Figure 2. Interstitial fibrosis with sclerosing glomeruli and destruction or loss of renal tubuli in the kidney of rhesus monkey offspring.

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Defects of the third molar teeth in rhesus monkeys prenatally and lactationally exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

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Introduction

Teeth are targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[1,2]. In humans also tooth abnormalities were reported among populations exposed to dioxins[3]. We reported that pre- and postnatal exposure to TCDD affected development of deciduous and permanent teeth in rhesus monkeys[4]. The main abnormalities detected in stillborn and early postnatallyd died offspirng were missing deciduous incisors and molars. Observation upto approximatly 4 years of age revealed missing permanent incisors and premolars. In addition, cone-shaped or maldirected premolars were detected. This paper describes additional tooth findings in surviving 4.5-year-old offspirng .

Materials and Methods

Details of the materials and methods for this study are given elsewhere[4]. Briefly, adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg were used. Female monkeys were mated with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating periodwas disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups, each consisting of approximately 20 animals. They were allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PND0). TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on GD20 at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy andlactation until PND90.

Surviving offspring were examined at approximately 4.5 years of age for this study. They were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

In addition to the abnormal dental findings reported previously[4], the present observation revealed missing third molars in the 300 ng/kg. In controls all the permanent teeth including the third molars were radiographically recognizable at 4.5 years of age, although the third molars were not fully erupted. Figure 1 is a radiograph of the right molar portion of the lower jaw of the offspring No. 1 (PND1718) in the control group. The first and second molars have been fully erupted, and their crowns with cusps and roots are clearly visible. Although only a mesial half of the third molar (arrow) appears in this radiograph, its already calcified crown is clearly observable. In contrast, at least two (No. 31, PND1710 and No. 66, PND1618) of the eight surviving offspring in the 300 ng/kg group had missing third molars. In the offspring No. 31, the third molars on the left side in the upper jaw and on both sides in the lower jaw were not seen in radiographs which include enough areas distal to the second molars. Figure 2 is a radiograph of the right molar portion of the lower jaw of the offspring No. 31. In the presumptive position of the third molar (arrow), neither calcified crown nor root is observable. The radiograph of the left upper jaw did not reveal the position of the third molar. In the offspring No. 66, the presence of the upper left third molar could be confirmed, whereas the lower right one was apparently missing. There were two other cases (No. 39, PND1690 and No. 44, PND1695) with

possible missing third molars which could not be definitely confirmed. The size of the CCD unit, 23 x 38 mm, is fairly large when compared with the size of the oral cavity of a rhesus monkey at the age of 4.5 years. Therefore it was not easy to get clear radiographs of the distal portion of the upper and lower dental arches by placing the unit on the labial side of the molars.

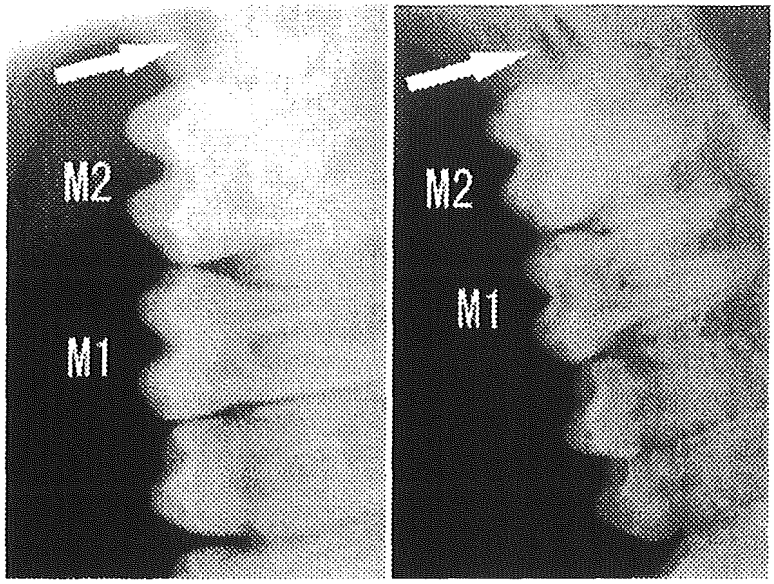

Figure 1.Lower right molars in No. 1 Figure 2. Lower right molars in No. 31

(Control group, PND 1718) (300 ng/kg group, PND 1710)

M1: the first molar; M2: the second molar; arrow: position of the third molar

The third molars in the rhesus monkey are still growing at the age of 4.5 years. Hence the final diagnosis of missing third molar should be done at the time of autopsy of these offspring.

In humans missing third molars are common among the general population. However, the third molars were invariably present in the parent monkeys used in the present study. Therefore missing third molars observed in the present study is considered to be caused by TCDD exposure.

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Attenuation of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cleft palate by dimethyl sulfoxide

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent experimental teratogen in mice. The most prominent effects induced by TCDD in fetus are cleft palate and hydronephrosis in mice. Recently, it has been reported that several compounds inhibited cleft palate in mice. For example, dimethylsulfoxide (DMSO) inhibited the secalonic acid-induced cleft palate in mice[1]. Methionine has been reported to inhibit all-trans retinoic acid (RA) or glucocorticoid (GC)-induced cleft palate in mice[2]. Therefore, in this experiment, the effects of these antiteratogenic compounds on the TCDD-induced cleft palate were examined. In addition, DMSO is known to be a hydroxyradical scavenger, the effect of dimethylthiourea (DMTU), a similar hydroxyradical scavenger[3], was examined. The effect of DMSO on RA-induced cleft palate was also examined.

Methods and Materials

2,3,7,8-TCDD was purchased from Radian International, Cambridge Isotope Laboratories, Inc. DMSO and L-methionine (Met) were purchased from Sigma Chemical Co. DMTU was purchased from Aldrich Co. RA was purchased from Wako Pure Chemicals Co. Female C57BL/6 mice were obtained from SLC Co. (Hamamatsu, Japan). TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to a working concentration in corn oil. The mice were given rodent chow (CRF-1, Oriental Co.) and distilled water *ad libtum* and housed under controlled conditions of temperature and light (12-h light; 12-h dark cycle). On gestation day (GD) 12.5, the mice were given single oral administration of TCDD (10μg/kg bw). DMSO was administered by gavage at 5-10 ml/kg bw /day on GD 13.5 to 14.5 or 13.5 to 17.5. Met was dissolved in a vehicle of 0.9% sodium chloride and was administered by i.p. at 200mg/kg bw/day on GD 13.5 and 14.5. DMTU was dissolved in distilled water and administered by gavage at 200 mg/kg bw/day on GD 13.5 and 14.5. RA was suspended in corn oil and administered by gavage at 200mg/kg bw on GD 12.5. On GD18.5, the dams were killed and fetuses were examined to evaluate the incidence of cleft palate.

Results and Discussion

TCDD had induced cleft palate in 50-70% of the fetuses after administration of 10μg/kg bw on GD 12.5. The TCDD-induced cleft palate was partially attenuated by additional administration of DMSO at 10 ml/kg bw/day (Table 1) or 5ml/kg bw/day (Table 2) from GD 13.5. The critical period for this DMSO antiteratogenic effects seems to be around GD 13.5 and 14.5, since the pretreatment of DMSO at GD 11.5 and the treatment of DMSO on GD 11.5 and GD 13.5 did not show the antiteratogenic effect on the TCDD-induced cleft palate (data not shown), and the longer administration from GD13.5 to 17.5 did not decrease to cleft palate compared to the shorter administration on GD13.5 and 14.5 (Table 3). On the other hand, no protective effect of Met treatment on GD13.5 and 14.5 on the TCDD-induced cleft palate was observed (Table 4). Contrary to the effect of DMSO, a similar hydroxyradical scavenger DMTU treatment on GD13.5 and 14.5 increased the TCDD-induced cleft palate (Table 5). It is suggested from this data that the hydroxyradical scavenging property may not be involved in the antiteratogenic effect of DMSO. The mechanism of increase of cleft palate by DMTU is unknown. Contribution of the maternal toxicity of DMTU indicated by two dead cases in the TCDD+DMTU-treated group remains unclear, since same mortality was found in the TCDD+DMSO-treated group. To investigate the action of DMSO, we examined the effect of GD13.5-14.5 DMSO administration on the GD 12.5 RA-induced cleft palate. DMSO turned out to be non-protective in this study (Table 6). Additional to the Met, which counter acts against RA and GC, but not against the TCDD, DMSO would be the second chemical that can be used as a probe to dissect the multiple pathways of chemically-induced cleft palate. At this time, the mechanisms of antiteratogenic effects of DMSO against TCDD induced-cleft palate are unknown. Recently, Dhulipara VC et al.[4] reported that the relevance of protein kinase A pathway to the protective effect of

DMSO against the secalonic acid D-induced cleft palate. Therefore, there may be a possibility that the PKA pathway is involved in the TCDD-induced cleft palate, and DMSO is acting through the pathway. In conclusion, this is the first to demonstrate that DMSO attenuated the TCDD-induced cleft palate in mice.

