Identification of candidate predictive markers of anticancer drug sensitivity using a panel of human cancer cell lines

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We previously investigated the correlations between the expression of 9216 genes and various chemosensitivities in a panel of 39 human cancer cell lines[1)] and found that the expression levels of *AKR1B1* and *CTSH* were correlated with sensitivity and resistance to multiple drugs, respectively. To validate these correlations, we investigated the expression of these two genes and the chemosensitivities in 12 additional gastric cancer cell lines. The expression of *AKR1B1* in the additional cell lines exhibited significant correlations with sensitivities to 8 of the 23 drugs examined, while that of *CTSH* displayed a significant negative correlation with only one (MS-247) of the 27 drugs examined. Their expressions were weakly correlated with sensitivity and resistance, respectively, to the remainder of the drugs. Moreover, when the 12 cell lines were divided into high-expressing and low-expressing groups, a comparison of these groups using Mann-Whitney's *U* test revealed that high expression levels of *AKR1B1* and *CTSH* were related to sensitivity to 21 of the drugs and resistance to 8 of the drugs, respectively. The present results suggest that *AKR1B1* and *CTSH* may be good markers for prediction of sensitivity to certain drugs and that our panel of 39 cell lines has the potential to identify candidate predictive marker genes. (Cancer Sci 2003; 94: 1074–1082)

Chemotherapy plays an important role in cancer treatment; however, the efficacy of anticancer drugs can vary significantly among individual patients. Differences with respect to the effectiveness of anticancer drugs among patients have been associated with variations in gene expression profiles in cancer cells.[2, 3)] Recently, various DNA microarray technologies have been developed that greatly facilitate genome-wide gene expression analysis of cancer cells. Taking advantage of such technologies, many clinical cancer samples for which extensive clinical and pathological information was available, such as tissue of origin, tumor grade, prognosis and response to anticancer drugs, have been examined.[2, 3)] There are several reports indicating that anticancer drug response can be predicted on the basis of gene expression in cancer cells from individual patients.[4, 5)] However, since the response of individual patients to anticancer agents can be influenced by local drug concentration, as well as hepatic metabolic enzymes involved in activation and inactivation of drugs, it is clear that drug responses *in vivo* are not determined solely by the gene expression profiles of cancer cells. Indeed, the activities of various metabolic enzymes are well known to be altered by a single nucleotide polymorphism (SNP) within the genes encoding these enzymes.[6)]

To exclude the issue of complicated pharmacokinetics within the body, we used a set of 39 established human cancer cell lines and examined drug response *in vitro*.[7, 8)] This approach has merit in that the intrinsic sensitivity of cancer cells to anticancer drugs can be determined precisely, without requiring consideration of pharmacokinetic issues. To identify genes that determine the sensitivity of cancer cells to anticancer drugs, we previously investigated, using cDNA microarrays, the expression of 9216 genes in the above-mentioned 39 cell line series and developed an integrated database of gene expression and drug sensitivity profiles.[1)] A similar database was originally developed by Weinstein and colleagues using 60 human cancer cell lines.[9, 10)] Recently, Blower *et al*. introduced a systematic approach to identify a gene set associated with a particular class of compounds with similar structures.[11)] Using our database, we extracted sets of genes associated with each of the 55 anticancer drugs examined.[1)] While some genes commonly correlated with sensitivities to various classes of anticancer drug, other genes correlated only with sensitivity to certain drugs displaying similar mechanisms of action. To generalize the relationship between chemosensitivity to particular drugs and expression patterns of particular genes previously observed in the 39 cell line series, validation should be performed in another set of cancer cell lines. In the present study, we focused on the two genes, *AKR1B1* and *CTSH*, which had displayed significant correlations with multiple drugs in the 39 cell line series. To verify whether the expressions of these two genes are related to drug responsiveness, we examined 12 additional gastric cancer cell lines for gene expression and chemosensitivities.

Materials and Methods

Cell lines and cell culture. The human cancer cell line panel has been described previously,[7)] and consists of the following 39 human cancer cell lines: lung cancer, NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273 and DMS114; colorectal cancer, HCC-2998, KM-12, HT-29, HCT-15 and HCT-116; gastric cancer, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74 and St-4; ovarian cancer, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3; breast cancer, BSY-1, HBC-4, HBC-5, MDA-MB-231 and MCF-7; renal cancer, RXF-631L and ACHN; melanoma, LOX-IMVI; glioma, U251, SF-295, SF-539, SF-268, SNB-75 and SNB-78; and prostate cancer, DU-145 and PC-3. The additional 12 gastric cancer cell lines are GCIY, GT3TKB, HGC27, AZ521, 4-1st, NUGC-3, NUGC-3/5FU, HSC-42, AGS, KWS-1, GMK-2 and GMK-5. GCIY, GT3TKB and HGC27 cells were obtained from the RIKEN cell bank and were described previously.[12, 13)] The AZ521[14)] cell line was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. The 4-1st cell line is derived from an adenocarci-

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noma of a 51-year-old female.[15] The GMK-2 cell line was established from an adenocarcinoma of a 52-year-old male and the GMK-5 cell line from an adenocarcinoma of a 69-year-old male. The NUGC-3 cell line and the 5-FU-resistant NUGC-3 variant cell line, NUGC-3/5FU, were described previously.[16, 17] The AGS[18] cell line was purchased from the American Type Culture Collection (ATCC). HSC-42[19, 20] and KWS-1[21] cells were generously provided by Dr. Masahiko Nishiyama, Hiroshima University, and Dr. Teiichi Motoyama, Yamagata University School of Medicine, respectively. All cell lines were cultured in RPMI-1640 supplemented with 5% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 mg/ml) at 37°C in humidified air containing 5% CO_2, with the sole exception that GMK-5 cells were cultured in D160 medium instead of RPMI-1640.

