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Gene Expression Profiling in Chemoresistant Variants of Three Cell Lines of Different Origin

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Abstract. *Background: Drug resistance is a major problem in clinical cancer chemotherapy. Several mechanisms of resistance have been identified, but the underlying genomic changes are still poorly understood. Materials and Methods: Gene expression profiling, using cDNA microarray, was performed in eight cell lines (K562 leukemia, MCF-7 breast cancer and S1 colon cancer) with acquired resistance against five cytostatic drugs; daunorubicin (DNR), doxorubicin (DOX), vincristine (VCR), etoposide (VP) and mitoxantrone (MX). Results: The resistant cell lines clustered together based on their type of origin. Several genes encoding ABC transporters were highly up-regulated, most notably ABCB1 (MDR1) and ABCB4 in several cell lines and ABCG2 (MXR) specifically in MX-resistant cell lines. A pronounced down-regulation of several histones was noted in the MCF-7-derived resistant sublines. Altered expression was also seen in, e.g., GSTs, topoisomerases, caveolins, annexins and CD44. Conclusion: These results will constitute a platform for further studies on specific pathways and biological processes involved in chemotherapy resistance.*

Cellular drug resistance is a major problem in the chemotherapeutic treatment of cancer patients. There are many different mechanisms for drug resistance, such as decreased drug uptake, increased efflux, intracellular detoxification, DNA repair and altered activity of target proteins. Drug resistance is often multifactorial, with many genes involved, but its detailed regulation on the genomic level is still poorly understood. With the introduction of cDNA microarray technology, the global gene expression profile can be analyzed in one single experiment. In recent years, this powerful method has become widely used in attempts to increase our understanding of chemotherapy resistance and mechanisms of action (1-3).

In the current study, the gene expression profiles in three different cell lines; [K562 (chronic myelogenous leukemia), MCF-7 (breast cancer) and S1 (colon cancer)] with induced resistance against five different cytostatic drugs [daunorubicin (DNR), doxorubicin (DOX), mitoxantrone (MX), etoposide (VP), and vincristine (VCR)] were investigated. These drugs have different modes of action: DNR, DOX and MX all intercalate with DNA.VP, DNR and DOX interact with topoisomerase II. VCR binds to tubulin and inhibits the microtubular function during mitosis. All of these drugs are known substrates for ABCB1 (formerly known as MDR1), which confers cross-resistance against numerous chemotherapeutic agents by pumping them out through the cell membrane (4).

The overall purpose was to study the genomic similarities and differences between these cell lines. One specific aim was to explore whether drug-specific fingerprints could be identified, regardless of tumor cell origin. The next step was to explore which genes and cellular functions are most important for the determination of different resistant phenotypes.

Materials and Methods

Cell culture. K562 leukemia cells and S1 colon cancer cells were cultured in Hepes buffered RPMI 1640 containing 5% NCS, 1% L-glutamine, and 100 U penicillin and 100 mg/ml streptomycin. MCF-7 breast cancer cells were grown in Improved MEM Zinc containing 5% NCS, 1% L-glutamine, and 100 U penicillin and 100 mg/ml streptomycin. All culture media were purchased from Invitrogen/ Life Technologies, Taastrup, Denmark. Cell cultures were maintained at 37˚C in an atmosphere of 95% air and 5% CO_2. The resistant cells were kept in sub-lethal concentrations of drugs. Before RNA extraction for expression analysis, all cell lines were transferred

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Table I. *Resistant cell lines used in the present study. The relative level of resistance to the selecting agent is indicated in parenthesis. nd, no data available.*

	Induced resistance against:				
	DNR	DOX	MX	VCR	VP
K562	K-DNR (852x)	-----	K-MX (15x)	K-VCR (152x)	K-VP (20x)
MCF-7	-----	M-DOX (nd)	M-MX (nd)	-----	M-VP (25x)
S1	-----	-----	S-MX (21600x)	-----	-----

to fresh, drug-free media for 24 hours. For RNA extraction, the lysis buffer was added directly into the cell culture flask.

Drugs. The cytostatic drugs used in this study were Daunorubicin (DNR), Aventis Pharma, Horsholm, Denmark; Mitoxantrone (MX) and Vincristine (VCR), Wyeth Lederle, Glostrup, Denmark; Etoposide (VP) Bristol Myers Squibb, Denmark; and Doxorubicin (DOX) Pfizer Aps, Ballerup, Denmark.

