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Molecular Determinants of Response of Tumor Cells to Berberine

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Abstract. *Berberine, an alkaloid of Hydratis canadensis (Goldenseal), reveals profound cytotoxic activity against tumor cells. The inhibition concentration 50% (IC_{50}) values for berberine of 60 cell lines of the National Cancer Institute (NCI) were correlated with those of 43,177 compounds included into the NCI database by COMPARE analysis. Among the standard cytostatic drugs, the IC_{50} values for berberine correlated significantly with those for daunorubicin, vinblastine, and paclitaxel but not with those for platinum compounds (cisplatin, carboplatin), alkylating agents (melphalan, ifosfamide), DNA topoisomerase I inhibitors (camptothecin, topothecan), and antimetabolites (5-fluorouracil, methotrexate). Significant correlations were also found to phyllanthoside, dactinomycin, didemnin B, bisantrene, maytansine, rhizoxin, geldanamycin, tetrocarcin A, and chromomycin A, most of which are involved in the multidrug resistance phenotype. Since several ATP-binding cassette (ABC) transporters confer multidrug resistance, we correlated the IC_{50} values for berberine with the microarray-based mRNA expression values of 31 ABC transporter genes. The expression of 8 ABC transporters correlated with the IC_{50} values for berberine. Using CEM/VCR1000 leukemia cells, which over-express the ABCB1 (MDR1) gene, we exemplarily validated that this ABC transporter confers resistance to berberine. Furthermore, the IC_{50} values for berberine of the 60 NCI cell lines were associated with microarray-based mRNA expression of 9,706 genes. By COMPARE and hierarchical cluster analyses, 20 genes were identified which significantly predicted sensitivity or resistance of the cell lines to berberine. In conclusion, the response of tumor cells to berberine is multi-factorial in nature. Novel candidate genes were identified that might determine cellular response to berberine.*

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Drug resistance and severe adverse side-effects are major obstacles of cancer chemotherapy. Therefore, new drugs with improved features are urgently required. Natural sources such as marine and terrestrial plants and animals are a fertile ground to find novel drugs with anti-tumor activity. The long-lasting experience of traditional folk medicines may facilitate the identification of novel agents. In China, herbs have been used as foods and medicine for millennia. In recent years, the active principles of medicinal herbs have been increasingly discovered making the active chemical compounds accessible to pharmacological research (1-7). As compounds of traditional Chinese medicine (TCM) for cancer chemotherapy may have molecular targets different from those of standard anti-tumor drugs, they are attractive in the search for novel drugs suitable to treat otherwise drug-resistant tumors with reduced side-effects on normal organs.

Recently, we analyzed 22 compounds of TCM for their cytotoxic activity against tumor cells (8). Based on the results of this pilot study, we now focused our efforts on one of these compounds, berberine, the active principle of the Chinese herb *Hydratis canadensis* (Huanlian, Goldenseal). Berberine is used in traditional Chinese medicine for the treatment of dysentery and infectious diarrhea (9). In addition, a number of other pharmacological actions were reported including anti-microbial, antibiotic, immuno-stimulant, anti-tumor, anti-motility, anti-parasitic, and hypotensive properties, and relaxant effects on the *Corpus cavernosum* in erectile dysfunction (10-12). Considering this broad spectrum of pharmacological activities, it is noteworthy that berberine is relatively non-toxic to humans (13).

The aims of the present study were, first, to investigate, whether tumor cells resistant to standard cytostatic drugs and other investigational cytotoxic compounds also exert cross-resistance to berberine and, second, to elicit the molecular determinants of sensitivity and resistance of tumor cells to berberine. Using COMPARE analysis, we analyzed the cross-resistance pattern of 60 cell lines of the National Cancer Institute (NCI), USA between berberine chloride and 43,177 compounds of the NCI database (http://www.ncbi.nci.nih.gov). Then, we correlated the microarray-based mRNA expression values of 31 ATP-binding cassette (ABC) transporter genes with the IC_{50} values for berberine, in order to elicit the involvement of berberine in multidrug resistance phenomena. Finally, we performed microarray-based COMPARE analyses of 9,706 genes and subjected candidate genes to hierarchical cluster analyses to identify a mRNA expression profile, which predicts sensitivity and resistance of tumor cells the berberine.