Anticancer drugs in clinical use and under development. Melphalan, cytarabine, 5-fluorouracil, 6-mercaptopurine, 6-thioguanine, tamoxifen, colchicine (Sigma), aclarubicin, neocarzinostatin (Yamanouchi Pharmaceutical), bleomycin, estramustine, toremifene (Nippon Kayaku), daunorubicin, pirarubicin (Meiji), doxorubicin, epirubicin, mitomycin C, L-asparaginase (Kyowa Hakko Kogyo), etoposide, peplomycin (Nihon Kayaku), vinblastine, vincristine, interferon-γ (Shionogi), methotrexate, mitoxantrone (Wyeth Lederie Japan), amsacrine (Pfizer Pharmaceutical; formerly Warner Lambert), camptothecin, irinotecan, SN-38 (Yakult), paclitaxel (Bristol-Myers Squibb), docetaxel (Aventis Pharma), oxaliplatin (Asahi Kasei), carmofur (Nihon Schering), interferon-α (Sumitomo Pharmaceutical), -β (Daiichi Pharmaceutical) and gemcitabine (Eli Lilly Japan) were obtained from the company specified in parentheses. The anticancer agents under development, E7010, E7070 (Eisai), NC190 (Taisho Pharmaceutical), navelbine (Kyowa Hakko Kogyo), CNDAC, radicicol (Sankyo), NK109, NK611, flutamide (Nihon Kayaku), Dolastine10, TZT1027 (Teikoku Hormone MFG), KRN5500 (Kirin Brewery), TAS103 (Taiho Pharmaceutical), FR901228 (Fujisawa Pharmaceutical), 4-hydroperoxycyclophosphamide (Shionogi), and topotecan (Aventis Pharma) were kindly provided by the company specified in parentheses.

Growth inhibition assay and data processing. Growth inhibition was assessed as changes in total cellular protein after 48 h of drug treatment using a sulforhodamine B assay. The drug concentration required for 50% inhibition of growth (GI_{50}) was calculated as previously described.[7, 22] All experiments were performed at least 3 times, with the median value being used for calculation of GI_{50}. All GI_{50} values were log-transformed and the absolute values $|\log GI_{50}|$ were used for all analyses. Average-linkage hierarchical clustering with regard to drugs was performed as described previously[1] with the use of Genespring software (Silicon Genetics)

Determination of gene expression by RT-PCR. Cell lines grown as monolayers to the log phase were washed twice with PBS and total RNA was extracted with TRIzol reagent (Life Technologies, Inc.). After treatment with DNase I (Boehringer Mannheim) to remove contaminating genomic DNA, 1 μg of total RNA was reverse-transcribed with Superscript II reverse transcriptase (RT) and an aliquot of the RT reaction product was used as a template for the following polymerase chain reaction (PCR). PCR was performed using the RNA PCR core kit (PE Biosystems) according to the manufacturer's protocol. The oligonucleotide primers used are: *AKR1B1* forward, 5′-ctggactacctggacctctacct-3′; reverse, 5′-tttgaggcaaagagaagtctt-3′; *CTSH* forward, 5′-tactggtccctacccaccttc-3′; reverse, 5′-ggaggtgctcactcaatgtttat-3′. Detection of PCR amplification was accomplished by subjecting PCR products to agarose gel electrophoresis, followed by staining of DNA fragments with ethidium bromide. Quantification of generated PCR-amplified products was performed by NIH Image software. RT-PCR experiments were repeated at least twice, with reproducible results. All values were log-transformed before analysis.

Statistical analysis between gene expression and chemosensitivity profiles. We calculated the degree of similarity between drug sensitivity and gene expression profiles by using Pearson correlation coefficients according to the following formula:

$$r = \frac{\Sigma (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\Sigma (x_i - \bar{x})^2 \, \Sigma (y_i - \bar{y})^2}}$$

where x_i represents the relative expression level of gene x in cell i, while y_i is the log sensitivity ($|\log_{10} GI_{50}|$) of cell i to drug y. $\bar{x}$ represents the mean of relative expression levels of gene, and $\bar{y}$ represents the mean sensitivity ($|\log_{10} GI_{50}|$) of drug y. Similarly, we used Pearson correlation coefficients when we calculated the degree of correlation between cDNA microarray and RT-PCR results.

Another statistical method, Mann-Whitney's U test, was employed to compare chemosensitivities in the high-expression group and those in the low-expression group of a given gene. We calculated U and P values by use of the one-sided Mann-Whitney test, i.e., the number of samples that overlapped when arranged in the order of their sensitivity ($|\log_{10} GI_{50}|$).

Results

Gene expression patterns of *AKR1B1* and *CTSH* and response to 64 anticancer drugs in a panel of 39 human cancer cell lines. We have previously developed a combined database of gene expression and drug sensitivity within a panel of 39 human cancer cell lines. This combined database allowed us to explore putative gene sets involved in drug responsiveness of cancer cells. Based on our previous analysis with the 39 cell line series, we selected two genes for further study; aldo-keto reductase family

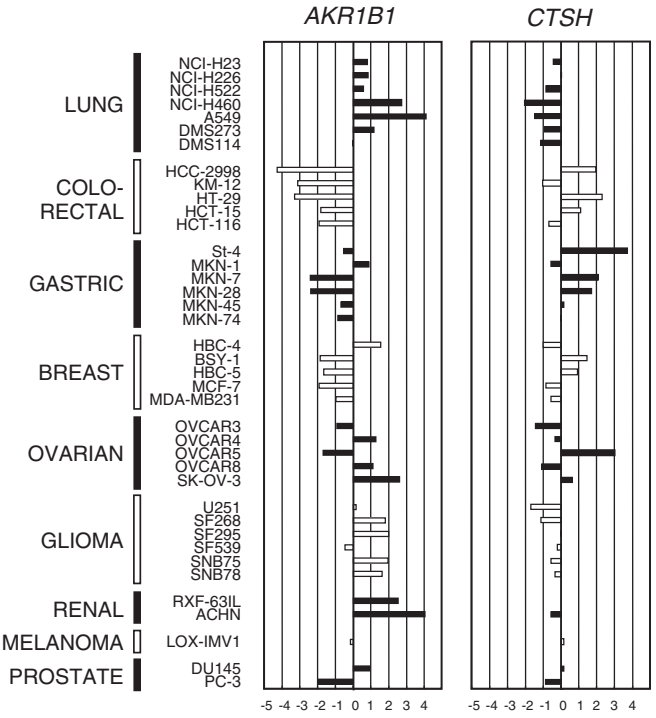

Fig. 1. Expression patterns of *AKR1B1* and *CTSH* genes determined by cDNA microarray analysis. The log expression ratio (log base 2) of a particular gene in each cell line is shown. Each expression ratio was centered so that "0" represents the mean expression level across the 39 cell lines. n.d.: not determined.