Induction of resistance. Wild-type human leukemia K562 cells were used for developing resistant cell lines. The cells were cultured in increasing drug concentrations for 35-40 passages. Culture conditions after resistance development: K-DNR 0.45 μM DNR, K-MX 0.44 μM MX, K-VCR 70 nM VCR and K-VP 3.4 μM VP. MX resistant S1-MX cells were obtained from Dr. Susan Bates and are identical to those designated S1-M1-80 by Miyake *et al.* (5). The cells were derived from the S1 clone of LS-180 colon carcinoma cells, and were advanced to high levels of resistance by exposure to increasing concentrations (up to 80 μM) of MX. The drug-resistant MCF-7 cells were also obtained from Dr. Susan Bates, and developed by culture in increasing drug concentrations of MX (80 nM), VP (4 μM) and DOX (3.7 μM), respectively. Drug resistance levels were measured by clonogenic assay (6) or sulphorhodamine B assay (7). The level of resistance against the selecting agent is indicated in Table I. We know, from initial experiments (data not shown), that K-DNR and K-VCR express P-glycoprotein (MDR1), both K562 control cells and resistant derivatives express MRP1 at about the same level, and K-MX expresses ABCG2. M-VP expresses high levels of MRP1 and is 6 to 10-fold cross-resistant to MX.

cDNA arrays. Bacterial clones containing plasmids with cDNAs representing 29623 human gene fragments were used to produce microarrays. The majority of these were clones purchased from Research Genetics (Huntsville, AL, USA). 1400 cDNA clones were obtained from Dr. Gerrit Los, UCSD Cancer Center, CA, USA. These clones were isolated from MCF-7 cells by subtractive suppression hybridization (8-9), representing genes that were significantly up- or down-regulated after treatment with DOX, cisplatin or paclitaxel.

Plasmids were isolated from bacteria, and PCR reactions were performed to amplify inserts. Purified PCR products were spotted onto Corning (Corning, NY, USA) CMT-GAPS glass slides by using a Biorobotics MG2 arrayer. Each gene was usually represented by one cDNA clone, but occasionally by two or three clones. Each clone was, for the most part, spotted once on the array, but some of them were spotted in duplicates or triplicates. Taken together, around 2/3 of the genes were represented once, while the remaining 1/3 of the genes appeared two or more times on each microarray slide.

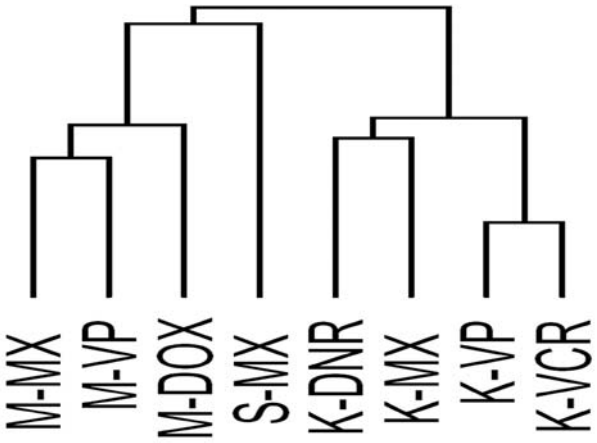

Figure 1. *Cluster analysis of the eight cell lines, based on differentially-expressed genes.*

RNA purification. Total RNA was isolated by the method of Chomczynski and Sacchi (10). RNA concentrations and purity were determined by spectrophotometer readings at 260 and 280 nm. The total RNA samples did not show any signs of degradation as visualized in formaldehyde-containing agarose gel electrophoresis.

Poly A RNA. Poly A RNA (mRNA) was purified from total RNA using NucleoTrap mRNA Mini Kit (Machery-Nagel GmBH & Co., Duren, Germany), following the manufacturer's instructions. The poly A concentrations in the eluates were quantified by spectrophotometry at 260 and 280 nm.

Control cells. For each hybridization, the unmodified sensitive counterpart was used as control, *e.g.,* K-MX, K-DNR, K-VP and K-VCR were all hybridized against K562, S-MX against S1, and M-MX, M-VP and M-DOX against MCF-7.

RT labelling of RNA. Hybridizations were performed in duplicates with dye-swap. Thus, in the first hybridization, 1 μg of mRNA from the test cells (resistant variants) was labelled with Cy3 and 1 μg of mRNA from control cells was labelled with Cy5. In the second hybridization, the dyes were swapped.