Materials and Methods

Cell lines. Human CCRF-CEM leukemia cells were maintained in RPMI medium (Gibco, Eggenstein, Germany) supplemented with 10% fetal calf serum in a humidified 7% CO_2 atmosphere at 37˚C. Cells were passaged twice weekly. All experiments were performed with cells in the logarithmic growth phase. The multidrug resistance gene 1 (*ABCB1, MDR1*)-expressing CEM/VCR1000 subline was maintained in 1000 ng/ml vincristine. The establishment of the resistant subline has been described (14). Sensitive and resistant cells were kindly provided by Dr. A. Sauerbrey (Dept. of Pediatrics, University of Jena, Jena, Germany).

The panel of 60 human tumor cell lines of the Developmental Therapeutics Program of the NCI consisted of leukemia (CCRF-CEM, HL-60, K-562, MOLT-4, RPMI-8226, SR), melanoma (LOX-IMVI, MALME-3M, M14, SK-MEL2, SK-MEL28, SK-MEL-5, UACC-257, UACC-62), non-small cell lung cancer (A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-460, NCI-H522), colon cancer (COLO205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), renal cancer (786-0, A498, ACHN, CAKI-1, RXF-393, SN12C, TK-10, UO-31) and ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3) cells, cells of tumors of the central nervous system (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251), prostate carcinoma (PC-2, DU-145), and breast cancer (MCF-7, NCI/ADR-Res, MDA-MB-231, Hs578T, MDA-MB-435, MDA-N, BT-549, T-47D). Their origin and processing have been previously described (15).

Sulforhodamine B assay. The determination of drug sensitivity in the NCI cell lines by the sulforhodamine B assay has been reported (16). The inhibition concentration 50% (IC_{50}) value for berberine chloride as well as for other established and investigational anti-cancer drugs have been deposited in the database of the database of the Developmental Therapeutics Program of the NCI (http://dtp.nci.nih.gov).

Growth inhibition assay. The *in vitro* response to drugs was evaluated by means of a growth inhibition assay as described (8). Aliquots of $5x10^4$ cells/ml were seeded in 24-well plates and berberine chloride (Sigma-Aldrich, Taufkirchen, Germany) was added immediately at different drug concentrations to allow calculation of the IC_{50} values. Cells were counted seven days after treatment with the drugs. The resulting growth data represent the net outcome of cell proliferation and cell death.

Statistical analyses. The mRNA expression values of 60 cell lines of the genes of interest were selected from the NCI database (http://dtp.nci.nih.gov). The mRNA expression has been determined by microarray analyses (17, 18).

This database has been searched, first, for genes identified by COMPARE analysis and, second, for genes of the ABC transporter family. COMPARE analyses were performed to produce rank-ordered lists of genes expressed in the 60 NCI cell lines. The methodology has been described previously in detail (19). Briefly, every gene of the NCI microarray database is ranked for similarity of its mRNA expression to the IC_{50} values for berberine chloride. To derive COMPARE rankings, a scale index of correlations coefficients (R-values) is created. In the standard COMPARE approach, greater mRNA expression in cell lines correlates with enhanced drug sensitivity, whereas in reverse COMPARE analyses, greater mRNA expression in cell lines indicates drug resistance. For validation, the mRNA expression values of ABC transporter genes analyzed by real-time RT-PCR (20) have been correlated to the mRNA expression values obtained by microarray analysis by Kendall's τ-test.

