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Transcriptome Analysis of Different Multidrug-resistant Gastric Carcinoma Cells

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Abstract. *Multidrug resistance (MDR) of human cancers is the major cause of failure of chemotherapy. To better understand the molecular events associated with the development of different types of MDR, two different multidrug-resistant gastric carcinoma cell lines, the MDR1/P-glycoprotein-expressing cell line EPG85-257RDB and the MDR1/P-glycoprotein-negative cell variant EPG85-257RNOV, as well as the corresponding drug-sensitive parental cell line EPG85-257P, were used for analyses of the mRNA expression profiles by cDNA array hybridization. Of more than 12,000 genes spotted on the arrays, 156 genes were detected as being significantly regulated in the cell line EPG85-257RDB in comparison to the non-resistant cell variant, and 61 genes were found to be differentially expressed in the cell line EPG85-257RNOV. Seventeen genes showed a differential expression level in both multidrug-resistant gastric carcinoma variants. The impact of these alterations in gene expression levels in different multidrug-resistant gastric carcinoma cell variants is discussed.*

Gastric cancer was the fourth most common malignancy in the world in 2000, with an estimated 870,000 new cases and 650,000 deaths per year (1). Most gastric cancers are diagnosed at locally advanced stages. Although a radical subtotal gastrectomy appears to be the only curative treatment, the risk of regional relapse and of hematogenic metastasis is high. Thus, adjuvant treatments (radiotherapy and/or chemotherapy) are frequently used. The available data, however, do not allow definite conclusions regarding the relative values of radiotherapy or chemotherapy. The use of chemotherapy and the combination of chemotherapy with radiation is a standard procedure in the treatment of advanced cancer. These therapeutic protocols produce an overall response rate of about 20% at best using a single-agent, and up to 50% using combination chemotherapy. Thus, a large number of malignancies are incurable. Generally, gastrointestinal cancers, including gastric carcinoma, are naturally resistant to many anticancer drugs. Additionally, these tumors are able to develop acquired drug resistance phenotypes, which include the multidrug resistance (MDR) phenomenon.

The MDR phenotype is characterized by simultaneous resistance of tumor cells to various antineoplastic agents which are structurally and functionally unrelated. Besides the classical MDR phenotype, mediated by the enhanced expression of the ATP-binding cassette (ABC) transporter (2) P-glycoprotein (MDR1/P-gp) (3), alternative forms of multidrug-resistant tumor cells have been described. The commonly used terms to designate this phenomenon are atypical MDR or non-MDR1/P-gp-mediated MDR.

Some of the mechanisms leading to atypical MDR have been identified. These mechanisms include enhanced expression of alternative ABC-transporters, such as MRP1-MRP9 (4) or BCRP (5). However, since all these mechanisms do not explain the MDR phenotype of all drug-resistant cells, other additional resistance mechanisms must be operating in cancer cells. Furthermore, the current concept of MDR is based on the hypothesis that MDR is a multifactorial event caused by different molecular mechanisms being switched on and off and temporarily being active together.

Consequently, the identification of factors involved in the drug-resistant phenotypes of gastric carcinoma is urgent in order to develop new treatment modalities and improve response rates in advanced tumors. In order to obtain more information about the cellular mechanisms that confer different types of MDR to gastric carcinomas, we previously established a model system *in vitro* (6). By treatment of the drug-sensitive human gastric carcinoma cell line EPG85-257P with the anthracycline daunorubicin, we selected a stable classical MDR variant EPG85-257RDB overexpressing MDR1/P-gp. Exposure of EPG85-257P cells to mitoxantrone revealed the atypical multidrug-

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Table I. *Genes found to be significantly up- or down-regulated in both MDR gastric carcinoma variants.*