Table 1

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO
No. of litters	6	6
No. of dead mice	0	0
No. of live fetuses	46	46
No. of resorbed fetuses	0	0
No. of dead fetuses	1	0
No. of fetuses with cleft palate	25	11
Cleft palate (%)	54.3	23.9**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO was administered by gavage at 10 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 2

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO
No. of litters	6	7
No. of dead mice	0	0
No. of live fetuses	46	61
No. of resorbed fetuses	0	0
No. of dead fetuses	0	0
No. of fetuses with cleft palate	25	21
Cleft palate (%)	54.3	34.4*

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO was administered by gavage at 5 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 3

Effects of DMSO on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMSO
No. of litters	8	6
No. of dead mice	0	2
No. of live fetuses	69	32
No. of resorbed fetuses	1	1
No. of dead fetuses	1	1
No. of fetuses with cleft palate	42	10
Cleft palate (%)	60.9	31.2**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMSO was administered by gavage at 10 ml/kg /day on days 13.5,14.5, 15.5, 16.5 and 17.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 4

Effects of Methionine (Met) on TCDD induced-cleft palate in mice

	TCDD	TCDD+Met
No. of litters	6	7
No. of dead mice	0	0
No. of live fetuses	51	59
No. of resorbed fetuses	0	0
No. of dead fetuses	1	0
No. of fetuses with cleft palate	33	37
Cleft palate (%)	64.7	62.7

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and Met was administered by I.p. at 200mg/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving TCDD alone (*p<0.05, **P<0.01)

Table 5

Effects of dimethylthiourea (DMTU) on TCDD induced-cleft palate in mice

	TCDD	TCDD+DMTU
No. of litters	9	7

No. of dead mice	0	2
No. of live fetuses	74	43
No. of resorbed fetuses	0	0
No. of dead fetuses	3	2
No. of fetuses with cleft palate	57	42
Cleft palate (%)	77.0	97.7**

TCDD (10μg/kg) was administered by gavage on day 12.5 of gestation and DMTU

was administered by gavage at 200mg/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

TCDD alone (*p<0.05, **P<0.01)

Table 6

Effects of DMSO on Retinoic acid (RA) induced-cleft palate in mice

	RA	RA+DMSO
No. of litters	5	5
No. of dead mice	0	0
No. of live fetuses	38	32
No. of resorbed fetuses	0	0
No. of dead fetuses	11	10
No. of fetuses with cleft palate	18	13
Cleft palate (%)	47.4	40.6

RA (200mg/kg) was administered by gavage on day 12.5 of gestation and DMSO

was administered by gavage at 5 ml/kg /day on days 13.5 and 14.5 of gestation.

Asterisks indicate values that were significantly different from the group receiving

RA alone (*p<0.05, **P<0.01)

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Chemosensitivity profile of cancer cell lines and identification of genes determining chemosensitivity by an integrated bioinformatical approach using cDNA arrays

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Abstract

We have established a panel of 45 human cancer cell lines (JFCR-45) to explore genes that determine the chemosensitivity of these cell lines to anticancer drugs. JFCR-45 comprises cancer cell lines derived from tumors of three different organs: breast, liver, and stomach. The inclusion of cell lines derived from gastric and hepatic cancers is a major point of novelty of this study. We determined the concentration of 53 anticancer drugs that could induce 50% growth inhibition (GI_{50}) in each cell line. Cluster analysis using the GI_{50}s indicated that JFCR-45 could allow classification of the drugs based on their modes of action, which coincides with previous findings in NCI-60 and JFCR-39. We next investigated gene expression in JFCR-45 and developed an integrated database of chemosensitivity and gene expression in this panel of cell lines. We applied a correlation analysis between gene expression profiles and chemosensitivity profiles, which revealed many candidate genes related to the sensitivity of cancer cells to anticancer drugs. To identify genes that directly determine chemosensitivity, we further tested the ability of these candidate genes to alter sensitivity to anticancer drugs after individually overexpressing each gene in human fibrosarcoma HT1080. We observed that transfection of HT1080 cells with the *HSPA1A* and *JUN* genes actually enhanced the sensitivity to mitomycin C, suggesting the direct participation of these genes in mitomycin C sensitivity. These results suggest that an integrated bioinformatical approach using chemosensitivity and gene expression profiling is useful for the identification of genes determining chemosensitivity of cancer cells. [Mol Cancer Ther 2005;4(3):399–412]

Introduction

Predicting the chemosensitivity of individual patients is important to improve the efficacy of cancer chemotherapy. An approach to this end is to understand the genes that determine the chemosensitivity of cancer cells. Many genes have been described that determine the sensitivity to multiple drugs, including drug transporters (1–3) and metabolizing enzymes (4–6). Genes determining the sensitivity to specific drugs have also been reported. For example, increased activities of γ-glutamyl hydrolase (7) and dihydrofolate reductase (8) are resistant factors for methotrexate; increased activities of thymidylate synthase (9), metallothionein (10), and cytidine deaminase (11) are resistant factors for 5-fluorouracil (5-FU), cisplatin, 1-β-D-arabinofuranosylcytosine, respectively; and increased activity of NQO1 (12) is a sensitive factor for mitomycin C (MMC). However, the chemosensitivity of cancer cells is not determined by a handful of genes. These genes are not sufficient to explain the variation of the chemosensitivity of cancer cells.

Recently, attempts were made to predict the chemosensitivity of cancers using genome-wide expression profile analyses, such as cDNA microarray and single nucleotide polymorphisms (13–18). For example, Scherf et al. (18) and Zembutsu et al. (15) reported the analysis of genes associated with sensitivity to anticancer drugs in a panel of human cancer cell lines and in human cancer xenografts, respectively. Tanaka et al. (17) presented prediction models of anticancer efficacy of eight drugs using real-time PCR expression analysis of 12 genes in cancer cell lines and clinical samples. We also analyzed chemosensitivity-related genes in 39 human cancer cell lines (JFCR-39; ref. 19) and validated the association of some of these genes to chemosensitivity using additional cancer cell lines (20). These genes can be used as markers to predict chemosensitivity. Moreover, some of these genes may directly determine the chemosensitivity of cancer cells.

In the present study, we established a new panel of 45 human cancer cell lines (JFCR-45) derived from tumors from three different organs: breast, liver, and stomach. Using JFCR-45, we attempted to analyze the heterogeneity of chemosensitivity in breast, liver, and stomach cancers. We assessed their sensitivity to 53 anticancer drugs and

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developed a database of chemosensitivity. Then, we analyzed gene expression in 42 human cancer cell lines using cDNA arrays and stored them in the gene expression database. Using these two databases, we extracted genes whose expression was correlated to chemosensitivity. We further screened them to identify genes that could change the sensitivity to anticancer drugs using an *in vitro* gene transfection assay.

Materials and Methods

Cell Lines and Cell Cultures

We established a panel of JFCR-45 that included a portion of JFCR-39 and the 12 stomach cancer cell lines described previously (19, 20). They consist of the following cell lines: breast cancer cells HBC-4, BSY1, HBC-5, MCF-7, MDA-MB-231, KPL-3C (21), KPL-4, KPL-1, T-47D (22), HBC-9, ZR-75-1 (23), and HBC-8; liver cancer cells HepG2, Hep3B, Li-7, PLC/PRF/5, HuH7, HLE, HLF (24), HuH6 (25), RBE, SSP-25 (26), HuL-1 (27), and JHH-1 (28); and stomach cancer cells St-4, MKN1, MKN7, MKN28, MKN45, MKN74, GCIY, GT3TKB, HGC27, AZ521 (29), 4-1ST, NUGC-3, NUGC-3/5-FU, HSC-42, AGS, KWS-1, TGS-11, OKIBA, ISt-1, ALF, and AOTO. The AZ521 cell line was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The 4-1ST, OKIBA, and AOTO cell lines were provided by Dr. Tokuji Kawaguchi (Department of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan). All cell lines were cultured in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) with 5% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37°C under 5% CO_2.

Determination of the Sensitivity to Anticancer Drugs

Growth inhibition experiments were done to assess the chemosensitivity to anticancer drugs. Growth inhibition was measured by determining the changes in the amounts of total cellular protein after 48 hours of drug treatment using a sulforhodamine B assay. The GI_{50} values, which represent 50% growth inhibition concentration, were evaluated as described before (30, 31). Several experiments were done to determine the median GI_{50} value for each drug. Absolute values were then log transformed for further analysis.

Anticancer Drugs and Compounds

Actinomycin D, 5-FU, tamoxifen, cytarabine, radicicol, melphalan, 6-mercaptopurine, 6-thioguanine, and colchicine were purchased from Sigma (St. Louis, MO). The anticancer agents in clinical use were obtained from the company specified in parentheses, and those under development were kindly provided by the company specified as described below: aclarubicin and neocarzinostatin (Yamanouchi Pharmaceutical, Tokyo, Japan); oxaliplatin (Asahi Kasei, Tokyo, Japan), HCFU (Nihon Schering, Osaka, Japan); doxifluridine (Chugai Pharmaceutical, Tokyo, Japan); toremifene, bleomycin, and estramustine (Nippon Kayaku, Tokyo, Japan); daunorubicin and pirarubicin (Meiji, Tokyo, Japan); doxorubicin, epirubicin, MMC, vinorelbine, and L-asparaginase (Kyowa Hakko Kogyo, Tokyo, Japan); peplomycin, etoposide, NK109, and NK611 (Nippon Kayaku); vinblastine, vincrinstine, IFN-γ, and 4-hydroperoxycyclophosphamide (Shionogi, Tokyo, Japan); carboplatin and cisplatin (Bristol-Myers Squibb, New York, NY); mitoxantrone and methotrexate (Wyeth Lederie Japan, Tokyo, Japan); cladribine (Janssen Pharmaceutical, Titusville, NJ); amsacrine (Pfizer Pharmaceutical, formerly Warner Lambert, Plymouth, MI); camptothecin, irinotecan, and SN-38 (Yakult, Tokyo, Japan); paclitaxel (Bristol-Myers Squibb); docetaxel and topotecan (Aventis Pharma, Strasbourg, France); IFN-α (Sumitomo Pharmaceutical, Osaka, Japan); IFN-β (Daiichi Pharmaceutics, Tokyo, Japan); gemcitabine (Eli Lilly Japan, Kobe, Japan); E7010 and E7070 (Eisai, Tokyo, Japan); dolastatine 10 (Teikoku Hormone MFG, Tokyo, Japan); and TAS103 (Taiho Pharmaceutical Co., Tokyo, Japan).