The RNA samples were concentrated in Microcon YM-30 columns (Amicon, Billerica, Mass, USA) to a volume of 12 μl and then annealed with anchor primer (oligo T primer). Samples were converted to cDNA and directly labelled using dye coupled

Table II. *Gene Ontology. The most overrepresented ($p<0.01$) biological processes or molecular functions among up- and down-regulated genes in the different cell lines.*

	K-DNR	K-MX	K-VCR	K-VP
Up	metal ion homeostasis vitamin A metab. cation homeostasis gas/oxygen transport	porphyrin metab. pigment metab. transaminase activity fatty acid metab. chloride channel activity	cell adhesion lipid metabolism neurogenesis phospholipid metab.	ABC transporter activity cell communication aldehyde dehydrogenase activity transferase activity cell adhesion
Down	receptor activity cell communication signal transduction immune response cell adhesion JNK cascade	immune response signal transduction cell communication receptor activity carbohydrate binding cell adhesion	immune response signal transduction cell communication RAS signaling glutathione transferase activity receptor activity	cell communication signal transduction immune response phospholipase C activity phagocytosis receptor activity
	M-DOX	M-MX	M-VP	S-MX
Up	calmodulin binding nitrogen metab. tetrahydrobiopterin metab. response to drug transferase activity	ribosomal activity Wnt receptor activity selenium binding	glutathione transferase activity lipid transport transmembrane ATPase activity transmembrane hydrolase activity histone acetyltransferase insulin-like growth factor binding	steroid metabolism cell adhesion cell-cell signaling positive chemotaxis apoptosis inhibitor activity
Down	chromatin assembly immune response DNA packaging actin polymerization	chromatin assembly immune response receptor activity DNA packaging cell adhesion caspase activation	chromatin assembly DNA packaging DNA metabolism glucose metabolism transferase activity	aldo-keto reductase activity mitosis bile acid transporter activity ER to Golgi transport

nucleotides. For test and control samples, Cy3 and Cy5 coupled nucleotides were used, respectively. After purification, the labelled test and control samples were pooled and concentrated to a volume of 26 μl.

Hybridization. The sample was added to a blocking mix containing poly dA, yeast tRNA and Denhardt's blocking solution, along with SSC and SDS. This solution was applied to the array slide, covered with a cover slip and hybridized in a hybridization chamber at 65˚C overnight. The slides were then washed x3 with SSC and dried.

Scanning and image analysis. The fluorescent signals were captured using a Gene Pix 4000A scanner (Axon Instruments, Union City, CA, USA) and images were analyzed with GenePix Pro software (Axon Instruments).

Data analysis. Signal intensities for Cy3 and Cy5 were calculated using local background corrected median pixel intensities. Subsequently, for each hybridization, spots automatically flagged by the image analysis software, as well as spots manually by visual examination for artifacts or debris, were excluded. For the remaining data, each intensity value below 20 was corrected to 20 in order to minimize erroneous large ratios for low intensity spots solely due to background correction. Ratio values were calculated as resistant test sample intensity over the nonresistant control sample intensity. Within hybridizations, ratios were normalized using a BASE (11) implementation of the intensity-based LOWESS normalization method described by Yang *et al.* (12).

The accordance between the duplicates was generally very good, with the exception of one of the K-DNR hybridizations. This particular hybridization suffered from poor technical quality and was excluded from further analysis. Data from the remaining dye-swap hybridizations were merged to form per spot geometric mean ratios and then normalized again. Thus, the total data set was made up of fifteen hybridizations; duplicates from seven of the eight different sublines and one of the K-DNR hybridizations.

Intensity-dependent estimation of differential expression was performed using a BASE implementation of the method described by Yang *et al.* (13) and expressed as standard deviations (SDs). An SD value was thus calculated for each spot using a sliding window range along spot intensity. We considered a gene as significantly up-regulated if its corresponding spot had >2-fold increase in ratio in the resistant variant compared to the control, and SD≥2. Correspondingly, a gene was considered significantly down-regulated if its corresponding spot had >2-fold decrease in ratio in the resistant variant compared to the control, and SD≤–2.

Comparison of the global gene expression profiles in the different cell lines was visualized using the hierarchical clustering plugin available in BASE. Pearson correlation was used to

Table III. *Top 5 most up- and down-regulated genes in the different cell lines. Ratios between expression levels in resistant vs. senstive cell lines are given in the up-regulated genes and sensitive vs. resistant in the down-regulated genes. Genes appearing more than once in one top 5 list are presented only as the transcript with the highest ratio. Transcripts with unknown function were excluded. Gene symbols are shown. For information on gene identity see [www.ncbi.nlm.nih.gov/UniGene].*