Kendall's τ-test was used to calculate significance values and rank correlation coefficients as a relative measure for the linear dependency of two variables. This test was implemented into the WinSTAT Program (Kalmia, Cambridge, MA, USA). Kendall's τ-test determines the correlation of rank positions of values. Ordinal or metric scaling of data are suited for the test and are transformed into rank positions. There is no condition regarding normal distribution of the data set for the performance of Kendall's τ-test.

The χ^2 test was used to implement the WinSTAT program (Kalmia) to prove bivariate frequency distributions for pairs of nominal scaled variables for dependencies.

In addition to the calculation of P- and R-values, the problem of multiple hypothesis testing was addressed. Significance tests might be prone to type I errors (false conclusions of significance) (21). Therefore, a step-up re-sampling multi-comparison procedure was applied to control the false discovery rate (FDR) among the significant correlations at a given significance level. This program was developed by Reiner *et al.* (22) and is available on http://www.math.tau.ac.il. The FDR is the expected proportion α of erroneous rejections among all rejections of the null-hypothesis.

Objects were classified by calculation of distances according to the closeness of between-individual distances by means of hierarchical cluster analysis. All objects were assembled into a cluster tree (dendrogram). The merging of objects with similar features leads to the formation of a cluster, where the length of the branch indicates the degree of relation. The distance of a subordinate cluster to a superior cluster represents a criterion for the closeness of clusters as well as for the affiliation of single objects to clusters. Thus, objects with tightly related features appear together, while separation in the cluster tree increases with progressive dissimilarity. Recently, cluster models have been validated for gene expression profiling and for approaching molecular pharmacology of cancer (17, 23). Cluster analyses applying the complete-linkage method were done with the WinSTAT program (Kalmia). Missing values were automatically

Table I. *Correlation of log_{10} IC_{50} values for berberine log_{10} IC_{50} values for standard anti-tumor drugs and investigational drugs identified by COMPARE analysis in 60 NCI cell lines.*

	p-value*
Standard Drugs:	
Tubulin Poisons:	
Vinblastine	0.001
Paclitaxel	3.80×10^{-4}
Topo II Inhibitors:	
Daunorubicin	0.022
Etoposide	n.s.
Topo I Inhibitors:	
Camptotecine	n.s.
Topotecan	n.s.
Platin Derivatives:	
Cisplatin	n.s.
Carboplatin	n.s.
Alkylating Agents:	
Melphalan	n.s.
Ifosfamide	n.s.
Antimetabolites:	
5-Fluorouracil	n.s.
Methotrexate	n.s.
Drugs identified by COMPARE analysis:	
Phyllanthoside	7.06×10^{-6}
Dactinomycin	6.81×10^{-5}
Didemnin B	0.025
Bisantrene	0.005
Maytansine	0.0031
Rhizoxin	0.036
Geldanamycin	2.42×10^{-4}
Tetrocarcin A	6.04×10^{-4}
Chromomycin A3	0.001

*Kendall's τ-test
n.s., not significant ($p>0.05$)

omitted by the program, and the closeness of two joined objects was calculated by the number of data points they contained. In order to calculate distances between all variables included in the analysis, the program automatically standardizes the variables by transforming the data with a mean = 0 and a variance = 1.

Results

Cross-resistance profile of the 60 NCI cell lines. To analyze determinants of cellular response to berberine, we first investigated the cross-resistance profile of the 60 NCI cell lines. For this reason, we compared the IC_{50} values obtained by the sulforhodamine assay for berberine with those of other drugs. Among the standard cytostatic drugs, the IC_{50} values for berberine correlated significantly with those for the multidrug resistance-type drugs daunorubicin, vinblastine, and paclitaxel but not with those for platinum compounds (cisplatin, carboplatin), alkylating agents (melphalan, ifosfamide), DNA topoisomerase I inhibitors (camptothecin, topothecan), and anti-metabolites (5-fluorouracil, methotrexate). Then, we performed a COMPARE analysis with 43,177 compounds included in the NCI database to identify further drugs whose IC_{50} values correlate with those of berberine. Compounds with COMPARE correlation coefficients of $R>0.4$ are listed in Table I. These compounds are from different classes, *e.g.*, anti-microtubule agents (maytansine, rhizoxin), RNA inhibitors (dactinomycin), inhibitors of heat shock protein 90 (geldanamycin), and others (tetrocarcin A, chromomycin A3, bisantrene, didemnin B, phyllanthoside). Interestingly, all of the drugs except for tetrocarcin A are involved in the multidrug resistance phenotype (24-30).