Gene Expression Profiles by cDNA Array

Expression profiles of 3,537 genes in 42 human cancer cell lines were examined using Atlas Human 3.6 Array (BD Biosciences Clontech, Inc., Franklin Lakes, NJ) in duplicates. Experiments were done according to the manufacturer's instructions. Briefly, cell lines were harvested in log phase. Total RNA was extracted with TRIzol reagent (Invitrogen, Inc., Carlsbad, CA) and purified with Atlas Pure Total RNA Labeling System. Purified total RNAs were converted to ^{32}P-labeled cDNA probe by SuperScript II (Invitrogen). cDNA probe was hybridized to the Atlas Array overnight at 68°C and washed. Hybridized array was detected with PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). Scanned data were transformed to the numerical value with Atlas Image 2.0 software (BD Biosciences Clontech) and normalized by dividing by the value of 90% percentile of all genes in each experiment. Then, the intensities of the genes were defined by the average of intensities of duplicate results. The genes whose expression levels differed more than twice between the duplicates were eliminated from subsequent analysis. When the intensities of gene expression in both arrays were below the threshold value, they were given the value of threshold and were used for analysis. We determined the values of threshold of the normalized data as 30% of the value of 90% percentile. Then, $\log_2$ was calculated for each expression value.

Hierarchical Clustering

Hierarchical clustering using average linkage method was done by "Gene Spring" software (Silicon Genetics, Inc., Redwood, CA). Pearson correlation coefficients were used to determine the degree of similarity.

Correlation Analysis between Gene Expression and Chemosensitivity Profiles

The genes whose expressions were observed in >50% of all cell lines examined were selected for the correlation analysis. The degree of similarity between chemosensitivity and gene expression were calculated using the following Pearson correlation coefficient formula:

$$r = \frac{\sum_i (x_i \quad x_m)(y_i \quad y_m)}{\sqrt{\sum_i (x_i \quad x_m)^2 \sum_i (y_i \quad y_m)^2}}$$

where x_i is the log expression data of the gene x in cell i, y_i is the log sensitivity $|\log_{10}GI_{50}|$ of cell i to drug y, x_m is the mean of the log expression data of the gene x, and y_m is the mean sensitivity $|\log_{10}GI_{50}|$ of drug y. A significant correlation was defined as $P < 0.05$.

Screening of the Genes That Determine Chemosensitivity

Candidate genes related to the chemosensitivity were cloned into the vector pcDNA3.1/*myc*-His A (Invitrogen). Transfection of HT1080 cells with the plasmid DNA was carried out using LipofectAMINE Plus reagent (Invitrogen). The transfection efficiency was monitored by green fluorescent protein fluorescence. The fluorescence of green fluorescent protein was observed in >90% of the green fluorescent protein–transfected HT1080 (data not shown). Twenty-four hours after the transfection, proper concentrations of MMC were added and the cells were treated for 24 hours. Efficacies of anticancer drugs were determined by measuring the growth inhibition. Cell growth was measured by following [^{3}H]thymidine incorporation. [^{3}H]thymidine (0.067 MBq) was added to each well and incubated at 37°C for 45 minutes. Cells were washed with prewarmed PBS( ) and fixed with 10% TCA on ice for 2 hours. After fixing, cells were washed with 10% TCA and lysed with 0.1% SDS-0.2 N NaOH solution. After incubation at 37°C, the lysed mixture was neutralized with 0.25 mol/L acetic acid solution. [^{3}H]thymidine incorporated into the cells was determined using scintillation counter. All experiments, except for interleukin (IL)-18, were done four times.

Results

Sensitivity of JFCR-45 to 53 Anticancer Drugs

Sensitivity to 53 drugs was assessed as described in Materials and Methods. The known modes of actions and the value of $|\log_{10}GI_{50}|$ of 53 anticancer drugs in each of the 45 cell lines are summarized in Table 1. The $|\log_{10}GI_{50}|$ indicated here is the median value of multiple experiments. The chemosensitivity of the cell lines differed even among those derived from the same organ. These data were stored in a chemosensitivity database. Figure 1 shows the classification of the anticancer drugs by hierarchical clustering analysis based on chemosensitivity, $|\log_{10}GI_{50}|$, of JFCR-45. As shown, the 53 drugs were classified into several clusters, each consisting of drugs with similar modes of action [e.g., one cluster included topoisomerase (topo) I inhibitors, such as camptothecin, topotecan, and SN-38]. The second cluster comprised tubulin binders, including taxanes and *Vinca* alkaloids. 5-FU and its derivatives were also clustered into a single group. These results indicated that our system using JFCR-45 was able to classify the drugs based on their modes of action, which is in agreement with previous findings using NCI-60 and JFCR-39 (18, 19, 32).

Classification of 42 Human Cancer Cell Lines According to Gene Expression Profiles

Using a cDNA array, we examined the expression of 3,537 genes in 42 cell lines of JFCR-45. Based on these expression profiles, hierarchical clustering was done. In a few experiments, cell lines derived from the same organ were clustered into a group (Fig. 2). Breast cancer cell lines, except KPL-4, formed one cluster. Liver and stomach

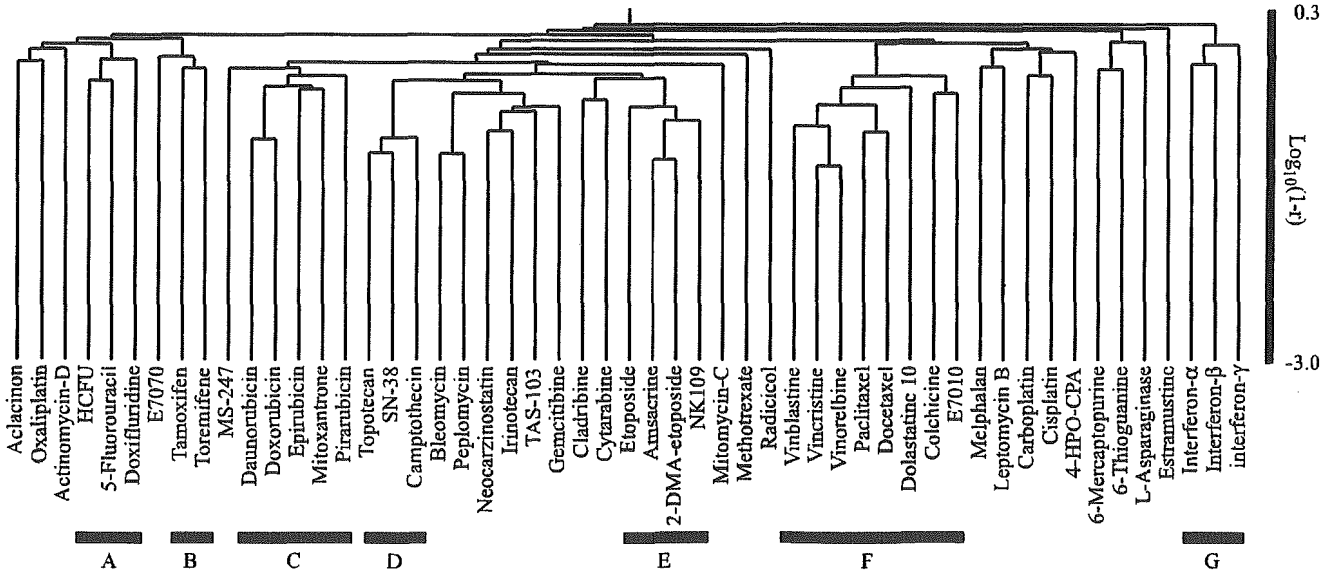

Figure 1. Hierarchical clustering of 53 anticancer drugs based on their activity on 45 human cancer cell lines. Hierarchical clustering method was "average linkage method" using Pearson correlation as distance. Fifty-three drugs were classified into several clusters, each consisting of drugs with similar modes of action or targets: (**A**) 5-FU derivatives, (**B**) estrogen receptor, (**C**) DNA synthesis/topo II inhibitors, (**D**) topo I inhibitors, (**E**) topo II inhibitors, (**F**) tubulin binders, and (**G**) IFN.

Table 1. The mode of actions and the median value of $|\log_{10}GI_{50}|$ of 53 anticancer drugs in each of the 45 cell lines