Table 1. List of 64 anticancer drugs and their known targets

JCI #	Drug name	Target/mode of action	*AKR1B1*		*CTSH*	
			r	*P*	*r*	*P*
Conventional drugs (41)						
−2	Melphalan	DNA cross-linker	0.45	0.005**	▲ 0.40	0.015*
−5	Cytarabine	Antimetabolite/Pyrimidine	0.31	0.052	▲ 0.38	0.017*
−6	Doxifluridine	Antimetabolite/Pyrimidine	0.07	0.679	▲ 0.17	0.324
−8	5-Fluorouracil	Antimetabolite/Pyrimidine	▲ 0.07	0.670	▲ 0.08	0.618
−10	Aclarubicin	DNA/RNA synthesis	▲ 0.14	0.410	0.02	0.927
−11	Actinomycin D	RNA synthesis	▲ 0.38	0.016*	▲ 0.02	0.904
−12	Bleomycin	DNA synthesis	0.53	<0.001***	▲ 0.45	0.004**
−13	Daunorubicin	DNA synthesis/Topo II	0.28	0.090	▲ 0.48	0.003**
−14	Doxorubicin	DNA synthesis/Topo II	0.32	0.050*	▲ 0.46	0.003**
−15	Epirubicin	DNA synthesis/Topo II	0.28	0.092	▲ 0.43	0.007**
−16	Mitomycin C	DNA alkylator	0.41	0.010**	▲ 0.34	0.036*
−17	Etoposide	Topo II	0.40	0.013*	▲ 0.35	0.030*
−18	Vinblastine	Tubulin	▲ 0.16	0.317	▲ 0.27	0.093
−19	Vincristine	Tubulin	▲ 0.23	0.151	▲ 0.22	0.170
−23	6-Mercaptopurine	Antimetabolite/Purine	▲ 0.18	0.298	▲ 0.18	0.284
−24	Methotrexate	DHFR[4]	0.08	0.635	▲ 0.22	0.187
−25	6-Thioguanine	Antimetabolite/Purine	0.06	0.707	▲ 0.24	0.160
−26	Neocarzinostatin	DNA synthesis	0.34	0.040*	▲ 0.39	0.016*
−27	Peplomycin	DNA synthesis	0.52	0.001**	▲ 0.47	0.003**
−28	Pirarubicin	DNA synthesis/Topo II	0.34	0.038*	▲ 0.38	0.019*
−29	Amsacrine	Topo II	0.58	<0.001***	▲ 0.51	0.001**
−30	Camptothecin	Topo I	0.41	0.011*	▲ 0.37	0.023*
−31	Irinotecan	Topo I	0.26	0.116	▲ 0.41	0.010*
−32	Carboplatin	DNA cross-linker	0.27	0.112	▲ 0.21	0.219
−33	Cisplatin	DNA cross-linker	0.44	0.005**	▲ 0.30	0.064
−35	Paclitaxel	Tubulin	▲ 0.25	0.122	▲ 0.17	0.306
−36	Docetaxel	Tubulin	▲ 0.34	0.036*	0.02	0.890
−37	Oxaliplatin	DNA cross-linker	▲ 0.01	0.964	▲ 0.16	0.352
−46	Carmofur	Antimetabolite/Pyrimidine	▲ 0.32	0.056	▲ 0.15	0.369
−47	Mitoxantrone	DNA synthesis	0.24	0.161	▲ 0.32	0.053
−48	L-Asparaginase	Protein synthesis	▲ 0.04	0.797	0.01	0.948
−49	Estramustine	(Estradiol/Nitrogen mustard mixture)	0.37	0.021*	▲ 0.26	0.111
−50	Interferon-α	Biological response modifier	▲ 0.16	0.342	0.02	0.889
−51	Interferon-β	Biological response modifier	▲ 0.05	0.754	0.02	0.897
−52	Interferon-γ	Biological response modifier	0.04	0.821	0.03	0.857
−86	Gemcitabine	Pyrimidine	0.42	0.008**	▲ 0.44	0.005**
−95	Tamoxifen	Estrogen receptor	▲ 0.47	0.003**	0.20	0.238
−96	Toremifene	Estrogen receptor	▲ 0.51	0.001**	0.26	0.126
−99	SN-38	Irinotecan derivative	0.41	0.009**	▲ 0.45	0.004**
−125	Cladribine	DNA synthesis	0.19	0.273	▲ 0.30	0.075
−202	Tomudex	Antimetabolite/Pyrimidine	0.32	0.048*	▲ 0.42	0.008**
	Positive correlation (P<0.05)		15/41		0/41	
	Negative correlation (P<0.05)		4/41		17/41	
Additional drugs (23)						
−34	Colchicine	Tubulin	▲ 0.16	0.339	▲ 0.25	0.136
−38	E7010	Tubulin	▲ 0.29	0.069	▲ 0.10	0.553
−40	NC-190	Topo II	0.23	0.165	▲ 0.29	0.080
−53	[1]	Antimetabolite/Cytosine	0.47	0.003**	▲ 0.47	0.003**
−54	Navelbine	Tubulin	▲ 0.26	0.108	▲ 0.30	0.062
−65	CNDAC	Antimetabolite/Cytosine	0.33	0.048*	▲ 0.40	0.014*
−66	Radicicol	Tyrosine kinase	▲ 0.36	0.028*	0.01	0.975
-70	DMDC	Antimetabolite/Cytosine	0.37	0.023*	▲ 0.45	0.006**
-72	NK109	Topo II	0.39	0.018*	▲ 0.39	0.018*
-73	2-DMA-etoposide[2]	Topo II	0.38	0.021*	▲ 0.36	0.026*
−79	Dolastatine 10	Tubulin	▲ 0.26	0.118	▲ 0.13	0.442
−87	KRN5500	Antibiotics	▲ 0.05	0.777	0.14	0.404
−94	Leptomycin B	Cell cycle inhibitor (crm1)	▲ 0.08	0.635	0.16	0.339
−97	TZT-1027 (Soblidotin)	(Dolastatin 10 derivative)	▲ 0.17	0.315	▲ 0.18	0.294
−98	TAS-103	Topo I/II	0.52	<0.001***	▲ 0.56	<0.001***
−119	[1]	Serine/Threonine kinase	0.51	0.001**	▲ 0.33	0.044*
−200	Flutamide	Androgen receptor	▲ 0.10	0.539	▲ 0.27	0.102
−204	FR901228	Histone deacethylase	▲ 0.01	0.948	▲ 0.21	0.191
−330	4-OOHCPA[3]	DNA alkylator	0.22	0.180	▲ 0.40	0.012*
10749	Topotecan	Topo I	0.41	0.009**	▲ 0.44	0.006**
10461	TNP-470	Angiogenesis	▲ 0.06	0.734	▲ 0.15	0.367
10315	MS-247	DNA synthesis	0.21	0.202	▲ 0.45	0.004**
10156	E7070	Cell cycle inhibitor	▲ 0.06	0.714	▲ 0.05	0.781
	Positive correlation (P<0.05)		8/23		0/23	
	Negative correlation (P<0.05)		1/23		10/23	
Total (64)						
	Positive correlation (P<0.05)		23/64		0/64	
	Negative correlation (P<0.05)		5/64		27/64	