Sequence name(s)	Sequence description	Accession #	Fold change RDB	RNOV
ADM	adrenomedullin	S73906	3.29	2.62
CUGBP2	CUG triplet repeat, RNA-binding protein 2	NM_006561	–3.19	–3.51
RPS16; RPL31	Human DNA sequence from clone RP3-483K16. Contains (parts of) two novel genes, RPS16 (40S Ribosomal protein S16) and RPL31 (60S Ribosomal protein L31).	AL034374	–5.09	–7.25
FGD1	faciogenital dysplasia (Aarskog-Scott syndrome)	U11690	–2.62	–2.66
HOXD1	homeo box D1	Z49995	–2.55	–2.56
Human DNA	*H.sapiens* DNA for muscle nicotinic acetylcholine receptor gene promoter, clone ICRFc105F02104.	Z79610	–2.69	–2.95
LOC51603	CGI-01 protein	NM_015935	–6.82	–3.68
LOC51657	map kinase phosphatase-like protein MK-STYX	AF069762	2.67	2.97
MYC	v-myc avian myelocytomatosis viral oncogene homolog	X00364	–2.51	–2.62
NRG1	neuregulin 1	U02328	–9.03	–4.05
PCDH9	protocadherin 9	AF169692	11.24	15.75
PLS3	plastin 3 (T isoform)	M22299	4.11	3.08
PRAME	preferentially expressed antigen in melanoma	AI017284	7.89	5.06
SERPINF1	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor).	U29953	–7.56	–4.2
SLIT2	slit (*Drosophila*) homolog 2	AF133270	–7.93	–3.31
TRIP6	thyroid hormone receptor interactor 6	AF000974	5.65	2.56
USP1	ubiquitin specific protease 1	AB014458	4.9	–3.17

RDB, classical MDR gastric carcinoma cell line EPG-85-257RDB
RNOV, atypical MDR gastric carcinoma cell line EPG-85-257RNOV

resistant cell line EPG85-257RNOV. Previously, it was demonstrated that these atypical MDR cells show a vesicular compartmentalization of the antitumor agent accompanied by a decreased cellular drug accumulation (7). It was reported that, in EPG85-257RNOV cells, the expression level of the mRNA encoding the ABC-transporter BCRP is much more pronounced as compared to the parental cells (8). Moreover, evidence was provided that the heterodimeric ABC-transporter TAP contributes to the atypical MDR phenotype in EPG85-257RNOV cells (9). Additionally, it was demonstrated that these atypical MDR gastric carcinoma cells show altered DNA topoisomerase IIα expression (10) and an elevated expression level of glypican-3 (GPC3) (11).

During recent years, powerful techniques have been developed for transcriptome analyses, *i.e.* the simultaneous detection of the expression of many different mRNAs in a given sample. These techniques include differential display reverse transcription-polymerase chain reaction (DDRT-PCR) (12), serial analysis of gene expression (SAGE) (13), subtractive suppression hybridization (SSH) (14) and the application of cDNA arrays (15). Various advantages and disadvantages are linked to these approaches. In earlier experiments, we applied cDNA arrays containing 588 cDNA sequences, known to be involved in toxicological responses, to analyze differential gene expression in a panel of drug-resistant gastric carcinoma cells (16). To complete this experimental strategy and for evaluation of different array-based mRNA profiling techniques, in this study arrays containing more than 12,000 verified human cDNA sequences were used to compare the mRNA expression pattern of sensitive, classical and atypical multidrug-resistant gastric carcinoma cells.

Materials and Methods

Gastric carcinoma cell lines and cell culture. The human gastric carcinoma cell line EPG85-257P and its drug-resistant sublines had been previously established in our laboratory (6, 7). The classical MDR cell line, EPG85-257RDB, exhibits a MDR1/P-gp-dependent 1,857-fold resistance against daunorubicin; the atypical MDR cell variant shows a 457-fold resistance against mitoxantrone. These gastric carcinoma-derived cell lines were grown in Leibovitz L 15 medium (Bio Whittaker, Grand Island, NY, USA), 1 mM L-glutamine, 6.25 mg/ml fetuin, 80 IE/l insulin, 2.5 mg/ml transferrin, 1 g/l glucose, 1.1 g/l $NaHCO_3$, 1% minimal essential vitamins and 20,000 kIE/l trasylol, in a humidified atmosphere of 5% CO_2 at 37˚C. Additionally, classical MDR cells were grown with 2.5 μg/ml (4.43 μM) daunorubicin (Farmitalia Carlo Erba, Freiburg, Germany) and atypical MDR cells were grown in the presence of 0.2 μg/ml (0.39 μM) mitoxantrone (Lederle, Wolfratshausen, Germany).