Drug name	Target/ mode of action	Breast											
		HBC-4	BSY1	HBC-5	MCF-7	MDA-MB-231	KPL-3C	KPL-4	KPL-1	T-47D	HBC-9	ZR-75-1	HBC-8
Aclarubicin	DNA/RNA synthesis	7.04	8.69	7.92	7.86	7.83	7.11	7.63	7.95	7.39	7.08	8.03	7.93
Oxaliplatin	DNA cross-linker	5.79	5.75	5.40	5.69	4.75	5.04	5.20	4.78	5.17	4.10	5.08	6.17
Actinomycin D	RNA synthesis	9.20	9.10	8.85	9.45	8.71	8.90	9.05	9.04	8.89	8.24	8.98	9.60
HCFU	Pyrimidine	4.36	5.17	4.44	5.13	4.57	4.65	5.55	4.41	4.97	4.22	4.68	4.84
5-FU	Pyrimidine	4.43	4.87	4.40	5.12	4.18	4.00	5.23	4.00	4.13	4.00	4.70	5.11
Doxifluridine	Pyrimidine	4.00	4.42	4.00	4.00	4.00	4.00	4.09	4.00	4.00	4.00	4.14	4.19
E7070	Cell cycle inhibitor	4.50	6.20	4.22	4.50	4.35	4.94	5.01	4.74	4.69	4.00	4.38	4.98
Tamoxifen	Estrogen receptor	4.95	5.42	5.01	5.04	4.90	5.14	5.49	4.93	5.31	4.90	4.95	5.53
Toremifene	Estrogen receptor	4.81	5.12	4.87	4.96	4.85	4.93	5.13	4.88	5.17	4.89	4.88	4.86
MS-247	DNA synthesis	6.08	6.79	5.32	6.78	5.98	6.09	6.16	5.86	6.63	6.42	6.88	6.71
Daunorubicin	DNA synthesis/topo II	6.96	7.34	6.82	7.68	6.83	6.77	7.25	6.84	7.41	6.92	7.39	7.97
Doxorubicin	DNA synthesis/topo II	7.13	7.26	6.85	7.58	6.66	6.74	7.38	6.76	7.36	6.94	7.12	7.85
Epirubicin	DNA synthesis/topo II	6.08	6.90	6.59	7.08	6.42	6.50	7.03	6.83	7.26	6.73	7.90	7.19
Mitoxantrone	DNA synthesis	6.28	7.12	6.00	8.06	6.50	6.40	6.83	6.38	7.11	6.96	8.02	7.44
Pirarubicin	DNA synthesis/topo II	8.97	9.00	8.34	9.00	8.47	8.62	9.00	8.39	9.00	8.22	9.00	9.00
Topotecan	Topo I	5.84	6.57	5.10	8.00	5.55	6.37	6.71	5.90	7.51	6.18	7.20	7.61
SN-38	Topo I	7.98	7.52	5.56	8.56	6.12	6.75	7.40	6.60	8.25	6.13	7.92	7.75
Camptothecin	Topo I	5.92	6.57	6.04	7.63	5.86	6.67	6.60	6.70	7.12	5.80	7.21	6.92
Bleomycin	DNA synthesis	4.81	4.89	4.00	4.48	4.00	4.00	5.59	4.00	5.46	4.46	4.22	4.37
Peplomycin	DNA synthesis	4.90	5.84	4.00	5.22	4.27	4.61	6.29	4.08	5.37	4.52	4.72	5.25
Neocarzinostatin	DNA synthesis	7.35	8.00	6.03	8.17	6.55	6.42	7.61	6.18	7.26	7.06	7.26	8.10
Irinotecan	Topo I	4.86	5.09	4.00	5.46	4.28	4.30	4.91	4.11	5.21	4.15	4.47	5.24
TAS103	Topo	6.81	7.22	6.37	7.66	6.57	6.45	7.20	6.17	7.25	6.16	7.13	7.60
Gemcitabine	Pyrimidine	6.74	5.62	4.00	8.00	5.20	4.00	7.25	4.00	7.18	5.15	4.71	5.75
Cladribine	Pyrimidine	4.00	4.00	4.00	5.41	4.05	4.60	4.73	4.00	4.83	4.23	4.00	4.68
Cytarabine	Pyrimidine	4.00	4.00	4.00	6.40	4.00	4.00	5.02	4.00	4.00	4.00	4.00	4.54
Etoposide	Topo II	4.88	5.48	4.39	6.15	4.66	4.00	5.42	4.68	5.93	4.48	5.11	4.72
Amsacrine	Topo II	5.20	5.78	5.29	6.56	5.25	4.89	5.69	4.93	5.97	5.14	6.56	5.70
2-Dimethylaminoetoposide	Topo II	4.67	4.82	4.02	6.02	4.48	4.00	5.03	4.00	5.05	4.89	5.74	4.71
NK109	Topo II	5.69	5.88	5.27	6.37	6.04	5.49	6.31	5.56	6.30	5.57	6.08	5.81
MMC	DNA alkylator	5.90	6.68	5.68	6.99	5.14	5.46	6.40	5.50	5.42	5.49	5.74	6.69
Methotrexate	DHFR	7.11	5.19	4.00	7.53	4.00	4.00	7.53	5.25	4.00	4.00	4.00	4.00
Radicicol	HSP90/Tyr kinase	5.55	5.80	5.17	7.28	6.55	5.19	6.13	5.28	7.43	5.39	6.18	6.62
Vinblastine	Tubulin	9.22	9.76	9.22	9.68	8.67	9.17	9.77	9.13	9.15	6.00	7.58	7.99
Vincristine	Tubulin	8.77	9.72	9.29	9.42	8.67	9.12	9.57	9.31	9.22	6.00	8.41	6.20
Vinorelbine	Tubulin	8.45	9.23	8.51	8.85	8.23	8.33	9.35	8.93	8.41	6.00	8.16	6.00
Paclitaxel	Tubulin	7.30	8.43	7.94	7.72	7.37	7.38	8.20	7.53	7.90	6.00	7.05	6.59
Docetaxel	Tubulin	8.41	8.98	8.23	8.52	7.88	8.18	8.82	8.19	8.56	6.00	7.15	8.28
Dolastatine 10	Tubulin	9.15	10.83	11.19	10.26	9.07	10.02	10.74	9.44	9.95	8.00	9.46	8.67
Colchicine	Tubulin	6.06	8.68	6.33	6.48	7.24	7.58	8.48	7.89	6.64	5.00	7.84	6.59
E7010	Tubulin	4.37	6.56	4.00	6.14	5.07	5.38	6.69	5.71	6.29	5.50	6.04	4.72
Melphalan	DNA cross-linker	4.20	4.92	4.42	5.09	4.33	4.67	4.04	4.66	4.38	4.08	4.45	4.57
Leptomycin B	Cell cycle inhibitor	9.35	9.64	9.33	9.44	8.91	9.59	9.47	9.63	9.26	8.96	9.78	9.74
Carboplatin	DNA cross-linker	4.00	4.34	4.12	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Cisplatin	DNA cross-linker	4.90	5.69	5.65	5.09	4.56	4.72	5.52	4.63	4.56	5.35	4.71	5.39
4-Hydroperoxycyclo-phosphamide	DNA alkylator	4.78	4.85	5.41	5.58	4.68	4.78	4.54	4.74	4.86	5.18	4.76	4.78
6-Mercaptopurine	Purine	5.41	4.73	4.15	5.88	5.17	5.11	4.50	5.02	6.00	4.27	4.05	4.50
6-Thioguanine	Purine	4.59	5.85	5.40	5.86	5.80	5.92	5.55	5.91	5.81	4.53	5.21	5.66
L-Asparaginase	Protein synthesis	6.55	6.63	4.00	6.43	6.01	6.03	7.20	6.18	6.10	5.49	6.07	6.36
Estramustine	Estradiol	4.09	4.51	4.00	4.00	4.66	4.85	4.56	4.31	4.17	4.74	4.00	4.73
IFN-α	Biological response	4.00	7.71	4.00	4.00	4.23	4.00	4.00	4.00	4.00	4.00	4.00	5.02
IFN-β	Biological response	4.00	8.00	4.00	4.00	6.40	4.23	7.08	4.00	4.00	4.00	4.00	4.56
IFN-γ	Biological response	7.69	7.93	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

(Continued on the following page)

Table 1. The mode of actions and the median value of $|\log_{10}GI_{50}|$ of 53 anticancer drugs in each of the 45 cell lines (Cont'd)