	K-DNR		K-MX		K-VCR		K-VP	
Up	ABCB1	170.2	CHST3	20.7	SPOCK2	388.2	ABCB1	151.2
	CA2	24.3	ALDH1A2	15.8	ABCB1	36.4	CD36	58.3
	STAR	18.8	STAR	13.3	STAR	25.2	STAR	30.6
	CA1	18.4	TPD52L1	13.3	ALDH1A2	16.2	ALDH1A2	24.3
	ALDH1A2	11.3	COL15A1	12.6	SLC2A12	15.5	COL12A1	23.9
Down	K6IRS4	213	CD44	124.7	HDAC5	66.7	CD44	97.7
	CD44	85.9	PTPN7	46.2	CD44	44.3	ARHGDIB	68.3
	FZD4	70.7	GAGE5	41	CUBN	27.5	AIF1	32.8
	MFAP2	60.8	FYB	40.2	AIF1	21.5	FYB	29.1
	LOC81691	54.7	ELMO1	33.9	XRCC1	17.9	ELMO1	21.1
	M-DOX		M-MX		M-VP		S-MX	
Up	ABCB1	124.5	ABCG2	109.9	MGP	28.3	ABCG2	244.8
	TCTE1L	67.1	EFEMP1	30	ABCC6	15.5	KIT	35.3
	CA2	48.3	KLHL8	27.1	EXT1	13.4	GOLPH4	31.2
	XK	38.8	TM4SF1	25.9	NDE1	10.7	SMOC2	28
	SH3GL3	23.8	IGFBP5	23.9	LKAP	10.1	RAB40C	21.6
Down	NDUFA11	77.1	GALC	57.5	DHRS2	30.6	EREG	21.1
	KIF5A	65.2	ENG	50.3	GALC	24.7	PR1	13.7
	ENG	60.1	IFIT1	48	IFIT1	13.8	MAN1A1	12.6
	MLAT4	51.1	G1P3	38	HIST1H2AL	13.1	CAPN6	11.7
	BM039	48.1	KIF5A	37.2	BASP1	12.5	PTPRD	11.7

calculate the distance metric and a bottom-up approach was used where the two closest points are merged, and the new cluster is represented by a weighted (center of mass) average of the two points in gene expression space.

To investigate possible over-representation within specific Gene Ontology (14) categories among up- and down-regulated genes, we employed the software tool EASE (15). The web-sites (www.ncbi.nlm.nih.gov/LocusLink) and (www.geneontology.org) were used for this process.

General outline of the analysis steps. The gene expression data were analyzed in several steps. The first step was to generate cluster analyses for comparison of the global gene expression profiles in the different cell lines (Figure 1), based on genes that were classified as significantly up- or down-regulated. The next part of the study aimed at identifying which genes were responsible for the differences and similarities in genomic profiles between the cell lines. All differentially-expressed genes were classified according biological function, by Gene Ontology search, in order to identify which biochemical processes were altered in the resistant cell lines (Table II). Genes with the highest and lowest expression ratios were listed (Table III). Lastly, we examined the gene expression pattern in a number of genes with established or assumed roles in chemotherapy resistance. These genes were picked up by a PubMed literature search, for articles published during the last five years, entering "resistance" and any of the five drugs DNR, DOX, VCR, VP, or MX as search words. Genes that came out of this search process, along with other genes in the same gene family, were analyzed specifically for their expression ratio in all of the resistant cell lines (Figure 2).

Results

Cluster analysis. A general feature seen in the global cluster analysis was that the resistant variants clustered together based on their origin. Thus, the four resistant subtypes of K562 clustered together and clearly separated from S1 and MCF7 cell lines, respectively (Figure 1). There was very sparse similarity between different cell lines resistant to the same drug, *e.g.,* the three MX-resistant sublines, in this unsupervised analysis of the global gene expression pattern.

Number of differentially-expressed genes. The number of gene spots that were significantly up-regulated (ratio >2x increase and SD>+2) varied between 242 and 845 in the different resistant sublines and between 297 and 736 gene spots were down-regulated (ratio >2x decrease and SD <–2).

Gene Ontology. In order to get a general idea of their biological significance, all of the differentially-expressed genes were classified according to the Gene Ontology™ into biological functions. The groups that were significantly over-represented among the up- or down-regulated genes in different cell lines are presented in Table II. A general difference could be noted in that the K562 sublines all showed down-regulation of genes associated with immune response, cell communication and signal transduction. These groups were composed of genes encoding interferon-inducible proteins, interleukins, protein tysine kinases, integrins *etc.* Another general feature was the down-regulation of genes involved in chromatin assembly and DNA packaging in the MCF-7-derived cell lines, mostly due to a significant decrease in the expression of several histones.