Table II. *Genes found to be exclusively significantly up- or down-regulated in the classical MDR gastric carcinoma cell line EPG85-257RDB.*

Fold change	Sequence name(s)	Sequence description	Accession #
55.02	GATA3	GATA-binding protein 3	NM_002051
21.39	CARP	cardiac ankyrin repeat protein	X83703
18.15	olfactory receptor	pINCY	AAF03329
16.93	DOC1	down-regulated in ovarian cancer 1	NM_014890
15.53	GATA3	GATA-binding protein 3	X58072
13.22	LOC54104	hypothetical protein	AF007130
12.35	GPM6A	glycoprotein M6A	D49958
7.67	MACS	myristoylated alanine-rich protein kinase C substrate (MARCKS, 80K-L)	AI755068
7.23	*Homo sapiens* cDNA	*Homo sapiens* cDNA FLJ13268 fis, clone OVARC1000971	AK023330
7.05	A2M	alpha-2-macroglobulin	M11313
5.91	MEIS2	Meis (mouse) homolog 2	AF017418
5.65	TRIP6	thyroid hormone receptor interactor 6	AJ001902
5.25	PTPRC	*Homo sapiens* T200 leukocyte common antigen precursor (PTPRC) gene, exon 33 and partial cds.	M23492
5.17	GRB14	growth factor receptor-bound protein 14	NM_004490
4.98	GSTM2	glutathione S-transferase M2 (muscle)	AW386100
4.90	USF1	upstream transcription factor 1	AB017568
4.71	GSTM3	glutathione S-transferase M3 (brain)	J05459
4.63	ESTs	*Homo sapiens* PAC clone RP5-978E18 from 7p21	AA127809
4.53	GSTM3	glutathione S-transferase M3 (brain)	C21373
4.38	GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	NM_002069
4.38	DKFZp761P1010	hypothetical protein DKFZp761P1010	AL353940
4.33	IQGAP1	IQ motif containing GTPase activating protein 1	AW341463
4.30	TES	testin	AK021575
4.23	ANGPT1	angiopoietin 1	U83508
4.22	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	X69111
4.22	ABCB4	ATP-binding cassette, sub-family B, member 4	NM_000443
4.20	PTPRC	protein tyrosine phosphatase, receptor type, C	Y00062
4.18	ISL1	ISL1 transcription factor, LIM/homeodomain, (islet-1)	U07559
4.11	LOC81569	actin like protein	U20582
4.03	FLNC	filamin C, gamma (actin-binding protein-280)	AF146692
3.92	PYGL	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	M14636
3.73	sperm protein Sp17	*Papio hamadryas* sperm protein Sp17 mRNA.	U75209
3.65	PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	Z29090
3.63	TRIP4	thyroid hormone receptor interactor 4	L40371
3.52	PCDH7	BH-protocadherin (brain-heart)	AB006757
3.45	HTATIP2	Tat-interacting protein (30kD)	U69161
3.45	NMU	neuromedin U	NM_006681
3.28	PDLIM1	PDZ and LIM domain 1 (elfin)	AI824089
3.25	SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	NM_006379
3.20	Sequence 30 .	Sequence 30 from Patent WO9947669.	AX017277
3.20	*Homo sapiens* cDNA	*Homo sapiens* cDNA FLJ10229 fis, clone HEMBB1000136	AK001091
3.15	EPLIN	epithelial protein lost in neoplasm beta	AF198454
3.13	GAS1	growth arrest-specific 1	L13698
3.11	EMP1	epithelial membrane protein 1	Y07909
3.09	CDKN2C	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	AF041248
3.07	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	J04456
3.06	STXBP2	syntaxin binding protein 2	U63533
3.00	TNK1	tyrosine kinase, non-receptor, 1	AF097738
2.98	TNFRSF6	tumor necrosis factor receptor superfamily, member 6	M67454
2.95	UGP2	UDP-glucose pyrophosphorylase 2	AW958467
2.86	KIAA0961 protein	pINCY	BAA76805
2.85	STXBP2	syntaxin binding protein 2	NM_006949
2.79	TG737	Probe hTg737 (polycystic kidney disease, autosomal recessive)	NM_006531
2.76	C5	complement component 5	M65134
2.73	MEIS2	Meis (mouse) homolog 2	AF179896