Drug name	Target/ mode of action	Liver											
		HepG2	Hep3B	Li-7	PLC/ PRF/5	HuH7	HLE	HLF	HuH6	RBE	SSP-25	HuL-1	JHH-1
Aclarubicin	DNA/RNA synthesis	8.13	7.77	7.39	7.68	8.29	7.49	7.86	7.70	7.87	7.39	7.97	8.23
Oxaliplatin	DNA cross-linker	7.07	5.39	5.78	5.61	6.44	4.90	4.75	5.60	5.19	4.58	6.04	6.01
Actinomycin D	RNA synthesis	9.03	8.61	8.24	8.04	8.99	8.13	8.45	8.75	8.25	8.47	8.78	9.00
HCFU	Pyrimidine	5.28	4.80	4.79	4.56	4.99	4.67	4.70	4.50	4.92	4.69	4.87	4.63
5-FU	Pyrimidine	5.27	4.20	4.26	4.21	5.08	4.00	4.19	4.00	4.60	4.00	5.29	4.72
Doxifluridine	Pyrimidine	4.49	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.04	4.00
E7070	Cell cycle inhibitor	5.47	4.99	4.77	4.44	5.36	4.61	4.43	4.74	5.09	4.29	4.29	4.87
Tamoxifen	Estrogen receptor	5.45	5.30	5.23	4.79	5.09	5.02	4.97	5.38	4.90	5.11	4.87	4.97
Toremifene	Estrogen receptor	5.06	4.97	4.92	4.82	4.99	5.09	4.91	4.95	4.92	5.00	4.80	5.10
MS-247	DNA synthesis	6.33	5.84	6.35	5.23	6.02	6.58	6.42	5.82	5.66	6.37	5.67	6.82
Daunorubicin	DNA synthesis/topo II	7.48	7.10	6.83	6.39	7.29	7.55	7.49	6.98	7.18	6.73	7.08	7.51
Doxorubicin	DNA synthesis/topo II	7.29	6.77	6.88	5.83	7.04	7.39	7.25	6.87	6.89	6.68	6.89	7.31
Epirubicin	DNA synthesis/topo II	7.33	6.86	6.87	6.29	7.31	7.21	7.25	6.91	6.84	6.73	6.74	7.03
Mitoxantrone	DNA synthesis	7.95	6.51	7.88	6.51	6.76	7.60	7.67	6.71	7.37	7.59	6.11	7.15
Pirarubicin	DNA synthesis/topo II	9.00	8.58	9.00	8.26	9.00	9.00	9.00	8.59	8.98	9.00	8.95	9.00
Topotecan	Topo I	7.93	5.81	7.70	5.64	6.07	7.73	7.73	5.72	6.83	6.74	5.30	6.99
SN-38	Topo I	8.43	6.37	8.21	6.03	6.75	8.28	8.31	5.91	7.05	7.47	5.69	7.74
Camptothecin	Topo I	7.44	6.19	7.48	5.86	6.35	7.42	7.53	6.10	6.69	6.79	6.16	6.92
Bleomycin	DNA synthesis	6.02	4.38	5.66	4.00	4.85	6.04	6.59	4.15	4.73	4.97	5.10	4.94
Peplomycin	DNA synthesis	6.73	4.72	6.40	4.45	5.46	5.86	6.56	4.01	5.12	5.83	5.35	5.34
Neocarzinostatin	DNA synthesis	8.22	6.72	7.81	6.34	6.92	7.60	7.80	6.57	7.27	7.53	6.67	7.09
Irinotecan	Topo I	5.18	4.36	5.61	4.00	4.33	5.25	5.13	4.11	4.37	4.64	4.05	4.78
TAS103	Topo	7.56	6.57	7.68	6.64	6.95	7.81	7.87	6.55	7.32	6.89	6.95	6.94
Gemcitabine	Pyrimidine	8.00	4.63	8.00	4.00	6.16	7.83	8.00	4.19	6.56	7.24	5.60	5.85
Cladribine	Pyrimidine	6.30	4.00	4.86	4.00	4.00	5.85	5.45	4.00	4.86	5.30	4.00	4.00
Cytarabine	Pyrimidine	6.22	4.00	4.00	4.00	4.00	5.22	5.41	4.00	4.00	4.00	4.00	4.00
Etoposide	Topo II	5.62	4.86	5.56	4.60	4.92	5.80	5.70	5.05	4.85	5.35	5.35	5.09
Amsacrine	Topo II	6.41	5.56	6.66	5.47	5.77	6.58	6.61	5.43	5.90	5.98	5.71	5.46
2-Dimethylaminoetoposide	Topo II	5.56	4.66	5.70	4.54	4.73	5.75	5.84	4.57	5.20	5.54	4.75	4.66
NK109	Topo II	6.56	5.96	6.72	5.85	6.05	6.83	6.77	5.84	6.24	6.39	5.92	6.09
MMC	DNA alkylator	6.56	5.04	7.09	5.63	5.73	6.15	6.31	5.38	5.32	6.20	5.50	5.99
Methotrexate	DHFR	7.47	4.00	6.11	4.00	6.12	6.64	6.83	4.00	6.71	4.06	4.00	5.13
Radicicol	HSP90/Tyr kinase	7.87	7.08	6.43	6.16	6.46	6.63	6.83	6.03	5.52	5.61	5.94	5.68
Vinblastine	Tubulin	8.18	6.50	9.30	7.73	9.35	9.73	9.20	7.22	6.00	9.51	9.11	9.66
Vincristine	Tubulin	7.93	6.00	7.70	6.00	8.52	8.76	8.40	6.00	6.00	8.27	8.38	9.11
Vinorelbine	Tubulin	7.98	6.00	8.15	6.00	8.43	8.75	8.28	7.05	6.00	8.51	8.65	9.21
Paclitaxel	Tubulin	7.35	6.84	7.41	6.48	7.44	7.50	7.27	6.00	6.73	7.80	8.22	7.94
Docetaxel	Tubulin	8.08	7.11	7.83	6.80	8.23	8.09	8.08	6.00	6.14	8.50	8.54	8.50
Dolastatine 10	Tubulin	10.42	8.94	10.71	9.50	10.12	10.19	9.94	8.60	8.00	10.30	9.68	10.61
Colchicine	Tubulin	7.16	5.40	7.25	6.43	7.62	7.77	7.39	5.54	5.00	7.50	7.45	8.17
E7010	Tubulin	6.28	4.62	6.38	6.23	6.35	6.47	6.35	4.79	4.00	6.50	6.44	6.50
Melphalan	DNA cross-linker	4.76	4.47	4.62	4.00	4.44	4.59	4.81	4.03	4.39	4.40	4.84	4.86
Leptomycin B	Cell cycle inhibitor	9.67	9.32	9.44	9.19	9.10	9.31	9.37	9.00	9.29	9.51	9.54	9.66
Carboplatin	DNA cross-linker	4.18	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.53
Cisplatin	DNA cross-linker	5.53	5.32	5.51	4.75	5.63	5.36	5.45	5.26	4.73	4.94	5.41	5.86
4-Hydroperoxycyclo-phosphamide	DNA alkylator	4.92	4.74	4.88	4.65	4.84	4.87	5.04	4.82	4.69	4.90	4.76	5.30
6-Mercaptopurine	Purine	5.01	4.10	5.12	4.42	4.00	4.17	4.49	4.90	5.29	4.58	4.82	5.10
6-Thioguanine	Purine	5.08	4.57	5.23	5.37	4.70	4.22	5.14	6.04	5.76	5.18	5.92	6.14
L-Asparaginase	Protein synthesis	6.40	4.78	8.00	6.49	4.00	6.91	6.63	4.00	6.35	8.00	6.61	4.42
Estramustine	Estradiol	4.00	4.00	4.27	4.24	4.05	4.37	4.03	4.10	4.14	4.18	4.09	4.14
IFN-α	Biological response	4.00	4.00	4.20	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
IFN-β	Biological response	4.00	4.00	7.15	6.17	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
IFN-γ	Biological response	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	7.93

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Table 1. The mode of actions and the median value of $|\log_{10}GI_{50}|$ of 53 anticancer drugs in each of the 45 cell lines (Cont'd)

Drug name	Target/ mode of action	Stomach											
		St-4	MKN1	MKN7	MKN28	MKN45	MKN74	GCIY	GT3 TKB	HGC27	AZ521	4-1ST	NUGC -3
Aclarubicin	DNA/RNA synthesis	7.88	8.09	7.73	7.25	8.59	7.43	8.00	7.86	7.13	8.49	7.96	9.04
Oxaliplatin	DNA cross-linker	4.75	5.04	4.42	4.58	6.84	4.93	5.71	5.31	5.10	6.16	5.17	6.18
Actinomycin D	RNA synthesis	7.99	8.74	8.77	9.02	9.39	9.20	8.24	9.12	8.76	9.55	8.80	8.85
HCFU	Pyrimidine	4.17	4.70	4.82	4.77	5.56	4.86	4.77	5.09	4.74	5.21	4.84	4.74
5-FU	Pyrimidine	4.35	4.40	4.26	4.27	5.46	4.22	4.60	5.09	4.34	5.12	4.04	4.67
Doxifluridine	Pyrimidine	4.00	4.00	4.01	4.00	4.20	4.00	4.00	4.00	4.00	4.00	4.00	4.02
E7070	Cell cycle inhibitor	4.43	6.03	4.90	5.48	4.55	5.20	5.04	4.82	5.69	6.02	4.88	5.75
Tamoxifen	Estrogen receptor	4.95	4.89	5.44	5.23	5.13	5.67	4.92	5.19	5.25	5.11	4.87	5.06
Toremifene	Estrogen receptor	4.81	4.92	4.90	4.82	4.93	5.23	4.85	4.92	5.07	5.09	4.87	4.96
MS-247	DNA synthesis	5.66	5.72	6.27	5.59	7.32	6.62	5.71	6.88	6.76	7.58	7.09	6.62
Daunorubicin	DNA synthesis/topo II	6.60	7.30	6.98	7.03	7.66	6.88	6.79	7.55	7.17	7.98	7.18	7.74
Doxorubicin	DNA synthesis/topo II	6.39	7.45	6.79	6.71	7.32	6.70	6.39	7.14	6.86	7.87	6.68	7.66
Epirubicin	DNA synthesis/topo II	7.21	7.53	6.85	6.60	7.35	6.60	6.53	7.10	6.71	8.00	7.02	7.68
Mitoxantrone	DNA synthesis	6.82	7.52	6.57	6.52	7.79	6.68	6.87	7.82	6.83	8.79	7.38	7.59
Pirarubicin	DNA synthesis/topo II	8.31	8.97	8.55	8.57	9.00	8.53	8.81	9.00	8.56	9.00	8.86	9.00
Topotecan	Topo I	7.21	6.27	5.54	5.81	8.00	5.62	6.61	7.83	5.64	7.74	8.00	7.68
SN-38	Topo I	6.83	6.63	6.16	6.16	8.71	6.17	6.89	8.49	6.04	8.49	8.78	8.28
Camptothecin	Topo I	7.13	6.39	5.82	5.50	7.99	5.62	6.81	7.53	5.49	7.61	7.75	7.73
Bleomycin	DNA synthesis	4.00	4.61	4.03	4.00	4.54	4.22	4.00	6.21	4.22	7.18	6.03	4.75
Peplomycin	DNA synthesis	4.00	4.80	4.56	4.09	5.18	4.82	4.39	5.96	4.68	7.32	6.16	4.92
Neocarzinostatin	DNA synthesis	6.17	6.92	6.58	6.47	8.38	7.19	6.95	7.74	6.92	8.58	7.59	8.00
Irinotecan	Topo I	4.00	4.41	4.29	4.02	5.41	4.26	4.44	5.24	4.00	5.58	5.39	5.41
TAS103	Topo	5.75	7.54	6.50	6.56	7.50	6.43	6.96	7.97	6.81	8.51	7.40	7.76
Gemcitabine	Pyrimidine	4.09	6.17	4.45	4.00	8.00	5.38	6.18	7.57	4.00	8.00	6.68	7.70
Cladribine	Pyrimidine	4.11	4.51	4.00	4.00	6.88	4.00	4.00	5.56	4.00	6.52	4.43	5.42
Cytarabine	Pyrimidine	4.00	4.00	4.00	4.00	6.41	4.00	4.00	6.38	4.00	6.56	5.68	5.76
Etoposide	Topo II	4.67	5.79	4.59	4.51	5.43	4.22	4.96	5.55	5.22	6.23	5.80	5.90
Amsacrine	Topo II	5.30	6.24	5.01	4.96	6.43	5.34	5.75	6.55	5.50	6.98	6.44	6.68
2-Dimethylaminoetoposide	Topo II	4.70	5.63	4.57	4.37	5.67	4.29	4.97	5.75	5.05	5.99	5.72	6.14
NK109	Topo II	6.02	6.66	5.88	5.76	6.51	5.62	6.58	6.92	6.29	6.90	6.66	6.78
MMC	DNA alkylator	4.93	5.00	5.33	5.10	7.09	5.56	5.75	6.17	5.74	6.45	5.99	7.28
Methotrexate	DHFR	7.27	7.04	4.00	4.00	7.15	4.00	7.06	7.04	7.49	7.37	7.33	7.32
Radicicol	HSP90/Tyr kinase	6.96	6.59	5.88	5.66	6.44	6.15	6.40	6.89	6.00	6.63	7.42	6.08
Vinblastine	Tubulin	6.17	9.62	7.60	9.64	9.04	9.25	8.58	9.88	9.37	9.76	9.85	9.53
Vincristine	Tubulin	6.37	9.36	8.60	8.58	8.42	9.13	8.12	9.30	8.91	9.36	9.61	8.94
Vinorelbine	Tubulin	6.00	8.60	8.51	8.59	8.42	8.53	7.96	9.22	8.37	8.89	8.83	8.87
Paclitaxel	Tubulin	6.87	7.68	7.50	7.48	7.89	7.16	6.77	8.15	7.70	8.09	7.86	8.15
Docetaxel	Tubulin	7.05	8.06	8.10	8.32	8.47	7.71	6.93	8.85	8.19	9.08	8.50	8.51
Dolastatine 10	Tubulin	9.41	9.56	10.27	10.18	9.75	10.29	10.51	10.60	9.23	10.42	10.53	10.35
Colchicine	Tubulin	7.76	7.99	7.28	7.90	7.75	7.51	7.34	7.78	7.65	7.70	8.69	7.53
E7010	Tubulin	6.06	6.21	6.26	6.35	6.02	6.15	6.39	6.69	6.08	6.69	6.67	6.40
Melphalan	DNA cross-linker	4.47	4.70	4.19	4.00	4.79	4.36	4.55	4.59	4.72	5.18	5.26	5.32
Leptomycin B	Cell cycle inhibitor	9.45	9.44	9.36	9.25	9.45	9.50	9.15	9.48	9.57	9.81	9.69	9.54
Carboplatin	DNA cross-linker	4.00	4.25	4.00	4.00	4.00	4.00	4.00	4.14	4.00	4.00	4.24	4.97
Cisplatin	DNA cross-linker	4.78	5.61	5.07	4.66	5.47	4.48	5.35	5.46	4.75	5.12	5.60	6.52
4-Hydroperoxycyclo-phosphamide	DNA alkylator	4.37	4.77	4.81	4.92	5.13	4.76	4.85	4.81	4.80	5.30	5.25	5.33
6-Mercaptopurine	Purine	4.21	5.58	4.67	5.21	5.39	5.86	4.45	5.21	5.47	5.54	5.97	5.03
6-Thioguanine	Purine	6.18	6.13	5.49	5.46	5.66	5.74	5.83	5.57	5.83	6.21	6.53	5.36
L-Asparaginase	Protein synthesis	6.32	6.41	6.64	6.54	6.65	6.91	5.30	6.70	5.78	6.72	6.34	6.51
Estramustine	Estradiol	4.21	4.26	4.00	4.00	4.20	4.72	4.29	4.45	4.34	4.20	5.11	4.48
IFN-α	Biological response	4.00	4.00	4.00	4.00	4.00	4.51	4.00	4.00	4.00	4.00	4.00	4.00
IFN-β	Biological response	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
IFN-γ	Biological response	4.00	4.07	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