Single most differentially-expressed genes. In the Gene Ontology categorization, all genes fulfilling the criteria for up- or down-regulation were treated alike, regardless of the level of differential expression. Another way of analyzing the data is to sort the genes by their expression ratio. Top 5 lists of the most up- and down-regulated genes are presented in Table III. The most striking finding was the marked increase in expression of different ABC-genes (ATP-binding cassette), which was seen in most of the resistant variants. Some genes were extremely up-regulated in more than one of the K562 lines: *STAR* (steroidogenic acute regulatory protein), *carbonic anhydrase II*, *aldehyde dehydrogenase* and different collagens. Among the most down-regulated genes, a marked decrease in expression was noted for *CD44* in all of the K562 cell lines.

Resistance genes. Expression levels of genes with established or suspected importance for resistance against the five chemotherapeutic agents DNR, MX, VCR, VP and DOX were analyzed, along with related genes of the same families, Figure 2. The findings are commented on in the "Discussion".

Discussion

In this cDNA microarray study, it was found that different resistant cell lines clustered together based on their cell type origin, *i.e.* all the K562 variants clustered together and were clearly separate from the MCF-7 and S1 cell lines. It should be noted that each resistant subline was hybridized against its non-resistant counterpart, *i.e.* a universal RNA pool was not used as control. This means that the cell line-specific cluster pattern did not merely reflect the inborn genomic differences between these cell lines of different origin, but rather indicates that different tumor types use different molecular strategies to deal with long-term exposure to cytostatic drugs. One of the main goals of this study was to identify drug-specific genomic fingerprints for resistance, regardless of tumor type. Such a finding would have been of clinical benefit for choosing the best drugs for individual patients. However, in the present study, no obvious drug-specific patterns could be found, with a few exceptions at the single-gene level.

In order to identify which genes and biological processes were of most importance for the different chemoresistant phenotypes, Gene Ontology search, unsupervised gene ranking by ratio and supervised search on genes with established relevance in resistance were performed.

It is well known that genes encoding ABC proteins play an important role in chemotherapy resistance. To date, 48 different ABC genes have been identified. Genomic profiling of the whole set of ABC transporters has recently been published (16, 17). At least 6 of these ABC genes have been associated with resistance, by causing an increased drug efflux from the tumor cell (4) . On our cDNA microarrays, 16 ABC genes were represented, of which 11 showed significantly altered expression in at least one of the resistant cell lines (Figure 2). ABCB1 (formerly known as MDR1) was the first ABC-transporter characterized. Its gene product, p-glycoprotein, is as transmembrane transporter of numerous chemotherapeutic agents, associated with multidrug resistance, MDR. The importance of ABCB1 as a resistant factor was strongly supported by the present study. *ABCB1* was in fact the most up-regulated gene in several of our resistant cell lines (Table III). Another well-characterized ABC-transporter is ABCG2 (formerly known as mitoxantrone-resistance protein (MXR) or breast cancer resistance protein (BCRP)) which has an established role in resistance against, *e.g.,* MX (4, 18). This was also confirmed in the present study, in which *ABCG2* was by far the most up-regulated gene in two of the MX-resistant cell lines, M-MX and S-MX (Figure 2 and Table III). In the third MX-resistant subline, K-MX, *ABCG2* was also slightly (1.6x) up-regulated. We also found a marked up-regulation in several of the other ABC genes (Figure 2), whose role in chemotherapy resistance is less established. However, a few studies have shown correlations between ABCB4(19), ABCC2 (4), ABCC4 (4), ABCC5 (4), ABCC6 (20) and resistance against chemotherapeutic agents.

Other factors that commonly have been linked to chemoresistance include glutathione S-transferases (21), metallothioneins (22), topoisomerases (23), erbb2 (24), p53, bcl-2 and bax. In the present study, the expression levels of the genes encoding these proteins varied over the cell lines, partially in accordance with literature findings.

Several of the genes encoding histones were clearly differentially-expressed, in a diverging but highly cell line-specific manner. Up-regulation of histones was seen most notably in K-VP and K-VCR, whereas a pronunced down-regulation of histones was observed in all three MCF-7-derived cell lines. This was also reflected in the gene