Table II. *Continued*

Fold change	Sequence name(s)	Sequence description	Accession #
2.71	MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax (*Drosophila*) homolog); translocated to, 4	U02478
2.70	LOC51189	ATPase inhibitor precursor	AK000934
2.66	RNASE4	ribonuclease, RNase A family, 4	D37931
2.64	75 kda infertility-related sperm protein	*Homo sapiens* infertility-related sperm protein mRNA, complete cds.	S58544
2.62	PFKP	phosphofructokinase, platelet	BE378739
2.60	TAF2J	TATA box binding protein (TBP)-associated factor, RNA polymerase II, J, 20kD	AI681931
2.57	RPL41	ribosomal protein L41	AF026844
2.55	TMEFF2	transmembrane protein with EGF-like and two follistatin-like domains 2	AF179274
2.53	RPS15	ribosomal protein S15	AW157053
2.52	BC-2	putative breast adenocarcinoma marker (32kD)	AF042384
2.51	MX2	myxovirus (influenza) resistance 2, homolog of murine	M33883
2.50	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	Y07958
–2.51	LAMR1	laminin receptor 1 (67kD, ribosomal protein SA)	J03799
–2.52	SULT2A1	Human dehydroepiandrosterone sulfotransferase (STD) gene, exon 6 & complete cds.	U13061
–2.53	TK1	thymidine kinase 1, soluble	M15205
–2.54	PBX3	pre-B-cell leukemia transcription factor 3	X59841
–2.54	MTHFD2	methylene tetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	NM_006636
–2.54	ATP6D	ATPase, H+ transporting, lysosomal (vacuolar proton pump), member D	BE206777
–2.54	SLC19A1	solute carrier family 19 (folate transporter), member 1	AI800935
–2.55	SIPA1	signal-induced proliferation-associated gene 1	AF029789
–2.58	USP9X	ubiquitin specific protease 9, X chromosome (*Drosophila* fat facets related)	NM_004652
–2.59	SCARB1	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 1	Z22555
–2.61	PPM1A	protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform	S87759
–2.63	DCTN1	dynactin 1 (p150, Glued (*Drosophila*) homolog)	X98801
–2.63	*Homo sapiens* cDNA	*Homo sapiens* cDNA FLJ12900 fis, clone NT2RP2004321	AK022962
–2.66	PTK9L	protein tyrosine kinase 9-like (A6-related protein)	AL136773
–2.66	PNN	pinin, desmosome associated protein	AF112222
–2.66	FZD2	frizzled (*Drosophila*) homolog 2	AB017364
–2.70	E2-EPF	ubiquitin carrier protein	AI571293
–2.72	GBA	glucosidase, beta; acid (includes glucosylceramidase)	M16328
–2.74	FUBP1	far upstream element (FUSE) binding protein 1	AW173306
–2.76	NCOR2	nuclear receptor co-repressor 2	AF125672
–2.77	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isoform	J04759
–2.78	SEPW1	selenoprotein W, 1	U67171
–2.82	RPL10	*Homo sapiens* cDNA: FLJ22915 fis, clone KAT06354, highly similar to HUMQM Human Wilm's tumor-related protein (QM) mRNA.	AK026568
–2.83	COL6A1	collagen, type VI, alpha 1	X99135
–2.85	MYPT1	myosin phosphatase, target subunit 1	AW847768
–2.87	Sequence 21.	Sequence 21 from Patent WO9954448.	AX014104
–2.88	NAPA	N-ethylmaleimide-sensitive factor attachment protein, alpha	BE314741
–2.90	YARS	tyrosyl-tRNA synthetase	U89436
–2.91	PBP	prostatic binding protein	NM_002567
–2.91	SCAP	SREBP cleavage-activating protein	D83782
–2.93	syntaphilin	*Homo sapiens* mRNA for 3'UTR of unknown protein	Y09836
–2.96	GLB1	galactosidase, beta 1	M27507
–2.96	MRPS18A	mitochondrial ribosomal protein S18A	AK001410
–2.97	UROS	uroporphyrinogen III synthase (congenital erythropoietic porphyria)	J03824
–2.99	PPIB	peptidylprolyl isomerase B (cyclophilin B)	BE386706
–3.02	LIG1	ligase I, DNA, ATP-dependent	M36067
–3.03	PRPF4B	serine/threonine-protein kinase PRP4 homolog	AB011108
–3.06	KIAA0290	KIAA0290 protein	AB006628
–3.08	INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kD	U96922
–3.10	LAMR1	laminin receptor 1 (67kD, ribosomal protein SA)	AW328280
–3.10	PROCR	protein C receptor, endothelial (EPCR)	AB026584
–3.11	Sequence 4727.	Sequence 47 from Patent WO9951727.	AX015383