(Continued on the following page)

Table 1. The mode of actions and the median value of $|\log_{10}GI_{50}|$ of 53 anticancer drugs in each of the 45 cell lines (Cont'd)

Drug name	Target/ mode of action	Stomach								
		NUGC -3/5-FU	HSC-42	AGS	KWS-1	TGS-11	OKIBA	ISt-1	ALF	AOTO
Aclarubicin	DNA/RNA synthesis	7.51	8.21	8.27	7.96	8.31	7.20	7.19	8.54	7.57
Oxaliplatin	DNA cross-linker	5.23	5.98	5.58	6.26	7.02	5.85	5.14	5.46	4.78
Actinomycin D	RNA synthesis	8.56	9.32	8.99	9.22	9.55	9.35	8.77	9.39	8.88
HCFU	Pyrimidine	4.36	4.89	5.00	4.71	4.27	5.10	4.15	4.23	4.44
5-FU	Pyrimidine	4.00	4.40	5.02	4.50	4.06	6.38	4.00	4.42	4.09
Doxifluridine	Pyrimidine	4.00	4.00	4.26	4.00	4.00	4.18	4.00	4.00	4.00
E7070	Cell cycle inhibitor	4.39	4.81	4.46	5.25	4.96	6.05	4.83	6.69	4.97
Tamoxifen	Estrogen receptor	4.86	4.89	5.59	4.93	5.20	5.58	4.93	5.43	5.13
Toremifene	Estrogen receptor	4.85	4.88	5.00	4.93	5.07	5.58	4.88	5.50	5.24
MS-247	DNA synthesis	5.64	7.11	7.01	6.74	6.67	6.20	5.70	5.70	5.63
Daunorubicin	DNA synthesis/topo II	6.85	7.57	7.42	6.99	6.93	7.59	6.37	6.94	6.80
Doxorubicin	DNA synthesis/topo II	6.47	7.33	7.53	6.91	6.90	8.00	6.01	6.34	6.54
Epirubicin	DNA synthesis/topo II	6.13	7.61	8.02	7.12	6.91	7.12	5.99	7.00	6.51
Mitoxantrone	DNA synthesis	6.18	7.70	7.75	7.21	6.74	8.56	5.76	6.14	6.37
Pirarubicin	DNA synthesis/topo II	8.65	9.00	9.00	8.99	8.58	8.81	8.16	8.68	8.57
Topotecan	Topo I	5.82	8.00	7.54	6.07	6.39	6.10	6.70	6.90	6.85
SN-38	Topo I	6.31	8.61	8.70	6.81	6.66	7.07	7.29	7.46	7.28
Camptothecin	Topo I	6.00	7.76	7.23	6.36	6.64	6.81	6.43	6.72	6.96
Bleomycin	DNA synthesis	4.00	5.66	5.19	4.00	4.00	5.55	4.00	4.81	4.58
Peplomycin	DNA synthesis	4.05	6.00	5.82	4.65	4.08	5.92	4.23	5.04	4.78
Neocarzinostatin	DNA synthesis	6.54	7.89	7.78	6.84	6.60	7.05	6.54	6.74	7.24
Irinotecan	Topo I	4.06	5.48	5.50	4.25	4.58	4.64	4.42	4.56	4.71
TAS103	Topo	6.45	7.66	7.98	6.94	6.45	6.89	6.24	6.45	7.74
Gemcitabine	Pyrimidine	4.00	6.77	6.65	4.00	4.06	6.76	4.86	5.82	7.27
Cladribine	Pyrimidine	4.00	4.46	4.56	4.00	4.00	6.41	4.00	4.00	4.24
Cytarabine	Pyrimidine	4.00	5.96	5.60	4.00	4.00	7.32	4.00	5.58	4.00
Etoposide	Topo II	4.72	6.11	6.13	5.13	4.41	8.00	4.73	5.10	5.79
Amsacrine	Topo II	4.91	6.53	6.30	5.71	4.99	6.60	5.06	5.57	6.29
2-Dimethylaminoetoposide	Topo II	4.12	5.94	5.17	4.78	4.36	6.25	4.57	4.80	5.75
NK109	Topo II	5.95	6.70	6.47	6.63	5.68	7.27	5.79	5.91	6.86
MMC	DNA alkylator	5.58	6.27	6.23	5.86	5.75	5.56	5.32	6.03	5.86
Methotrexate	DHFR	4.00	7.38	7.53	7.81	4.00	6.66	4.00	4.00	4.00
Radicicol	HSP90/Tyr kinase	5.71	7.63	7.07	6.78	6.80	6.80	5.76	6.38	6.74
Vinblastine	Tubulin	8.20	9.85	9.69	9.80	9.28	9.71	7.04	8.12	8.33
Vincristine	Tubulin	7.12	9.70	9.24	9.35	9.41	10.00	6.00	7.46	8.20
Vinorelbine	Tubulin	7.13	9.32	8.86	8.87	8.58	9.79	6.00	8.25	8.64
Paclitaxel	Tubulin	6.49	8.07	7.74	7.96	8.03	8.29	6.52	7.79	7.52
Docetaxel	Tubulin	7.21	8.86	8.63	8.47	8.49	8.46	7.33	8.68	8.27
Dolastatine 10	Tubulin	8.89	10.69	10.50	10.44	10.13	11.86	8.69	10.09	10.26
Colchicine	Tubulin	5.98	8.59	8.19	8.34	7.45	8.74	6.05	7.56	7.84
E7010	Tubulin	4.37	6.69	6.47	6.64	6.27	6.88	4.51	5.50	5.36
Melphalan	DNA cross-linker	4.56	5.34	5.27	4.00	5.00	4.62	4.15	4.73	4.67
Leptomycin B	Cell cycle inhibitor	9.12	9.64	9.53	8.66	9.16	9.71	8.82	9.76	9.49
Carboplatin	DNA cross-linker	4.00	4.36	4.16	4.00	4.00	4.62	4.00	4.00	4.26
Cisplatin	DNA cross-linker	4.80	5.64	5.55	4.74	5.71	5.79	5.43	5.57	5.51
4-Hydroperoxycyclo-phosphamide	DNA alkylator	4.78	5.50	5.44	4.70	4.68	5.17	4.61	4.66	4.78
6-Mercaptopurine	Purine	5.19	5.90	5.86	4.95	4.55	4.85	4.00	4.00	4.00
6-Thioguanine	Purine	5.50	6.54	5.61	5.79	5.92	6.10	4.00	4.46	4.36
L-Asparaginase	Protein synthesis	6.63	6.47	6.93	6.51	4.94	6.52	4.00	5.56	4.00
Estramustine	Estradiol	4.08	5.03	4.74	4.42	4.02	4.79	4.59	4.95	4.76
IFN-α	Biological response	4.00	4.00	4.00	4.00	4.51	4.20	4.62	4.62	4.16
IFN-β	Biological response	4.00	4.00	4.00	4.00	6.02	4.93	4.77	6.28	6.54
IFN-γ	Biological response	4.00	4.00	4.00	4.00	4.00	4.00	4.00	5.06	4.00