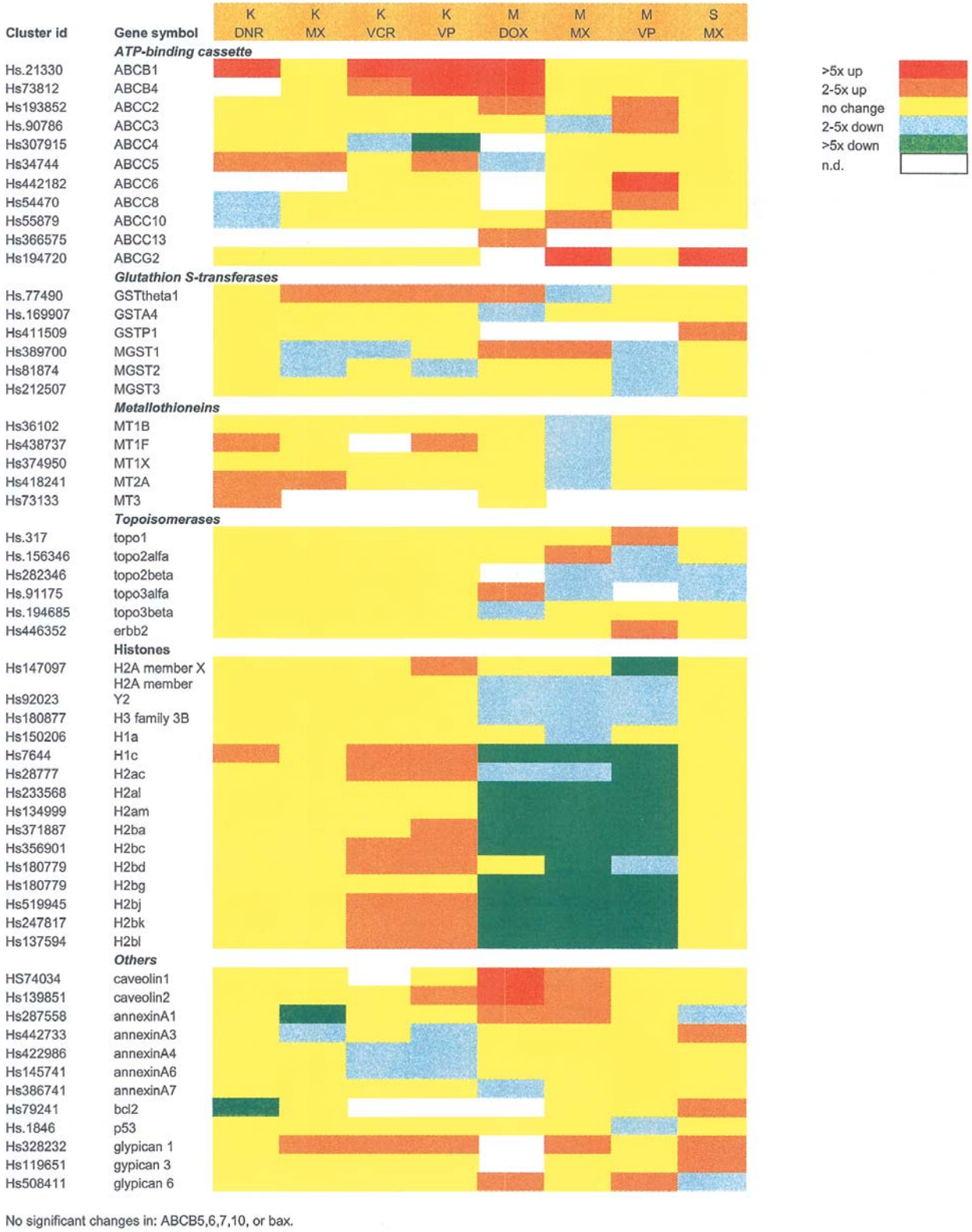

Figure 2. *Expression ratios in genes with an established or suggested role in resistance against these drugs, according to the literature. In cases with genes in duplicate or triplicate on the array, the mean ratio is presented. Only genes with >2-fold increase or decrease in expression level in at least one cell line are shown.*

ontology classification, showing chromatin assembly and DNA packaging to be the most down-regulated functions in the MCF-7-derived sublines (Table II). It has been shown that anticancer drugs have less effect in tumor cells with tightly condensed chromatin DNA, possibly by reducing the accessibility to their DNA-related target (25) . Histones are crucial not only for chromatin assembly, also being involved in several other cellular functions such as DNA repair (26) and apoptosis (27), which may explain our finding that both up- and down-regulation of histones can be associated with chemoresistance. Further studies are clearly needed to clarify these issues.

Caveolin-1 (28), and annexin-1 (29) are proteins that have been reported up-regulated specifically in DOX-resistant MCF-7 cells, tentatively by promoting drug efflux. It is of interest to note that the genes encoding these proteins were also highly up-regulated in our M-DOX cell line.

One factor that has been associated with decreased intracellular drug accumulation and resistance against MX is glypican 3 (30). The present study confirmed up-regulation of *glypican 3*, and *glypican 1*, in one of the MX-resistant cell lines, S-MX.