Table II. *Continued*

Fold change	Sequence name(s)	Sequence description	Accession #
–3.18	NOT56L	Not56 (D. melanogaster)-like protein	NM_005787
–3.19	PCDH10	protocadherin 10	AB037821
–3.20	TOB1	transducer of ERBB2, 1	NM_005749
–3.21	CRYGD	crystallin, gamma D	U66583
–3.21	NTE	neuropathy target esterase	NM_006702
–3.21	TKT	transketolase (Wernicke-Korsakoff syndrome)	AW006207
–3.23	CTNS	cystinosis, nephropathic	NM_004937
–3.23	GIP	gastric inhibitory polypeptide	M18185
–3.29	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	NM_005412
–3.30	ASNA1	arsA (bacterial) arsenite transporter, ATP-binding, homolog 1	NM_004317
–3.33	ACTG1	actin, gamma 1	BE314833
–3.35	AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	AF032455
–3.37	PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	U84573
–3.50	RHO7	GTP-binding protein Rho7	AI554560
–3.65	NSF	N-ethylmaleimide-sensitive factor	U03985
–3.79	RIMS1	rab3 interacting protein	AF263307
–3.82	MEF-2	myelin gene expression factor 2	AB037762
–3.85	TEK	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)	L06139
–4.02	NOLC1	nucleolar and coiled-body phosphprotein 1	NM_004741
–4.09	HRMT1L2	HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 2	Y10807
–4.17	MAAT1	melanoma-associated antigen recognised by cytotoxic T lymphocytes	NM_006428
–4.22	PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	NM_000935
–4.24	Human full length insert cDNA	*Homo sapiens* full length insert cDNA clone YX37A07.	AF087992
–4.25	TEK	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)	AL047086
–4.47	SSRP1	structure specific recognition protein 1	M86737
–4.56	caldesmon 1	*Homo sapiens* mRNA for caldesmon, 3' UTR	AJ223812
–4.76	ALY	transcriptional coactivator	AF047002
–5.91	KIAA0736	KIAA0736 gene product	AB018279
–8.16	GPC3	glypican 3	L47125
–19.11	Human glucose transporter 3	*Homo sapiens* glucose transporter 3 gene, exons 1 to 6.	AF274889

Gene expression analyses. After harvesting the cells, total RNA was isolated using the RNeasy kit from Qiagen (Hilden, Germany), according to the manufacturer's recommendations. The RNA quality was checked with the RNA 6000 Nano Lab Chips (Agilent, Palo Alto, CA, USA) using Agilents 2100 Bioanalyzer system. Cyanine-3 and cyanine-5-labelled cDNA targets were generated from 10 μg total RNA using the LabelStar kit (Qiagen). In each experiment, cyanine-3-labelled cDNA from the parental, drug-sensitive cell line EPG85-257P was mixed with cyanine-5-labelled cDNA from the multidrug-resistant cell variants (EPG85-257RNOV or EPG85-257RDB) and hybridized to the human-1 cDNA microarray from Agilent. The hybridization conditions were chosen according to the manufacturer's recommendations. The human-1 cDNA microarrays contain more than 12,000 sequence-verified cDNAs from the Incyte Genomics Human UniGene 1 and Human Drug Target clone sets. All hybridizations were done as fluor reversal pairs. For scanning of the microarrays, the Agilent dual laser microarrays scanner was used. After feature extraction with Agilent's Feature Extraction software, the data analysis was done using the Rosetta Resolver system (Rosetta Biosoftware, Seattle, WA, USA).