Role of ABC transporters for resistance to berberine. As a next step, we studied the role of ABC transporters for resistance of the 60 NCI cell lines to berberine. The mRNA expression of 31 ABC transporters (represented by 68 different clones with individual Genbank accession numbers) obtained by microarray analysis (http://dtp.nci.nih.gov) and the mRNA expression of ABC transporters obtained by real-time RT-PCR (20) were correlated to each other, in order to validate the microarray data by another independent method. We observed significant correlations for 18 ABC transporters (represented by 31 different clones) with $p<0.05$ by Kendall's τ-test. Subsequently, only the microarray-based mRNA expression values of these 18 ABC transporters were correlated with the IC_{50} values for berberine. By using FDR computation, we adjusted the significance level to 0.05 and found an α-value of 0.01392. This means that the mRNA expression of ABC transporter genes with $p<0.01392$ correlated significantly with the IC_{50} values for berberine at an error rate of <5%. These genes are listed in Table II. The expression of *ABCA3*, *ABCB1, ABCC3, ABCC6* and *ABCC6* correlated directly with the IC_{50} values for berberine. The mRNA expression of *ABCA5, ABCB6* and *ABCF1* correlated inversely with the IC_{50} values for berberine.

The causative role of one of these ABC transporters was exemplarily confirmed using a cell line, which selectively over-expresses the *ABCB1 (MDR1)* gene. The IC_{50} values calculated from the dose response curves shown in Figure 1 were 1 μM and 18.5 μM for sensitive CCRF-CEM and ABCB1 (MDR1)-over-expressing CEM/VCR1000 cells, respectively. The CEM/VCR1000 were 18-fold cross-resistant to berberine as compared to drug-sensitive parental CCRF-CEM cells.

COMPARE analysis of microarray data. Next, we performed COMPARE analyses of the IC_{50} values for berberine and the mRNA expression of 9,706 genes of the NCI cell lines to produce scale indices of correlation coefficients. We first

Table II. *Correlation of microarray-based mRNA expression of ABC transporters with IC_{50} values for berberine of 60 NCI cell lines.*

Symbol	Name	Genbank Acc. No.	*p*-value*
ABCA3	ATP-binding cassette, sub-family A (ABC1), member 3	U78735	0.014 (+)
ABCA5	ATP-binding cassette, sub-family A (ABC1), member 5	H26264	0.012 (-)
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	M14758	0.0006 (+)
ABCB6	ATP-binding cassette, sub-family B (MDR/TAP), member 6	AF070598	0.010 (-)
ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	AF085692	0.005 (+)
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	AF071202	0.0006 (+)
ABCC6	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	U66689	0.0003 (+)
ABCF1	ATP-binding cassette, sub-family F (GCN20), member 1	W90495	0.006 (-)

*Kendall's τ-test
(+), high mRNA expression correlates with high IC_{50} values (direct correlation)
(-), high mRNA expression correlates with low IC_{50} values (inverse correlation)