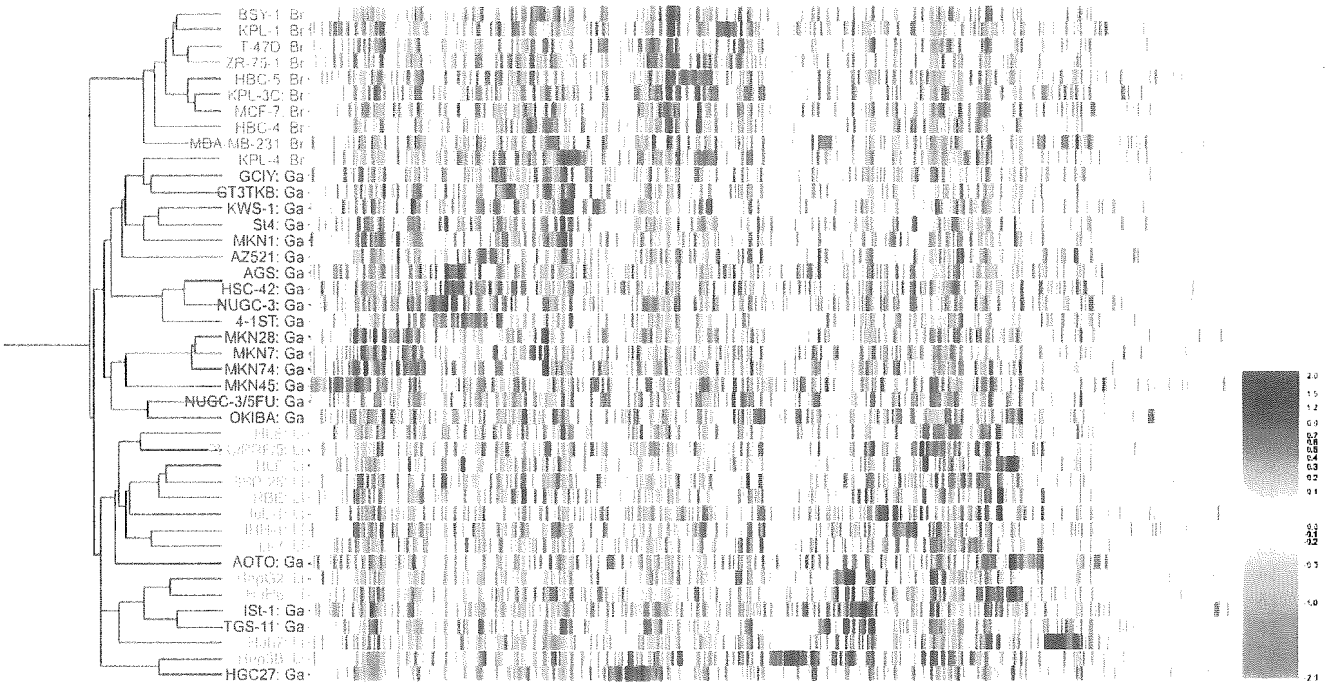

Figure 2. Hierarchical clustering of 42 human cancer cell lines based on their gene expression profiles. Gradient color indicates relative level ($\log_2$ transformed) of gene expression. *Red*, high expression of gene (2.0); *yellow*, normal expression of gene (0.0); *green*, low expression of gene (2.0). *Red* was expressed four times more than *yellow*. *Br*, *Ga*, and *Li*, breast, stomach, and liver cancer cell lines, respectively. Cell lines with the same tissue of origin tended to form a cluster.

cancer cell lines clustered separately from the breast cancer cell lines and formed tissue-specific subclusters. However, four stomach cancer cell lines, AOTO, ISt-1, TGS-11, and HGC27, were intercalated into a cluster of liver cancer cell lines. These results suggested that the established cell lines maintained characteristics of their organ of origin as far as the gene expression profile was concerned.

Correlation Analysis between Gene Expression Profiles and Chemosensitivity Profiles

To investigate genes that may be involved in chemosensitivity, we integrated the two databases and did a correlation analysis between gene expression and drug sensitivity. Comprehensive calculations for the Pearson correlation coefficients were done on the expression of 3,537 genes and sensitivity to 53 drugs in 42 cell lines. We selected genes that satisfied the following criteria: showing a P of correlation <0.05 between the expression of the gene and its sensitivity to a certain drug and being significantly expressed in >50% of the cell lines. We examined the data for the distribution by scatter graph analysis and removed those data showing a highly non-normal distribution. The higher the expression of the gene showing positive correlation, the higher the sensitivity was to the drug (i.e., this gene was a sensitive candidate gene). In contrast, genes that showed a negative correlation with chemosensitivity were resistant candidate genes. Consequently, different sets of genes were extracted with respect to each of the 53 drugs. Table 2 shows sets of genes whose expression was correlated with the sensitivity of 42 cell lines to MMC, paclitaxel, vinorelbine, and SN-38. As for MMC, 20 genes were extracted as sensitive genes and 10 genes were extracted as resistant candidate genes. Some of these genes (such as *JUN*, *EMS1*, and *NMBR*) are related to cell growth, whereas others included various types of genes (such as *SOD1*, *PELP1*, *SFRS9*, etc.). Similarly, many sensitive and resistant candidate genes were extracted with the other drugs tested. We further applied a Pearson correlation analysis to the cell lines originating from the same organ. Genes whose expressions were correlated with the MMC sensitivity in 10 breast cancer, 12 liver cancer, and 20 stomach cancer cell lines are shown in Table 3. As described previously (19, 20), these genes may predict chemosensitivity.

Identification of Genes That Change Cellular Chemosensitivity

These genes described above may include genes that directly determine chemosensitivity. To identify such genes, we established a screening system in which we could detect any change in the anticancer drug sensitivity by monitoring cell growth inhibition. [^{3}H]thymidine incorporation was used as a variable to measure cell growth. To detect small changes in sensitivity, a higher transfection efficiency was required. Therefore, the human fibrosarcoma cell line, HT1080, which reportedly showed high transfection efficiency, was selected for the subsequent experiments. Transfection efficiency of HT1080 cells

Table 2. Genes related to the sensitivity to MMC, vinorelbine, paclitaxel, and SN-38 in 42 human cancer cell lines

Rank	Gene	Genbank ID	*r*	*P*
A. MMC				
Sensitive				
1	*SF1*	D26121	0.566	0.001
2	*CBR3*	Ab004854	0.486	0.006
3	*EMS1*	M98343	0.480	0.010
4	*JUN*	J04111	0.473	0.015
5	*SFRS9*	U30825	0.448	0.010
6	*NMBR*	M73482	0.428	0.012
7	*RBMX*	Z23064	0.419	0.012
8	*SOD1*	M13267	0.418	0.024
9	*NOL1*	X55504	0.415	0.025
10	*PELP1*	U88153	0.405	0.019
11	*ARHA*	L25080	0.404	0.030
12	*AARS*	D32050	0.398	0.018
13	*NME1*	X17620	0.398	0.032
14	*HNRPA2B1*	M29065	0.390	0.044
15	*NME2*	L16785	0.378	0.025
16	*VAT1*	U18009	0.376	0.031
17	*SERPINB10*	U35459	0.372	0.028
18	*KIAA0436*	AB007896	0.353	0.041
19	*DRPLA*	D31840	0.350	0.049
20	*MC3R*	L06155	0.346	0.049
Resistant				
1	*SPTBN1*	M96803	0.450	0.013
2	*PET112L*	AF026851	0.425	0.027
3	*CAPN1*	X04366	0.421	0.032
4	*MEL*	X56741	0.414	0.028
5	*PACE*	X17094	0.380	0.035
6	*DVL2*	AF006012	0.370	0.034
7	*LOC54543*	AJ011007	0.366	0.022
8	*PAPOLA*	X76770	0.351	0.033
9	*RPLP2*	M17887	0.345	0.049
10	*ARF4L*	L38490	0.340	0.042
B. Vinorelbine				
Sensitive				
1	*ARHA*	L25080	0.534	0.003
2	*NME2*	L16785	0.521	0.001
3	*VIL2*	X51521	0.463	0.015
4	*YWHAQ*	X56468	0.450	0.011
5	*HK1*	M75126	0.449	0.016
6	*SATB1*	M97287	0.439	0.006
7	*CAMLG*	U18242	0.439	0.007
8	*CARS*	L06845	0.433	0.007
9	*CCNB1*	M25753	0.427	0.013
10	*U2AF1*	M96982	0.424	0.022
11	*PTMA*	M26708	0.423	0.018
12	*MLC1SA*	M31211	0.397	0.022
13	*NME1*	X17620	0.393	0.035
14	*SARS*	X91257	0.386	0.032
15	*CDC20*	U05340	0.385	0.029
16	*PPP4C*	X70218	0.385	0.039
17	*TNFAIP3*	M59465	0.384	0.023
18	*EEF1D*	Z21507	0.384	0.023
19	*PFKP*	D25328	0.365	0.028
20	*ENTPD2*	U91510	0.365	0.037
21	*CCL5*	M21121	0.358	0.035
22	*ACAT1*	D90228	0.352	0.048
23	*IQGAP1*	L33075	0.351	0.042
24	*PAX5*	M96944	0.342	0.038
25	*NRGN*	Y09689	0.336	0.042
26	*K-α-1*	K00558	0.328	0.048
27	*NDUFB7*	M33374	0.321	0.049
Resistant				
1	*HOXB1*	X16666	0.600	0.000
2	*F10*	K03194	0.514	0.002
3	*GPX2*	X53463	0.509	0.002
4	*NR1I2*	AF061056	0.498	0.002
5	*ANXA4*	M19383	0.481	0.005
6	*PDLIM1*	U90878	0.465	0.006
7	*LIPC*	X07228	0.464	0.004
8	*SERPINF2*	D00174	0.447	0.004
9	*HSD17B1*	M36263	0.443	0.014
10	*MAN2B1*	U60266	0.440	0.008
11	*LSS*	D63807	0.430	0.014
12	*PIK3CG*	X83368	0.415	0.010
13	*DBN1*	U00802	0.414	0.017
14	*NDUFA4*	U94586	0.410	0.038
15	*BDH*	M93107	0.399	0.024
16	*BCL2L1*	Z23115	0.385	0.039
17	*EEF1B2*	X60656	0.383	0.030
18	*F2*	V00595	0.382	0.026
19	*RARA*	X06614	0.369	0.029
20	*ITGB4*	X53587	0.367	0.042
21	*IMPA1*	X66922	0.367	0.042
22	*PACE*	X17094	0.367	0.042
23	*AGA*	M64073	0.361	0.042
24	*MVD*	U49260	0.353	0.038
25	*EHHADH*	L07077	0.346	0.039
26	*TFPI2*	D29992	0.343	0.035
27	*MARCKS*	M68956	0.342	0.045
28	*FGB*	J00129	0.334	0.035
29	*GPD1*	L34041	0.322	0.049
C. Paclitaxel				
Sensitive				
1	*ADH6*	M68895	0.513	0.002
2	*RAB28*	X94703	0.480	0.007
3	*U2AF1*	M96982	0.441	0.017
4	*GPC1*	X54232	0.440	0.013
5	*HK1*	M75126	0.439	0.020
6	*CARS*	L06845	0.436	0.006
7	*TNFAIP3*	M59465	0.433	0.009
8	*K-α-1*	K00558	0.418	0.010
9	*PFKP*	D25328	0.416	0.012
10	*GDI2*	D13988	0.411	0.033
11	*VIL2*	X51521	0.410	0.034
12	*RUNX2*	AF001450	0.409	0.038
13	*NME2*	L16785	0.407	0.015
14	*CDC20*	U05340	0.395	0.025
15	*GNAI2*	X04828	0.391	0.033