Results

In search of genes involved in different types of MDR, cDNA microarrays containing more than 12,000 verified cDNA sequences were used. In the first analysis, the mRNA expression profiles of the classical MDR gastric carcinoma cell line EPG85-257RDB were compared to those in the non-resistant parental cell line EPG85-257P. Likewise, differential gene expression patterns were analyzed by comparing the mRNA expression profiles of the atypical drug-resistant human gastric carcinoma cell line EPG85-257RNOV with those of the parental variant. Only genes with an expression change of more than ± 2.5-fold in the drug-resistant cell lines compared to the non-resistant cell line were considered as being significantly regulated.

Altogether, 17 genes were found to be differentially regulated in both drug-resistant cell variants, EPG85-257RDB and EPG-85RNOV (Table I). In the classical

Table III. *Genes found to be exclusively significantly up- or down-regulated in the atypical MDR gastric carcinoma cell line EPG85-257NOV.*

Fold change	Sequence name(s)	Sequence description	Accession #
6.74	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)	AI923532
5.55	OLR1	oxidised low density lipoprotein (lectin-like) receptor 1	AB010710
5.12	CSF1	colony stimulating factor 1 (macrophage)	M37435
4.89	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	X57522
4.73	CDH19	cadherin 19, type 2	AF047826
4.24	SYCP2	synaptonemal complex protein 2	Y08982
4.23	RGS5	regulator of G-protein signalling 5	AI674877
4.22	LHFP	lipoma HMGIC fusion partner	AF098807
3.89	SEMA3A	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	L26081
3.70	MYO6	myosin VI	AB002387
3.64	PASK	KIAA0135 protein	D50925
3.53	KIAA0537	KIAA0537 gene product	NM_014840
3.37	BMI1	murine leukemia viral (bmi-1) oncogene homolog	L13689
3.21	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	NM_001530
3.06	MPP1	membrane protein, palmitoylated 1 (55kD)	M64925
2.97	INPP1	inositol polyphosphate-1-phosphatase	L08488
2.91	RAB27A	RAB27A, member RAS oncogene family	AL120794
2.89	LAMA2	laminin, alpha 2 (merosin, congenital muscular dystrophy)	M59832
2.80	ADD3	adducin 3 (gamma)	D67031
2.77	PPP1R3D	protein phosphatase 1, regulatory subunit 6	Y18206
2.76	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	AA598526
2.70	DYRK4	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	Y09305
2.69	BTG1	B-cell translocation gene 1, anti-proliferative	AI364742
2.56	TRIP6	thyroid hormone receptor interactor 6	AF000974
2.50	PTPRK	protein tyrosine phosphatase, receptor type, K	L77886
–2.57	METTL1	methyltransferase-like 1	Y18643
–2.59	HOXA1	homeo box A1	S79869
–2.61	STIP1	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	NM_006819
–2.93	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	AI028488
–3.08	DCTD	dCMP deaminase	AA101974
–3.12	KRT16	keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	AF061809
–3.17	USP1	ubiquitin specific protease 1	AB014458
–3.17	CKMT1	creatine kinase, mitochondrial 1 (ubiquitous)	J04469
–3.32	VIM, DNMT2	Human DNA sequence. Contains the VIM gene for vimentin, the DNMT2 gene for DNA methyl transferase 2,	AL133415
–3.80	BMP7	bone morphogenetic protein 7 (osteogenic protein 1)	X51801
–4.25	NTS	neurotensin	AA992733
–4.68	BDH	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)	AW246790
–5.59	NTS	neurotensin	U91618
–5.96	NR5A2	nuclear receptor subfamily 5, group A, member 2	AB019246
–5.98	GAL	galanin	M77140
–6.04	HGF	hepatocyte growth factor (hepapoietin A; scatter factor)	X16323
–6.25	LPHH1	latrophilin	NM_012302
–6.60	TOP2B	topoisomerase (DNA) II beta (180kD)	X68060
–9.25	RCL	putative c-Myc-responsive	NM_006443
–17.74	ATA1	amino acid transporter system A1	AW960471
–26.82	ALDH1A1	aldehyde dehydrogenase 1 family, member A1	K03000

multidrug-resistant cell line EPG85-257RDB, collectively 156 genes were detected as being significantly regulated in comparison to the non-resistant cell line. Of these genes, 74 genes were found to be up-regulated and 89 genes were found to be down-regulated. One hundred and thirty-nine sequences of these 156 genes were exclusively differentially-regulated in the classical MDR variant (Table II). In the atypical MDR gastric carcinoma cell variant EPG85-257RNOV, a total of 61 genes were found to be differentially-expressed with 31 being up- and 30 down-regulated. Likewise, 46 of these 61 sequences were specific for the atypical MDR cell line (Table III).