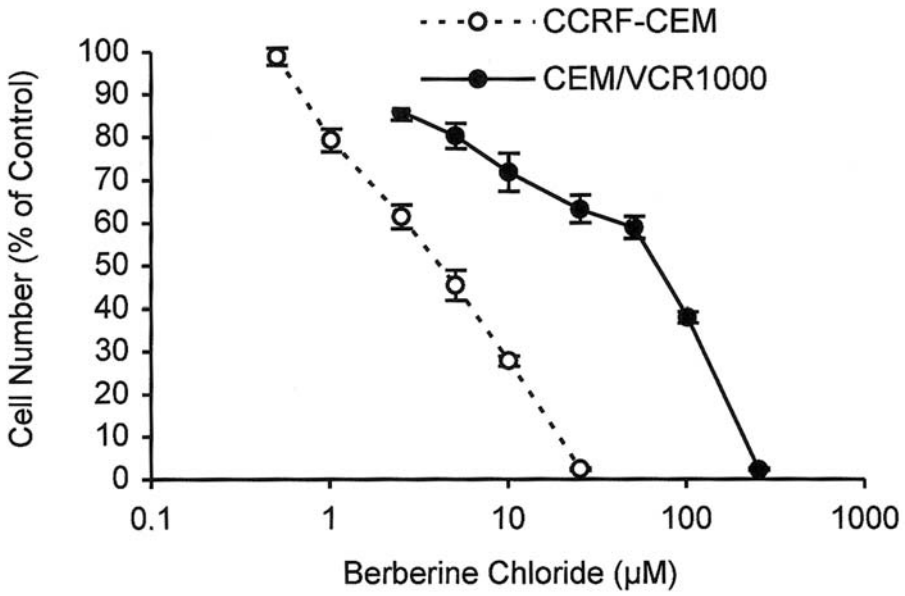

Figure 1. *Cross-resistance of multidrug-resistant and ABCB1(MDR1)-over-expressing CEM/VCR1000 leukemia cells to berberine as assayed by a growth inhibition assay. Values represent the mean (± SEM) of three independent experiments.*

performed a standard COMPARE analysis in which cell lines that were most inhibited by berberine (lowest IC_{50} values) were correlated with the highest mRNA expression levels of genes. The genes identified by this approach are shown in Table III. These genes may be considered as possible candidate genes, which determine cellular sensitivity to berberine. Afterwards, reverse COMPARE analyses were done, which correlated the most inhibited cell lines with the lowest gene expression levels (Table III). By using FDR computation, we adjusted the significance level to 0.05 and found an α-value of 0.007, which means that the 20 genes or expressed sequence tags (EST) with $p \leq 0.007$ have a probability of ≤5% to erroneously correlate to cellular response to berberine. This approach provided genes that might determine cellular resistance to berberine.

Hierarchical cluster analyses. The genes obtained by standard and reverse COMPARE analyses for berberine were subjected to hierarchical cluster analysis to obtain a dendrogram, where the cell lines are arranged according to their expression profile of these genes. The dendrogram for berberine can be divided into three major cluster branches (Figure 2).

Then, the median $\log_{10}$ IC_{50} values for berberine were used as cut-off threshold to define cell lines as being

Table III. *Correlation of the constitutive mRNA expression of genes identified by COMPARE analysis with IC_{50} values for berberine of 60 NCI cell lines.*