Forty-one drugs are common with the previous study and 23 drugs were not previously examined. Each number represents Pearson correlation coefficient between gene expression and chemosensitivity. Closed triangles represent negative correlation. *** P<0.001, ** P<0.01, * P<0.05.

1) The suppliers of these compounds did not provide the chemical names for reasons of commercial confidentiality.
2) 2-Dimethylamino-etoposide.
3) 4-Hydroperoxycyclophosphamide.
4) Dihydrofolate reductase.

1, member B1 (*AKR1B1*) was associated with sensitivity to multiple anticancer drugs, while cathepsin H (*CTSH*) was associated with resistance to multiple anticancer drugs. The relative expression levels of these two genes in the 39 cell lines are shown in Fig. 1. The expression levels of these genes did not vary greatly between cell lines of different tissue origin, with the exception of cell lines derived from colorectal cancer, which exhibited markedly low expression levels of *AKR1B1*. We next examined the correlations between the expression patterns of these genes and the chemosensitivity profiles of 64 drugs across 39 cell lines. Forty-one of the 64 drugs studied here were included in the collection of 55 drugs used in the previous study, and the remaining 23 drugs included certain anticancer agents currently under development. As shown in Table 1, the expression pattern of *AKR1B1* displayed significant positive correlations with chemosensitivity to multiple drugs (23 out of the 64 drugs examined); i.e., the higher the expression of *AKR1B1*, the higher the sensitivity of the relevant cell line to anticancer drugs. Interestingly, it has positive correlations with chemosensitivity to 8 of the 23 drugs that were additionally examined in this study. On the other hand, the expression pattern of *CTSH* showed significant negative correlations with the chemosensitivity patterns of multiple drugs (27 of the 64 drugs examined). It showed negative correlations with the chemosensitivity patterns of 10 of the 23 additional drugs studied, respectively. These results suggest that these two genes are associated with sensitivity to a wide spectrum of anticancer drugs with different modes of action.

Confirmation of cDNA microarray data by semi-quantitative RT-PCR. To confirm gene expression data obtained from the previous cDNA microarray study, we examined the expression of *AKR1B1* and *CTSH* by semi-quantitative RT-PCR in 11 of the original 39 cell line series (6 gastric and 5 colorectal cancer cell lines). As shown in Fig. 2, the expression data obtained by cDNA microarray analysis agreed quite well with that determined by RT-PCR. Similar results were obtained in cell lines derived from other tissues (data not shown). These results indicate that gene expression data determined by both cDNA microarray and RT-PCR analysis are reliable with regard to these genes.

Correlation analysis between mRNA expression and protein expression. We next examined the protein expression of aldose reductase (encoded by *AKR1B1*) and cathepsin H (encoded by *CTSH*) in the 39 cell lines by immunoblot analysis (Fig. 3A). In both cases, the protein expression levels significantly correlated with the mRNA expression levels determined by cDNA microarray analysis.

Correlation analysis between gene expression patterns of *AKR1B1* and *CTSH* and response to 64 anticancer drugs in another set of 12 gastric cell lines. To examine the generality of the relationship between expression of the above genes and chemosensitivity, we collected a dozen extra gastric cancer cell lines, in addition to the 6 conventional cell lines included in our previous study with the 39 cell line series, and determined the gene expression patterns of *AKR1B1* and *CTSH* in the 12 lines by semi-quantitative RT-PCR (Fig. 4). We next determined the relative chemosensitivities of all 18 gastric cancer cell lines to the 64 anticancer drugs under study here (Fig. 5). Using these data, we compared the gene expression patterns of these two genes with the chemosensitivity patterns in the additional 12 cell lines (Table 2). The expression pattern of *AKR1B1* across the 39 cell line series had significant correlations with the chemosensitivity patterns of up to 23 drugs ($P<0.05$, Table 1). Its expression pattern across the 12 gastric cancer cell lines also had signifi-

