidrug-resistant cell lines that also overexpress *MDR1*. When these genes are transfected into paclitaxel-sensitive cell lines, they directly induce paclitaxel resistance independent of *MDR1* [15–17]. Third, when studying the evolution of paclitaxel resistance in SKOV-3 using a self-organizing map technology, the first changes in *MDR1* expression were detected after moderate paclitaxel resistance was established. Thus, several hundred transcripts demonstrated paclitaxel-related transcriptional changes earlier than *MDR1* [5]. Indeed, the present study identified a diverse set of non-*MDR1* transcriptional changes in the three resistant cell lines, arguing that independent mechanisms of paclitaxel resistance exist. Fourth, the magnitude of multidrug resistance does not correlate closely with the level of *MDR1* overexpression [18]. More importantly, clinical studies by our group and others suggest that *MDR1* expression does not increase with acquired resistance to chemotherapy in the clinic [19, 20]. This observation is in concert with clinical trials of select MDR1 inhibitors,

which are not highly efficacious at restoring sensitivity to MDR substrates [21]. In toto, these four lines of evidence suggest that the recurrent, in vitro induction of MDR reflects a selection bias associated with extreme resistance and that this resistance does not mimic or define drug resistance in the clinic.

In addition to *MDR1*, there are seven genes overexpressed in all three resistant cell lines. Notably, none of these genes have yet to be associated with a drug resistance phenotype. *MGC4175* and *MGC14772* were recently described in the NIH Mammalian Gene Collection Project and encode hypothetical proteins. These two genes are located near *MDR1* on chromosome 7q21. This observation can be interpreted in two ways: (1) *MGC4175* and *MGC14772* overexpression is an epiphenomenon of *MDR1* overexpression, and (2) *MGC4175* and/or *MGC14772* overexpression is independent of *MDR1* overexpression. Indeed, if the second scenario proves correct, it is hypothesized that chromosome 7q21 is under considerable pressure for amplification in conditions of drug stress because it contains multiple genes involved in cell protection from chemotherapeutics. Indeed, further analysis is needed to confirm whether *MGC4175* and *MGC14772* are consistently overexpressed in drug-resistant cell lines. Preliminary evaluation of *MGC4175* confirms that its expression is associated with the multidrug-resistant phenotype in additional cell lines. This evaluation is the subject of an independent report (in press). More recently, *MGC4175* overexpression in multidrug-resistant cell lines has also been found in an independent study (95th AACR Meeting abstract, 2004).

Also of note is the overexpression of *MDR3* (*ABCB4*) in both $OVCAR8_{TR}$ (16-fold) and $MCF\text{-}7_{TR}$ (11-fold). MDR3 p-glycoprotein is normally involved in the transport of phospholipids from liver hepatocytes into bile, and can transport paclitaxel and vinblastine, albeit relatively inefficiently [22]. *MDR3* expression negatively correlates with a positive clinical outcome in individuals with acute and chronic leukemia [23]. Additional testing may clarify the role of *MDR3* in paclitaxel resistance.

The microenvironment has been shown to influence tumor cell phenotype with respect to growth, metastasis, and response to chemotherapy. FN genes and heat shock protein genes (HSPs) showed significantly decreased expression in resistant cell lines in this study. FN is a large multidomain glycoprotein found in connective tissue, on cell surfaces and in plasma and other body fluids. It interacts with a variety of macromolecules including components of the cytoskeleton and the extracellular matrix. FN is involved in many cellular processes, including tissue repair, embryogenesis and cell migration/adhesion. FN secreted from the peritoneum increases MMP-9 activity and expression, and in turn, invasiveness of ovarian cancer cells [24].

HSPs, also called stress proteins, are a group of proteins that are present in all cells across all phyla. They are induced when a cell undergoes various types of environmental stresses such as heat, cold and oxygen deprivation. It has been reported that *hsp27* overexpression in breast cancers may be involved in cell growth arrest and increased differentiation while, in contrast, *hsp70* may be involved in cell proliferation [25]. HSPs are thought to inhibit apoptosis and reduce the effectiveness of chemotherapy [26]. However, the relationship between HSPs and drug resistance to chemotherapy has not been well defined.

In summary, microarray technology identified an enormous and only minimally overlapping set of genes with altered expression in three paclitaxel-resistant ovarian and breast cancer cell lines. Therefore, we reason that a single mechanistic pathway cannot explain the genesis of paclitaxel resistance in these cell lines. Paclitaxel resistance is likely to involve the altered expression of multiple genes. Further, we have identified transcriptional changes not previously associated with the drug resistance phenotype. Efficient strategies for evaluating the functional significance of these changes and their role in clinical drug resistance are needed.

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