Symbol	GenBank	Name	Function	*p*-value*
Standard COMPARE:				
	R00266	unknown	unknown	0.001
GLS	W72090	glutaminase	catalyzes the first reaction of renal glutamine catabolism	7.7×10^{-4}
MPP5	AA015950	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)	tumor suppression, signal transduction	7.0×10^{-5}
RBSK	AA037228	ribokinase	unknown	5.3×10^{-4}
	H01822	unknown	unknown	1.6×10^{-5}
CD2AP	R80586	CD2-associated protein	CD2 clustering, cytoskeletal polarization	5.8×10^{-4}
ADCY8	R60892	adenylate cyclase 8 (brain)	transformation of ATP into cyclic AMP	1.9×10^{-5}
NK4	N90140	natural killer cell transcript 4	may play a role in lymphocyte activation	8.1×10^{-5}
GRB14	R24266	growth factor receptor-bound protein 14 signal transduction	inhibits autophosphorylated insulin receptor	1.1×10^{-4}
IL15	N59270	interleukin 15	stimulates proliferation of T-lymphocytes	1.4×10^{-4}
Reverse COMPARE:				
SDHB	AA044439	succinate dehydrogenase complex, subunit B, iron sulfor (Ip)	part of respiratory chain at the inner mitochondrial membrane	1.6×10^{-7}
	AA039882	*Homo sapiens* transcribed sequence with strong similarity to protein sp:P12814 *(H.sapiens)* AAC1	unknown	3.8×10^{-6}
FLJ31542fis	W73942	*Homo sapiens* cDNA FLJ31542 fis, clone NT2RI2000829	unknown	2.5×10^{-6}
	R37439	unknown	unknown	3.7×10^{-5}
RNF123	N67005	ring finger protein 123	unknown	1.2×10^{-4}
LOC92906	W45728	hypothetical protein BC008217	unknown	0.007
FLJ13052	AA057000	NAD kinase	signal transduction; putative inorganic poly phosphate/ATP kinase	1.6×10^{-4}
GC20	AA043856	translation factor sui7 homolog	probably involved in translation	2.2×10^{-5}
FLJ36874	N67562	hypothetical protein FLJ36874	unknown	1.9×10^{-5}
DXS9879E	AA034498	DNA segment on chromosome X (unique) 9879 expressed sequence	unknown	5.3×10^{-5}

* Kendall's τ-test

sensitive or resistant. As can be seen in Table IV, the distribution of sensitive or resistant cell lines was significantly different between the branches of the dendrograms, indicating that cellular response to berberine is predictable by these genes. The sensitivity and resistance of the cell lines to established drugs could be predicted for vinblastine, paclitaxel, phyllanthoside, dactinomycin, maytansine, and geldanamycin (Table IV), indicating that the set of genes which predicts berberine resistance also predicts resistance to these drugs.

Discussion

In the present investigation, we showed that berberine exerts profound cytotoxic activity towards tumor cell lines. The correlation of IC_{50} values for berberine of 60 NCI cell lines with those for daunorubicin, vincristine, paclitaxel, and other investigational drugs points to a possible involvement of berberine in the multidrug resistance phenotype. Given the relevance of ABC transporters for multidrug resistance (31, 32), a systematic analysis of the role of the ABC transporters for resistance to berberine was undertaken. The significant relationships between the IC_{50} values for berberine to the expression of several ABC transporters in the 60 NCI cell lines indicate that they might be candidate resistance genes for berberine. Among them were *ABCA3, ABCB1, ABCC3, ABCC4* and *ABCC6*, most of which are known to be involved in transport and resistance to established anti-cancer drugs (31). We chose one gene, *ABCB1 (MDR1),* to validate the results obtained by correlation analyses by using a cell model, which over-expresses this ABC transporter. We found that *ABCB1*