Discussion

The development of different types of MDR is a complex biological phenomenon. So far, various different factors have been identified as playing an important role in drug resistance. Besides the enhanced drug extrusion activity of various members of the superfamily of ABC-transporters, miscellaneous alternative factors, *e.g.* proteins involved in regulation of apoptosis and cell cycle, DNA repair systems, detoxifying enzymes, or DNA-topoisomerases, contribute to drug resistance of human cancer cells. However, since the hitherto identified drug resistance-associated proteins do not explain the complex cross-resistance pattern in various drug-resistant tumor cell lines, other additional biological mechanisms must be operating in such cells.

To identify new factors potentially involved in drug resistance, in this study a well characterized *in vitro* gastric carcinoma model consisting of a drug-sensitive, a classical MDR and an atypical MDR variant (6) was analyzed by cDNA array hybridization using the Agilent-based microarray platform with more than 12,000 human sequences. The most important advantage of array hybridization technology over alternative transcriptome analysis approaches is the ease and practicability of the experimental procedures. However, this technique is not suitable to identify new, hitherto unknown, genes and is limited to the eligibility of the cDNAs spotted on the array. Furthermore, the analyses of differential gene expression profiles do not provide direct evidence that the altered expression level of a given gene plays a critical role in antineoplastic drug resistance. In addition, these changes in gene expression might be downstream events triggered by unknown molecular mechanisms responsible for the drug-resistant phenotype.

Within the 17 genes differentially-regulated in both multidrug-resistant gastric carcinoma variants, no sequence could be identified that was previously known to be associated with drug resistance. For example, the gene exhibiting the most pronounced expression level in both multidrug-resistant cell variants is protocadherin 9 (PCDH9). Although no functional data are available for PCDH9, it is improbable that it is directly involved in drug resistance. Protocadherins represent a subgroup within the cadherin family of calcium-dependent cell-cell adhesion molecules (17). Many of the approximately 70 protocadherins in mammals are highly expressed in the central nervous system. Roles in tissue morphogenesis and formation of neuronal circuits during early vertebrate development have been inferred, but so far no association with cancer drug resistance has been demonstrated for any of the 70 adhesion molecules.

Likewise, in the list of genes exclusively differentially-regulated in the classical MDR gastric carcinoma variant EPG85-257RDB, none of the well-known drug resistance-associated factors could be detected. However, it was demonstrated that the expression level of MDR3/P-glycoprotein (ABCB4) was elevated in EPG85-257RDB cells, but this ABC-transporter does not play an important role in multidrug resistance of human neoplasms (2). On the other hand, the most important factor causing the multidrug-resistant phenotype in EPG85-257RDB cells (6,18), the ABC-transporter MDR1/P-gp (ABCB1), was not overexpressed in this cell line, although the human-1 cDNA microarray used contains a cDNA homologous to this ABC-transporter.

In the case of the atypical MDR gastric carcinoma cell line EPG85-257RNOV, two different factors, well-known as contributing to the drug-resistant phenotype, could be confirmed as differentially-regulated, the ABC-transporter TAP1 (ABCB2) (9) and DNA-topoisomerase IIβ (unpublished observation). However, the most important drug resistance gene known to be overexpressed in this cell line, the ABC-transporter BCRP (ABCG2) (8), was not found to be up-regulated by microarray hybridization. Moreover, glypican 3 (GPC3) and DNA-topoisomerase IIα, also known to be differentially-expressed in this cell line (10,11), likewise could not be identified by cDNA array hybridization. All other differentially-regulated genes in the cell line EPG85-257RNOV have not been previously associated with drug resistance.