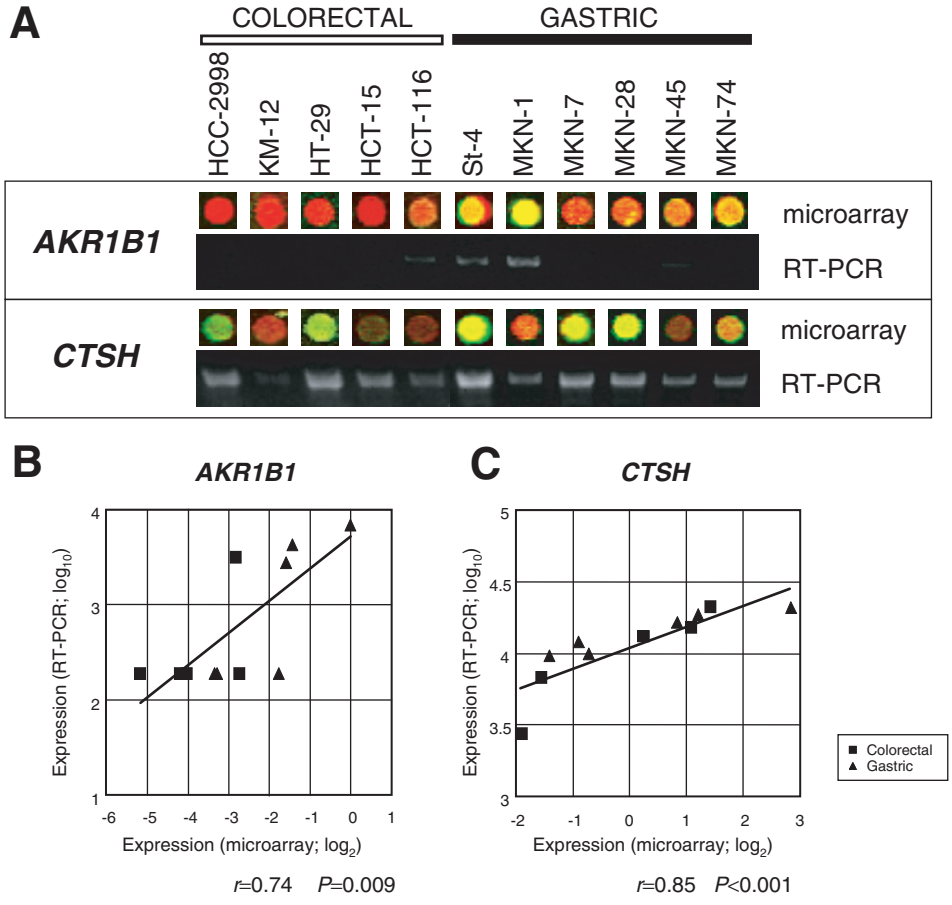

Fig. 2. Confirmation of reliability of the microarray data. A, Expression of *AKR1B1* and *CTSH* detected by cDNA microarray and RT-PCR in 6 gastric and 5 colorectal cancer cell lines. B and C, Correlations between expression levels determined by cDNA microarray and RT-PCR analysis. Each triangle represents a gastric cancer cell line and each square represents a colorectal cancer cell line. Pearson correlation coefficients (*r*) and *P* values (*P*) are shown.

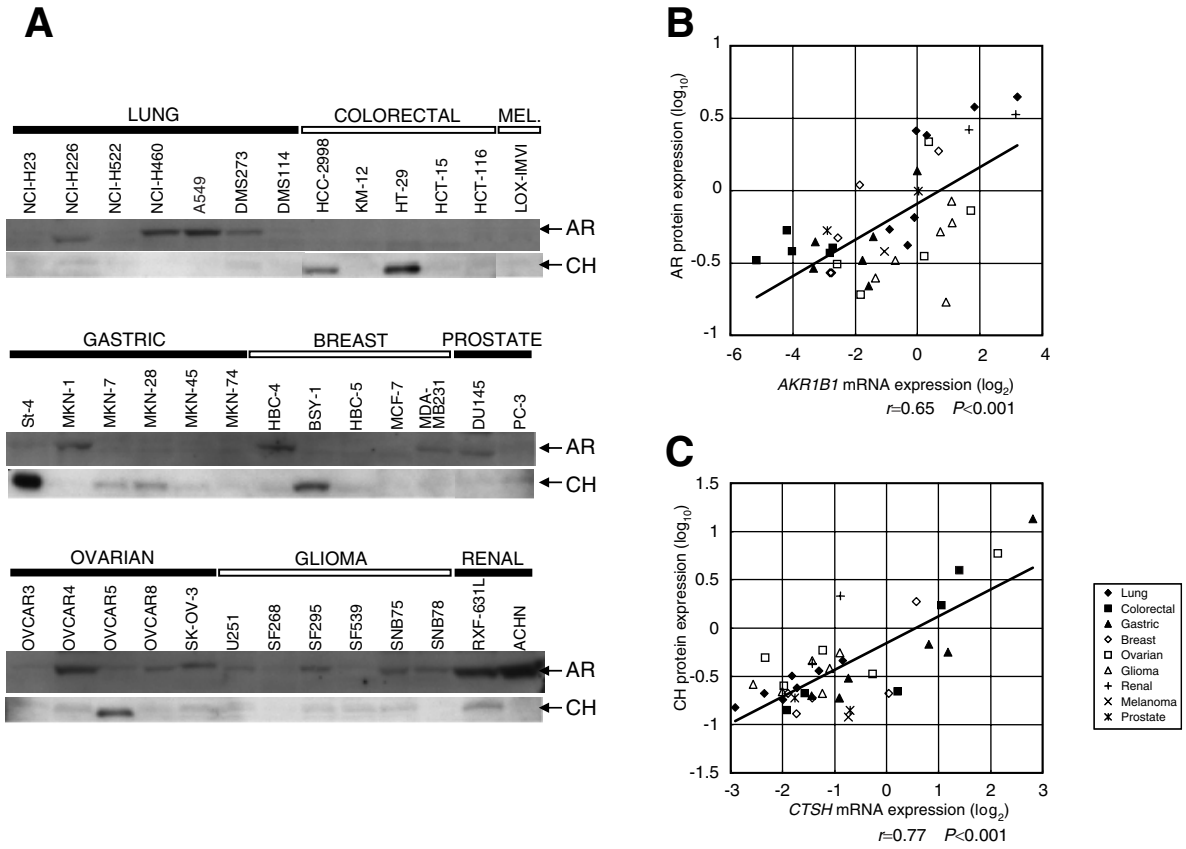

Fig. 3. Correlations between mRNA expression and protein expression. A, Protein expression of aldose reductase (AR) and cathepsin H (CH) in 39 human cancer cell lines. B and C, Scatter plots of protein expression levels determined by immunoblot analysis against mRNA expression levels determined by cDNA microarray. Each symbol represents a cell line. Pearson correlation coefficients (*r*) and *P* values (*P*) are shown.