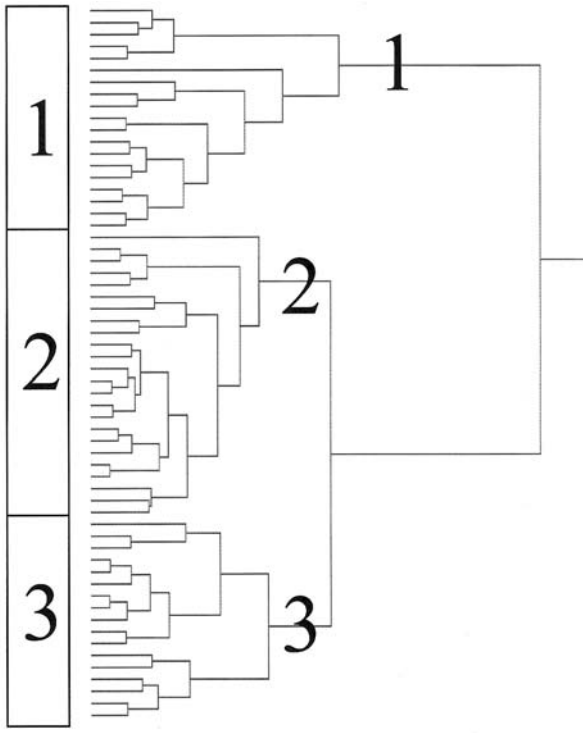

Figure 2. *Dendrogram of hierarchical cluster analysis (complete linkage method) obtained from mRNA expression of genes correlating with $log_{10}IC_{50}$ values for berberine. The dendrogram shows the clustering of 60 cell lines of the NCI's screening panel.*

(MDR1) confers resistance to berberine, which is in accord with previous results of ours and others (8, 33, 34). A causative role of the other ABC transporters for resistance to berberine remains to be established in the future. Interestingly, the expression of *ABCA5, ABCB6* and *ABCF1* correlated inversely with the IC_{50} values for berberine. Whether these genes are implicated in the sensitivity towards berberine remains open for future studies.

In addition to the role of ABC transporters for berberine resistance of human tumor cells, they are also important for berberine's function in plants and microorganisms. Berberine is a broad-spectrum anti-microbial with activity against bacteria, fungi, protozoans, and viruses as well as cytotoxicity against cancer cells (35-40). Berberine is synthesized in the roots of *Coptis japonica* and is transported by the ABC transporter, Cjmdr1, to the rhizome of the plant, where berberine protects from anti-microbial attacks (41). Gram-negative plant pathogens have developed an effective permeability barrier against plant anti-microbials including berberine by a membrane which restricts the penetration of amphipathic compounds and by MDR pumps which extrude toxins across this barrier (42, 43). *Berberis* plants produce both berberine as well as an ABC transporter inhibitor, 5-methoxy-hydnocarpin, to disable the bacterial resistance mechanisms against berberine (44).

The response of tumor cells to berberine is determined by multiple other factors in addition to ABC transporters, *i.e.*, activator protein (AP)-1, telomerase, DNA topoisomerase II, and nucleoplasmin/B23 (45-47). For this reason, we performed COMPARE and hierarchical cluster analyses of microarray-based mRNA expression values for 9,706 genes of the 60 NCI cell lines in an effort to gain deeper insight into the multi-factorial nature of cellular response to berberine. Apart from genes with still unknown function, we identified genes from different functional groups to be correlated with cellular response to berberine, *e.g.*, signal transducers *(MMP5, GRB14, FLJ13052),* interleukins (IL-15), and others *(GLS, CD2AP, ADCY8, NK4, SDHB, GC20)*. Although none of these genes have been assigned to drug sensitivity yet, the results obtained in this study suggest that these genes may also contribute to berberine resistance. Further studies are warranted to clarify their causative relevance for cellular drug response.

A recent study reported mRNA expression levels of 11,000 genes by microarray technology in relationship to cellular sensitivity to berberine (48). We compared our set of genes with that of these authors. None of the genes identified in the present study was among the genes identified by Iizuka *et al.* (48). Two explanations might account for the discrepancy in both microarray-based investigations: First, while our approach focused on the correlation of mRNA expression levels in 60 NCI cell lines of different tumor types (leukemia, colon cancer, lung cancer, breast cancer, ovarian cancer, CNS tumors, prostate cancer, and renal cancer) with IC_{50} values for berberine, Iizuka *et al.* (48) correlated the gene expression of 8 pancreatic cell lines with response to berberine. The different tumor types used in both studies may lead to different gene expression profiles. Second, the selection criteria in our approach were very stringent. We selected only genes with the highest COMPARE indices and the smallest probability of erroneously correlating with IC_{50} values for berberine by FDR calculation. Other genes identified by Iizuka *et al.* (48) might also correlate significantly with IC_{50} values for berberine in our approach, however, with smaller COMPARE correlation coefficients. Furthermore, the evaluation of gene expression in the study of Iizuka *et al.* (48) and in the present investigation was performed on a subset of 11,000 and 9,706 genes, respectively, which represents only a fraction of an estimated total number of 25,000 genes in the human genome. There may be many genes not included that may also play an important role in determining cell sensitivity to berberine.