Moreover, it is interesting to note that differences in gene expression detected with the human-1 cDNA microarray used in this study are not unambiguously in accordance with the differential mRNA expression profiles measured with a cDNA array containing 588 cDNA sequences known to be involved in toxicological responses (16). From nine sequences found to be differentially-regulated in that study, four genes, Hsp27, CCT5, prothymosin α and JNK2, did not show a differentially-regulated expression by applying the thresholds defined in this study. The genes of two factors, RcI and vimentin, were considerable down-regulated in the atypical MDR cell line EPG85-257RNOV by using the human-1 cDNA microarray used in this study, as well as by using the array with toxicology-associated factors (16). In contrast, aldehyde dehydrogenase 1, enhanced in expression in EPG85-257RDB cells and not altered in expression in the

cell line EPG85-257RNOV by using the array with toxicology-associated factors (16), showed no up-regulation in EPG85-257RDB cells and a dramatic down-regulation in EPG85-257RNOV cells when using the human-1 cDNA microarray used in this study. The cDNAs of two additional genes found to be differentially-regulated in the previous study (16), TxP-a and HLH IR21, were not spotted on the human-1 cDNA microarray.

These discrepancies in gene expression profiles by using different cDNA arrays may be partly explained by long-term mutations in the used cell culture models. On the other hand, these discrepancies demonstrate that different cDNA array technologies reveal different data. Thus, for assessment of the expression data, it is very important to consider the specific array technique used. Although, array technology is a very interesting tool to explore alterations in gene expression in different drug-resistant cancer cells, the data have to be evaluated by alternative methods.

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References

1 Stewart BM and Kleihus P (eds): World Cancer Report. IARC Press, Lyon, 2003.
2 Lage H: ABC-transporters: implications on drug resistance from microorganisms to human cancers. Int J Antimicrob Agents *22*: 188-199, 2003.
3 Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE and Gottesman MM: P-glycoprotein: from genomics to mechanism. Oncogene *22*: 7468-7485, 2003.
4 Kruh GD and Belinsky MG: The MRP family of drug efflux pumps. Oncogene *22*: 7537-7552, 2003.
5 Lage H and Dietel M: Effect of the breast-cancer resistance protein on atypical multidrug resistance. Lancet Oncol *1*: 169-75, 2000.
6 Lage H: Molecular analysis of therapy resistance in gastric cancer. Dig Dis *21*: 326-338, 2003.
7 Dietel M, Arps H, Lage H and Niendorf A: Membrane vesicle formation due to acquired mitoxantrone resistance in human gastric carcinoma cell line EPG85-257. Cancer Res *50*: 6100-6106, 1990.
8 Ross DD, Yang W, Abruzzo LV, Dalton WS, Schneider E, Lage H, Dietel M, Greenberger L, Cole SP and Doyle LA: Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. J Natl Cancer Inst *91*: 429-433, 1999.
9 Lage H, Perlitz C, Abele R, Tampe R, Dietel M, Schadendorf D and Sinha P: Enhanced expression of human ABC-transporter tap is associated with cellular resistance to mitoxantrone. FEBS Lett *503*: 179-184, 2001.
10 Kellner U, Hutchinson L, Seidel A, Lage H, Danks MK, Dietel M and Kaufmann SH: Decreased drug accumulation in a mitoxantrone-resistant gastric carcinoma cell line in the absence of P-glycoprotein. Int J Cancer *71*: 817-824, 1997.
11 Wichert A, Stege A, Midorikawa Y, Holm PS and Lage H: Glypican-3 is involved in cellular protection against mitoxantrone in gastric carcinoma cells. Oncogene *23*: 945-955, 2004.
12 Liang P and Pardee AB: Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science *257*: 967-971, 1992.
13 Velculesku VE, Zhang L, Vogelstein B and Kinzler KW: Serial analysis of gene expression. Science *270*: 484-487, 1995.
14 Diatchenko L, Lau YF, 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 or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA *93*: 6025-6030, 1996.
15 Marshall A and Hodgson J: DNA chips: an array of possibilities. Nat Biotechnol *16*: 27-31, 1998.
16 Ludwig A, Dietel M and Lage H: Identification of differentially expressed genes in classical and atypical multidrug-resistant gastric carcinoma cells. Anticancer Res *22*: 3213-3221, 2002.
17 Frank M, Kemler R: Protocadherins. Curr Opin Cell Biol *14*: 557-562, 2002.
18 Lage H, Jordan A, Scholz R and Dietel M: Thermosensitivity of multidrug-resistant human gastric and pancreatic carcinoma cells. Int J Hyperthermia *16*: 291-303, 2000.

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