Apart from the ABC genes, very few of the genes with most pronounced up- or down-regulation (Table III) have been previously linked to resistance against the drugs in question. One exception is *CD44* that was highly suppressed in all four K562-derived cell lines (Table III). CD44 plays an important role in tumor-endothelium interactions, cell migration, cell adhesion, tumor progression and metastasis (31). A decreased expression of CD44 has been reported in a multidrug-resistant melanoma cell line (32).

The present study describes the genomic profiles by using cDNA microarray. Our findings have not yet been confirmed by RT-PCR or by studies on the protein level. Therefore, our results on single genes should be interpreted with caution. However, the fact that several of the ABC transporters were highly up-regulated in an expected manner strengthens the relevance of the results.

In conclusion, this study showed that the expression levels of multiple genes were altered after induction of resistance against cytostatic drugs. The fact that the global genomic profiles showed cell line-specific patterns, could indicate that tumor cells of varying origin use different strategies to develop resistance. A general feature in the resistant K562 variants, but not in the other cell lines, was that many genes involved in immune response and general function of myeloid cells were differentially-expressed. It seems reasonable to assume that these differences reflect variations in the inborn ability to deal with stress and genotoxic exposure; K562 is a leukemia cell line, whereas MCF-7 and S1 both are carcinomas of epithelial origin. It is unclear which of these genomic changes were important for the acquisition of resistance and which alterations reflect secondary cellular processes. When it comes to previously established resistance factors, the current study strongly supports the importance of the ABC-transporters in the development of resistance against these chemotherapeutic agents, but also genes encoding GSTs, topoisomerases, caveolins and histones seem to be involved.

These kinds of exploratory investigations on global gene expression profiles will hopefully constitute important platforms for further functional studies on specific pathways and biological processes involved in chemotherapy resistance.

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References

1 Zembutsu H, Ohnishi Y, Tsunoda T, Furukawa Y, Katagiri T, Ueyama Y, Tamaoki N, Nomura T, Kitahara O, Yanagawa R, Hirata K and Nakamura Y: Genome-wide cDNA microarray screening to correlate gene expression profiles with sensitivity of 85 human cancer xenografts to anticancer drugs. Cancer Res *62*: 518-527, 2002.

2 Scherf U, Ross D, Waltham M, Smith L, Lee J, Tanabe L, Kohn K, Reinhold W, Myers T, Andrews D, Scudiero D, Eisen M, Sausville E, Pommier Y, Botstein D, Brown P and Weinstein J: A gene expression database for the molecular pharmacology of cancer. Nat Genet *24*: 236-244, 2000.

3 Cheok M, Yang W, Pui C, Downing J, Cheng C, Naeve C, Relling M and Evans W: Treatment-specific changes in gene expression discriminate *in vivo* drug response in human leukemia cells. Nat Genet *34*: 85-90, 2003.

4 Sparreboom A, Danesi R, Ando Y, Chan J and Figg W: Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Update *6*: 71-84, 2003.

5 Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T and Bates S: Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res *59*: 8-13, 1999.

6 Roed H, Vindelov L, Christensen I, Spang-Thomsen M and Hansen H: The cytotoxic activity of cisplatin, carboplatin and teniposide alone and combined determined on four human small cell lung cancer cell lines by the clonogenic assay. Eur J Cancer Clin Oncol *24*: 247-253, 1988.

7 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren J, Bokesch H, Kenney S and Boyd M: New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst *82*: 1107-1112, 1990.

8 Diatchenko L, Lau YFC, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED and Siebert PD: Suppression subtractive hybridization: a method for generating differentially regulated tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA *93*: 6025-6030, 1996.

9 Johnsson A, Zeelenberg I, Min Y, Hilinski J, Berry C, Howell S and Los G: Identification of genes differentially expressed in association with acquired cisplatin resistance. Br J Cancer *83*: 1047-1054, 2000.

10 Chomczymski P and Sacchi N: Single step method of RNA isolation by acid guanidium phenol-chloroform extraction. Anal Biochem *162*: 156-159, 1987.

11 Saal L, Troein C, Vallon-Christersson J, Gruvberger S, Borg A and Peterson C: BioArray Software Environment (BASE): a platform for comprehensive management and analysis of microarray data. Genome Biol *3*: software0003.0001-0003.0006, 2002.

12 Yang Y, Dudoit S, Luu P, Lin D, Peng V, Ngai J and Speed T: Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res *30*: e15, 2002.