Table IV. *Separation of clusters of 60 NCI cell lines obtained by the hierarchical cluster analysis shown in Figure 2 in comparison to drug sensitivity. The median $log_{10}IC_{50}$ value for each drug was used as a cut-off to separate tumor cell lines as being "sensitive" or "resistant".*

		Cluster 1	Cluster 2	Cluster 3	Number	χ^2 Test
Berberine	sensitive (<-4.678)	2	12	15		
	resistant (>-4.678)	16	12	1	58	$p=9.45 \times 10^{-6}$
Standard Drugs:						
Tubulin Poisons:						
Vinblastine	sensitive (<-9.057)	3	15	12		
	resistant (>-9.057)	16	9	5	60	$p=0.010$
Paclitaxel	sensitive (<-8.315)	5	14	11		
	resistant (>-8.315)	14	10	6	60	n.s.
Topo II Inhibitors:						
Daunorubicin	sensitive (<-7.113)	4	16	10		
	resistant (>-7.113)	15	8	7	60	$p=0.048$
Etoposide	sensitive (<-4.965)	7	16	7		
	resistant (>-4.965)	12	8	10	60	n.s.
Topo I Inhibitors:						
Camptotecine	sensitive (<-7.509)	7	15	8		
	resistant (>-7.509)	12	9	9	60	n.s.
Topotecan	sensitive (<-7.409)	4	16	7		
	resistant (>-7.409)	15	8	10	60	n.s.
Platin Derivatives:						
Cisplatin	sensitive (<-5.433)	10	12	8		
	resistant (>-5.433)	9	12	9	60	n.s.
Carboplatin	sensitive (<-3.890)	9	12	9		
	resistant (>-3.890)	10	12	8	60	n.s.
Alkylating Agents:						
Melphalan	sensitive (<-4.432)	6	17	7		
	resistant (>-4.432)	13	7	10	60	n.s.
Ifosfamide	sensitive (≤-3.389)	7	12	11		
	resistant (>-3.389)	12	12	6	60	n.s.
Anti-metabolites:						
5-Fluorouracil	sensitive (<-4.631)	10	10	10		
	resistant (>-4.631)	9	14	7	60	n.s.
Methotrexate	sensitive (<-7.185)	6	14	10		
	resistant (>-7.185)	13	10	7	60	n.s.
Drugs identified by COMPARE analysis:						
Phyllanthoside	sensitive (<-8.047)	2	15	13		
	resistant (>-8.047)	17	9	4	60	$p=0.001$
Dactinomycin	sensitive (≤-8.771)	2	15	14		
	resistant (>-8.771)	17	9	3	60	$p=4.13 \times 10^{-4}$
Didemnin B	sensitive (<-8.021)	6	13	11		
	resistant (>-8.021)	13	11	6	60	n.s.
Bisantrene	sensitive (<-6.903)	5	14	11		
	resistant (>-6.903)	14	10	6	60	n.s.
Maytansine	sensitive (<-7.677)	4	14	12		
	resistant (>-7.677)	15	10	5	60	$p=0.042$
Rhizoxin	sensitive (≤-13)	8	13	13		
	resistant (>-13)	11	10	4	59	n.s.
Geldanamycin	sensitive (<-6.381)	5	11	14		
	resistant (>-6.381)	14	12	3	59	$p=0.022$
Tetrocarcin A	sensitive (<-6.021)	5	13	12		
	resistant (>-6.021)	14	11	5	60	n.s.
Chromomycin A3	sensitive (≤-10.620)	5	15	10		
	resistant (>-10.620)	1	9	7	60	n.s.

n.s., not significant

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