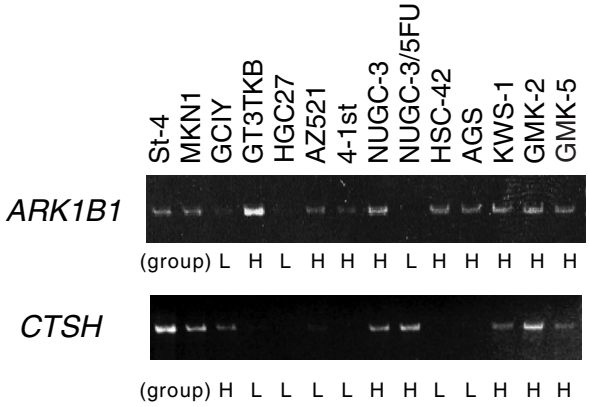

Fig. 4. Expression analysis of *AKR1B1* and *CTSH* genes in 12 gastric cancer cell lines by RT-PCR. "H" represents the high-expression group, while "L" represents the low-expression group.

cant positive correlations in regard to 8 of the of the drugs, including amsacrine, TAS-103, gemcitabine, SN-38, camptothecin, NK109 and 2-DMA-etoposide ($P<0.05$, Table 2a). The expression of *AKR1B1* also displayed weak (not significant) positive correlations in regard to the remainder of the drugs. On the other hand, the expression levels of *CTSH* displayed significant negative correlations with sensitivities to 27 drugs when the panel of 39 cell lines was examined. The expression pattern across the 12 gastric cancer cell lines displayed a significant negative correlation to only one drug (MS-247; $P<0.05$), though weak negative correlations with sensitivities to 25 of the drugs were seen (Table 2b).

Comparison between chemosensitivities in high-expressing cell lines and those in low-expressing cell lines in regard to *AKR1B1* and *CTSH* genes. We next examined whether cell lines highly expressing the *AKR1B1* gene display chemosensitivity compared to those not expressing or weakly expressing *AKR1B1*. As shown in Fig. 4, we separated the 12 gastric cancer cell lines into two groups: one group consisted of 3 cell lines (GCIY, HGC27 and NUGC-3/5FU) expressing the *AKR1B1* gene in low levels, while the other consisted of 9 cell lines highly expressing the *AKR1B1* gene. Then, we performed Mann-Whitney's *U* test to compare the chemosensitivities between these two groups. The cell lines highly expressing *AKR1B1* were significantly sensitive to 21 of the 23 drugs examined ($P<0.05$, Table 2a). Similarly, in regard to *CTSH*, we separated the 12 cell lines into two groups: a high-expressing group consisting of GCIY, NUGC-3, NUGC-3/5FU, KWS-1, GMS-2 and GMK5 and a low-expressing group consisting of GT3TKB, HGC27, AZ521, 4-1st, HSC-42 and AGS. The cell lines highly expressing *CTSH* were significantly resistant to 8 drugs, including peplomycin, MS-247, SN-38, bleomycin, topotecan, 4-OO-HCPA and melphalan ($P<0.05$, Table 2b). These results suggested that *AKR1B1* and *CTSH* could serve as predictive markers for the above-mentioned drugs.

Discussion

In the present study, we examined whether the expression levels of *AKR1B1* and *CTSH*, which showed significant correlations with chemosensitivity of cancer cells to various anticancer

drugs in a prior analysis of 39 human cancer cell lines, still displayed significant correlations to drug sensitivities in another set of 12 gastric cancer cell lines. The expression of *AKR1B1* in the additional cell lines also displayed significant correlations with sensitivities to 8 of the 23 drugs examined, while the expression of *CTSH* displayed a significant correlation with resistance to only one (MS-247) of the 27 drugs examined. Their expression levels displayed weak (not significant) correlations with sensitivities to the remainder of the drugs. However, cell lines highly expressing *AKR1B1* and *CTSH* genes displayed significant chemosensitivity to 21 drugs and chemoresistance to 8 drugs, respectively. The studies described here and elsewhere[23] indicate that prediction of chemosensitivity of cancer cells by expression profiling of particular genes is feasible. The present study supported the validity of the previous analysis with a series of 39 cell lines for the identification of candidate predictive marker genes. Among these genes, *AKR1B1* and *CTSH* are promising predictive markers for chemosensitivity and chemoresistance, respectively. Although the statistical analysis revealed that the expressions of these genes are associated with drug responsiveness, these associations do not represent proof of causal relationships between gene expression and chemosensitivity. The functional involvement of these genes in drug responsiveness should be examined in the future.

In the correlation analysis in the 12 additional gastric cancer cell lines, we observed significant correlations with regard to