13 Yang I, Chen E, Hasseman J, Liang W, Frank B, Wang S, Sharov V, Saeed A, White J, Li J, Lee N, Yeatman T and Quackenbush J: Within the fold: assessing differential expression measures and reproducibility in microarray assays. Genome Biol *11*: research0062, 2002.

14 Ashburner M, Ball C, Blake J, Botstein D, Butler H, Cherry J, Davis A, Dolinski K, Dwight S, Eppig J, Harris M, Hill D, Issel-Tarver L, Kasarskis A, Lewis S, Matese J, Richardson J, Ringwald M, Rubin G and Sherlock G: Gene ontology: tool for the unification of biology.The Gene Ontology Consortium. Nat Genet *25*: 25-29, 2000.

15 Hosack D, Dennis GJ, Sherman B, Lane H and Lempicki R: Identifying biological themes within lists of genes with EASE. Genome Biol *10*: R70, 2003.

16 Szakacs G, Annereau J, Lababidi S, Shankavaram U, Arciello A, Bussey K, Reinhold W, Guo Y, Kruh G, Reimers M, Weinstein J and Gottesman M: Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells. Cancer Cell *6*: 105-107, 2004.

17 Annereau J, Szakacs G, Tucker C, Arciello A, Cardarelli C, Collins J, Grissom S, Zeeberg B, Reinhold W, Weinstein J, Pommier Y, Paules R and Gottesman M: Analysis of ATP-binding cassette transporter expression in drug-selected cell lines by a microarray dedicated to multidrug resistance. Mol Pharmacol *66*: 1397-1405, 2004.

18 Litman T, Druley T, Stein W and Bates S: From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci *58*: 931-959, 2001.

19 Turton N, Judah D, Riley J, Davies R, Lipson D, Styles J, Smith A and Gant T: Gene expression and amplification in breast carcinoma cells with intrinsic and acquired doxorubicin resistance. Oncogene *20*: 1300-1306, 2001.

20 Belinsky M, Chen Z, Shchaveleva I, Zeng H and Kruh G: Characterization of the drug resistance and transport properties of multidrug resistance protein 6 (MRP6, ABCC6). Cancer Res *62*: 6172-6177, 2002.

21 Townsend D and Tew K: Cancer drugs, genetic variation and the glutathione-S-transferase gene family. Am J Pharmacogenomics *3*: 157-172, 2003.

22 Theocharis S, Margeli A, Klijanienko J and Kouraklis G: Metallothionein expression in human neoplasia. Histopathology *45*: 103-118, 2004.

23 Jarvinen T, Tanner M, Rantanen V, Barlund M, Borg A, Grenman S and Isola J: Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol *156*: 839-847, 2000.

24 Knuefermann C, Lu Y, Liu B, Jin W, Liang K, Wu L, Schmidt M, Mills G, Mendelsohn J and Fan Z: HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene *22*: 3205-3212, 2003.

25 Kim M, Blake M, Baek J, Kohlhagen G, Pommier Y and Carrier F: Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res *63*: 7291-7300, 2003.

26 Momota H, Ichimiya S, Kondo N, Kikuchi T, Torigoe T, Yamaki T, Houkin K and Sato N: Histone H2AX sensitizes glioma cells to genotoxic stimuli by recruiting DNA double-strand break repair proteins. Int J Oncol *23*: 311-315, 2003.

27 Konishi A, Shimizu S, Hirota J, Takao T, Fan Y, Matsuoka Y, Zhang L, Yoneda Y, Fujii Y, Skoultchi A and Tsujimoto Y: Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks. Cell *114*: 655-656, 2003.

28 Lavie Y, Fiucci G and Liscovitch M: Up-regulation of caveolae and caveolar constituents in multidrug-resistant cancer cells. J Biol Chem *273*: 32380-32383, 1998.

29 Wang Y, Serfass L, Roy M, Wong J, Bonneau A and Georges E: Annexin-I expression modulates drug resistance in tumor cells. Biochem Biophys Res Commun *314*: 565-570, 2004.

30 Wichert A, Stege A, Midorikawa Y, Holm P and Lage H: Glypican-3 is involved in cellular protection against mitoxantrone in gastric carcinoma cells. Oncogene *23*: 945-955, 2004.

31 Martin T, Harrison G, Mansel R and Jiang W: The role of the CD44/ezrin complex in cancer metastasis. Crit Rev Oncol Hematol *46*: 165-186, 2003.

32 Molinari A, Calcabrini A, Meschini S, Marra M, Stringaro A, Toccacieli L, Cianfriglia M and GA: What is the relationship between P-glycoprotein and adhesion molecule expression in melanoma cells? Melanoma Res *12*: 109-114, 2002.

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