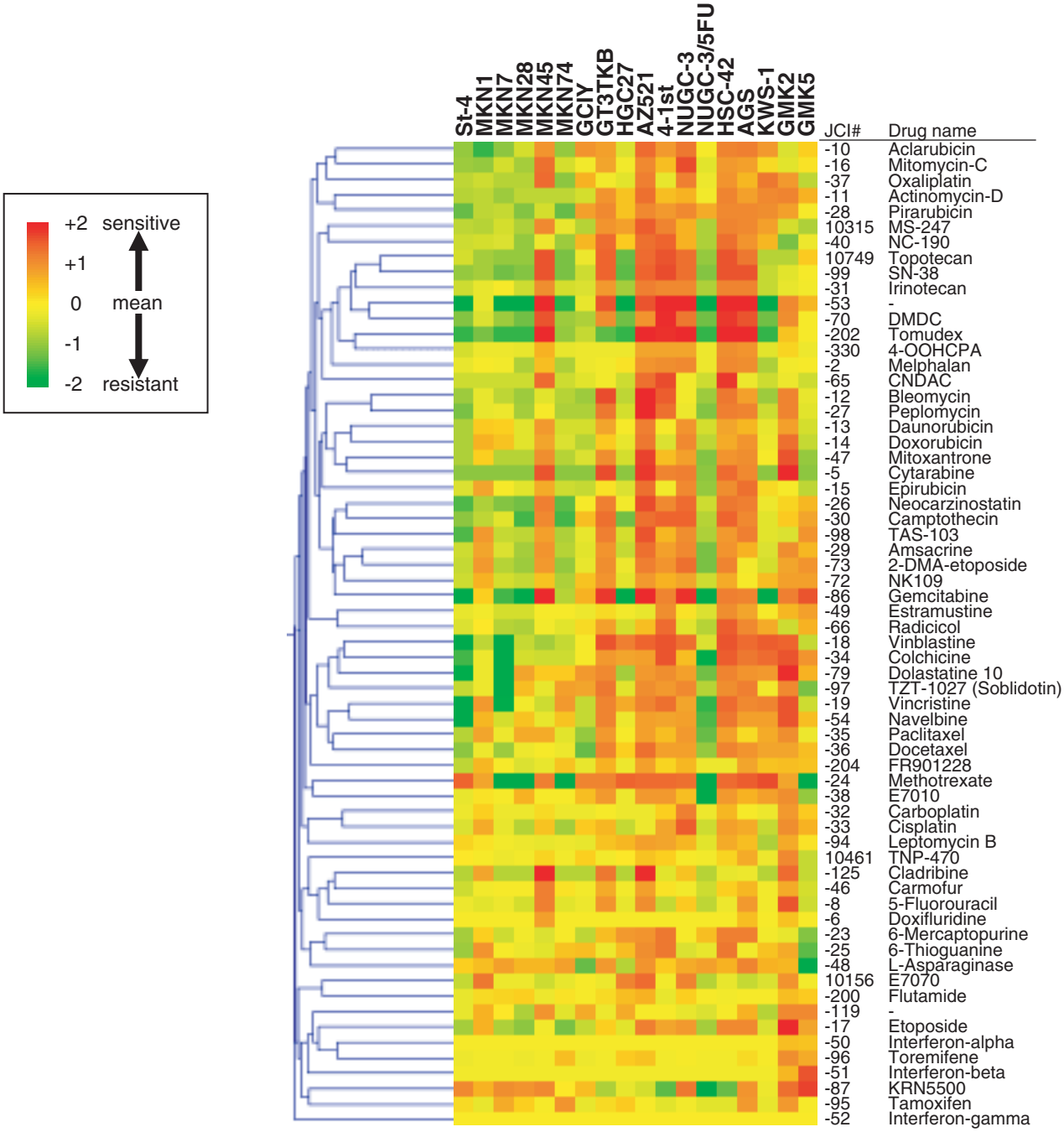

Fig. 5. Sensitivities to 64 anticancer drugs were determined in 18 gastric cancer cell lines. The patterns of GI_{50} values for the 64 drugs across the 18 cell lines were clustered. Colors on the image map represent the relative sensitivity of the cell line to the relevant drug. Numbers beside the color bar refer to the difference in |log GI_{50}| from the mean |log GI_{50}| for each drug.

Table 2. Validation of relationship between gene expression and chemosensitivity patterns across the 39 cell lines by using another set of 12 gastric cancer cell lines

a) *AKR1B1*

Rank	Drug	39 lines		12 gastric lines		Mann-Whitney (12 gastric lines)	
		r	*P*	*r*	*P*	*U*	*P*
1	Amsacrine	0.58	<0.001***	0.81	0.001**	1	0.009**
2	Bleomycin	0.53	<0.001***	0.45	0.141	2	0.018*
3	TAS-103	0.52	<0.001***	0.61	0.034*	2	0.018*
4	Peplomycin	0.52	0.001**	0.52	0.084†	1	0.009**
5	JCI#-119	0.51	0.001**	0.21	0.513	12	0.432
6	JCI#-53	0.47	0.003**	0.63	0.029*	2	0.018*
7	Melphalan	0.45	0.005**	0.19	0.55	6	0.105
8	Cisplatin	0.44	0.005**	0.55	0.065†	4	0.050*
9	Gemcitabine	0.42	0.008**	0.68	0.016*	2	0.018*
10	SN-38	0.41	0.009**	0.60	0.038*	1	0.009**
11	Camptothecin	0.41	0.011*	0.66	0.02*	2	0.018*
12	Topotecan	0.41	0.009*	0.55	0.063†	2	0.018*
13	Mitomycin-C	0.41	0.010*	0.42	0.176	3	0.032*
14	Etoposide	0.40	0.013*	0.53	0.074†	1	0.009**
15	NK109	0.39	0.018*	0.89	<0.001***	1	0.009**
16	2-DMA-etoposide	0.38	0.021*	0.75	0.005**	2	0.018*
17	DMDC	0.37	0.023*	0.55	0.063†	2.5	0.03*
18	Estramustine	0.37	0.021*	0.53	0.074†	2	0.018*
19	Pirarubicin	0.34	0.038*	0.45	0.143	4	0.050*
20	Neocarzinostatin	0.34	0.040*	0.57	0.051†	2	0.018*
21	CNDAC	0.33	0.048*	0.26	0.423	4	0.050*
22	Tomudex	0.32	0.048*	0.44	0.154	3	0.032*
23	Doxorubicin	0.32	0.050*	0.50	0.097†	2	0.018*

b) *CTSH*

Rank	Drug	39 lines		12 gastric lines		Mann-Whitney (12 gastric lines)	
		r	*P*	*r*	*P*	*U*	*P*
1	TAS-103	▲ 0.56	<0.001***	▲ 0.35	0.259	9	0.09†
2	Amsacrine	▲ 0.51	0.001**	▲ 0.20	0.527	13	0.242
3	Daunorubicin	▲ 0.48	0.003**	▲ 0.16	0.619	10	0.12
4	Peplomycin	▲ 0.47	0.003**	▲ 0.41	0.188	3	0.008**
5	JCI#-53	▲ 0.47	0.003**	▲ 0.37	0.237	7	0.047*
6	Doxorubicin	▲ 0.46	0.003**	0.01	0.983	13	0.242
7	MS-247	▲ 0.45	0.004**	▲ 0.61	0.037*	0	0.001**
8	SN-38	▲ 0.45	0.004**	▲ 0.45	0.142	6	0.032*
9	DMDC	▲ 0.45	0.006**	▲ 0.50	0.097†	8	0.066†
10	Bleomycin	▲ 0.45	0.004**	▲ 0.35	0.263	4	0.013*
11	Gemcitabine	▲ 0.44	0.005**	▲ 0.09	0.775	14	0.294
12	Topotecan	▲ 0.44	0.006**	▲ 0.43	0.168	7	0.047*
13	Epirubicin	▲ 0.43	0.007**	▲ 0.32	0.305	9	0.09†
14	Tomudex	▲ 0.42	0.008**	▲ 0.33	0.293	10	0.12
15	Irinotecan	▲ 0.41	0.010**	▲ 0.41	0.191	8	0.066†
16	CNDAC	▲ 0.40	0.014*	▲ 0.49	0.106	7.5	0.056†
17	4-OOHCPA	▲ 0.40	0.012*	▲ 0.44	0.149	7	0.047*
18	Melphalan	▲ 0.40	0.015*	▲ 0.49	0.104	7	0.047*
19	Neocarzinostatin	▲ 0.39	0.016*	▲ 0.36	0.244	8	0.066†
20	NK109	▲ 0.39	0.018*	0.02	0.948	16	0.409
21	Pirarubicin	▲ 0.38	0.019*	▲ 0.08	0.808	9	0.09†
22	Cytarabine	▲ 0.38	0.017*	▲ 0.12	0.712	11	0.155
23	Camptothecin	▲ 0.37	0.023*	▲ 0.22	0.484	9	0.09†
24	2-DMA-etoposide	▲ 0.36	0.026*	▲ 0.27	0.392	10	0.12
25	Etoposide	▲ 0.35	0.030*	▲ 0.00	1	11	0.155
26	Mitomycin-C	▲ 0.34	0.036*	▲ 0.06	0.846	8.5	0.078†
27	JCI#-119	▲ 0.33	0.044*	▲ 0.14	0.661	18	0.531

Drugs which had a significant correlation with *AKR1B1* (a) and *CTSH* (b) in the 39 cell lines were examined for the correlation between gene expression and drug response in the 12 gastric cancer cell lines studied here. Closed triangles represent a negative correlation. For comparison of chemosensitivity in high-expressing cell lines and in low-expressing cell lines of *AKR1B1* and *CTSH*, we calculated *U* values by applying the Mann-Whitney test. *** $P<0.001$, ** $P<0.01$, * $P<0.05$, † $P<0.1$.

only 8 of the 23 drugs for *AKR1B1* and 1 of the 27 drugs for *CTSH* ($P<0.05$), in contrast to our previous analysis in the 39 cell lines. This was probably due to the small sample size (12). In fact, when we re-examined these correlations in 18 cell lines by adding 6 gastric cancer cell lines from the 39 cell lines previously examined, we could confirm most of the significant correlations observed in the 39 cell lines (20 of the 23 drugs for *AKR1B1* and 15 of the 27 drugs for *CTSH*, $P<0.05$). Moreover, Mann-Whitney's *U* test revealed that high expression of these two genes was significantly related to chemosensitivities to 21 drugs and chemoresistance to 8 drugs, respectively ($P<0.05$).

Aldose reductase, which is encoded by the *AKR1B1* gene, is a member of the monomeric, NADPH-dependent aldo-keto reductase family. It catalyzes the reduction of a number of aldehydes, including the aldehyde form of glucose, which is reduced to the corresponding sugar alcohol, sorbitol.[24] Previous studies have shown that the overexpression of aldose reductase desensitizes liver cancer cells to daunorubicin by induction of carbonyl reduction.[25, 26] In this study, however, *AKR1B1* expression rather exhibited a weak positive correlation with daunorubicin sensitivity, although it was not statistically significant. Moreover, *AKR1B1* expression showed significant positive correlations to 20 out of the 64 drugs examined in this study. Differential effects of aldose reductase on each drug could explain this issue.[25, 26] On the other hand, overexpression of aldose reductase induces apoptosis by causing a redox imbalance.[27] Since aldose reductase was shown to mediate cytotoxic signals in response to hyperglycemia and TNF-α in lens epithelial cells,[28] aldose reductase could also mediate cytotoxic signals by anticancer drugs in cancer cells.

Cathepsin H is a protease which belongs to the cathepsin family. The major cathepsin activities include a group of cysteine-dependent proteases, cathepsins B, H, and L, which are structurally related to papain. The mature active forms of cathepsins are located predominantly in lysosomes, where they play an important role in regulating intracellular protein degradation and turnover.[29] Cathepsin H was shown to be highly expressed in both glioma and colon cancers as compared with normal tissue; moreover, its expression correlates with tumor progression and invasion.[30–32] In this study, the more that cancer cells express *CTSH*, the more these cells appear resistant to various anticancer drugs. Since malignant tumors exhibit chemoresistance in many cases, *CTSH* could play a role in such resistance phenomena. The causal relationship of these genes with chemoresistance is presently under investigation.

In summary, we conclude that the relationships between gene expression and drug responsiveness observed in the 39 cell line series were in part validated in the panel of 12 gastric cancer cell lines used here. This suggests that prediction of chemosensitivity is feasible through the use of *AKR1B1* and *CTSH*, as illustrated here, and may be improved by the use of other, as yet unvalidated candidate genes identified in our previous analysis by using the 39 cell-line series. The studies described here and elsewhere[11, 23] provide valuable information for drug sensitivity prediction, and should have great potential for future clinical application.

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