NOTE: Column 2 shows the name of the gene according to HUGO database. Column 4 shows Pearson correlation coefficient between chemosensitivity to drugs and gene expression. "Sensitive" indicates candidate genes sensitive to each drug. "Resistant" indicates genes resistant to each drug.

(Continued on the following page)

Table 2. Genes related to the sensitivity to MMC, vinorelbine, paclitaxel, and SN-38 in 42 human cancer cell lines (Cont'd)

Rank	Gene	Genbank ID	*r*	*P*
16	*ARHA*	L25080	0.381	0.041
17	*CNR2*	X74328	0.378	0.030
18	*PPP2R2B*	M64930	0.376	0.026
19	*SLC6A8*	L31409	0.374	0.046
20	*DDX9*	L13848	0.374	0.042
21	*ACAT1*	D90228	0.369	0.038
22	*PI3*	Z18538	0.329	0.047
Resistant				
1	*NAP1L1*	M86667	0.530	0.004
2	*HOXB1*	X16666	0.516	0.004
3	*PACE*	X17094	0.507	0.004
4	*MAN2B1*	U60266	0.486	0.003
5	*GPX2*	X53463	0.480	0.004
6	*DBN1*	U00802	0.469	0.006
7	*ANXA4*	M19383	0.468	0.007
8	*SERPINF2*	D00174	0.463	0.003
9	*AGA*	M64073	0.444	0.011
10	*BCL2L1*	Z23115	0.428	0.021
11	*LIPC*	X07228	0.401	0.015
12	*BDH*	M93107	0.393	0.026
13	*LSS*	D63807	0.384	0.030
14	*PDLIM1*	U90878	0.372	0.033
15	*ZNF161*	D28118	0.368	0.038
16	*UBE2E1*	X92963	0.363	0.032
17	*TLE1*	M99435	0.360	0.039
18	*RARA*	X06614	0.359	0.034
19	*PTPRN*	L18983	0.357	0.035
20	*APOE*	M12529	0.353	0.048
21	*F10*	K03194	0.348	0.040
22	*NR1I2*	AF061056	0.342	0.041
23	*UBE2L3*	X92962	0.332	0.045
24	*FGB*	J00129	0.313	0.049
D. SN-38				
Sensitive				
1	*EMS1*	M98343	0.573	0.001
2	*JUN*	J04111	0.564	0.003
3	*IL-6*	X04602	0.514	0.003
4	*RPL23*	X52839	0.495	0.004
5	*CDKN3*	L25876	0.455	0.017
6	*RPL3*	X73460	0.445	0.011
7	*TFPI*	J03225	0.442	0.009
8	*MRPL3*	X06323	0.437	0.009
9	*HLA-C*	M11886	0.424	0.014
10	*AARS*	D32050	0.419	0.012
11	*ARHGDIA*	X69550	0.416	0.031
12	*NOL1*	X55504	0.406	0.029
13	*SF1*	D26121	0.394	0.031
14	*SOD1*	M13267	0.389	0.037
15	*VEGF*	M32977	0.384	0.043
16	*EIF2S1*	J02645	0.382	0.034
17	*CDH5*	X79981	0.372	0.030
18	*FOSL1*	X16707	0.371	0.047
19	*IDS*	M58342	0.366	0.047
20	*PMVK*	L77213	0.364	0.044
21	*PPP2CB*	X12656	0.364	0.041
22	*NMBR*	M73482	0.362	0.035
23	*RPL26*	X69392	0.358	0.035
24	*PELP1*	U88153	0.356	0.042
25	*MC3R*	L06155	0.356	0.042
26	*RPS8*	X67247	0.355	0.036
Resistant				
1	*CAPN1*	X04366	0.496	0.010
2	*MEL*	X56741	0.478	0.010
3	*PACE*	X17094	0.443	0.012
4	*TIMP2*	J05593	0.433	0.019
5	*AOP2*	D14662	0.422	0.025
6	*ZNF174*	U31248	0.402	0.018
7	*ID3*	X69111	0.393	0.038
8	*KLF5*	D14520	0.384	0.036
9	*CALD1*	M64110	0.382	0.031
10	*LOC54543*	AJ011007	0.368	0.021
11	*PTPN3*	M64572	0.363	0.038
12	*ACTB*	X00351	0.362	0.025
13	*LY6E*	U42376	0.360	0.037
14	*ID1*	D13889	0.343	0.044

(Continued)

was >90% as evaluated by transfection of a plasmid expressing the enhanced green fluorescent protein (data not shown). To validate this screening system, we examined the effect of *NQO1* gene, coding DT-diaphorase that increases cellular sensitivity to MMC (12). As shown in Fig. 3B, cells transfected with NQO1 significantly enhanced growth inhibition by MMC compared with the mock-transfected and LacZ-transfected cells. We confirmed the cellular expression of the *NQO1* gene product by immunoblot (Fig. 3C). Thus, this screening system can be used to detect changes in chemosensitivity in HT1080 cells. Using this screening system, we examined whether the 19 genes, which were extracted in Tables 2 and 3, altered sensitivity to drug. Notably, the *HSPA1A* gene coding 70-kDa heat shock protein, whose expression was correlated with MMC sensitivity in the breast and liver cancer cell lines, significantly enhanced the MMC sensitivity in HSPA1A-transfected HT1080 cells (Fig. 3B). Similarly, the *JUN* gene encoding c-JUN, whose expression was correlated with MMC sensitivity, also enhanced the MMC sensitivity in JUN-transfected HT1080 cells (Fig. 3B). The expression of *myc*-tagged LacZ, 70-kDa heat shock protein, and JUN in the transfected cells was confirmed by immunoblotting with anti-*myc* antibody (Fig. 3C). Transfection with 17 other genes did not alter the MMC sensitivity. For example, transfection with the *IL-18* gene did not affect MMC sensitivity (Fig. 3B).

Discussion

The assessment system for determining pharmacologic properties of chemicals by a panel of cancer cell lines was first developed in the National Cancer Institute (33–35). We established a similar assessment system (JFCR-39;

Table 3. Genes related to MMC sensitivity in breast, liver, and stomach cancer cell lines

Rank	Gene	Genbank ID	*r*	*P*
A. Breast cancer				
Sensitive				
1	*INHBB*	M31682	0.972	0.000
2	*NK4*	M59807	0.838	0.018
3	*HSPA1A*	M11717	0.751	0.050
4	*LOC54557*	AF075050	0.735	0.024
5	*CD47*	Y00815	0.717	0.045
Resistant				
1	*RPN2*	Y00282	0.882	0.009
2	*ATP5O*	X83218	0.842	0.017
3	*CAST*	D50827	0.815	0.025
4	*HPCA*	D16593	0.776	0.024
5	*ZNF9*	M28372	0.774	0.024
6	*A2LP*	U70671	0.772	0.042
7	*IL-18*	D49950	0.747	0.033
8	*NRGN*	Y09689	0.727	0.041
B. Liver cancer				
Sensitive				
1	*EB1*	U24166	0.872	0.002
2	*JUN*	J04111	0.813	0.008
3	*EIF3S8*	U46025	0.772	0.015
4	*CTSD*	M11233	0.753	0.012
5	*SCYA5*	M21121	0.741	0.022
6	*PHB*	S85655	0.739	0.023
7	*HSPA1A*	M11717	0.729	0.026
8	*SPP1*	X13694	0.723	0.018
9	*TAB7*	X93499	0.712	0.021
10	*ACTN1*	X15804	0.692	0.039
11	*RXRB*	M84820	0.678	0.045
12	*PSME2*	D45248	0.673	0.047
13	*HLA-C*	M11886	0.647	0.043
14	*RPL19*	X63527	0.643	0.033
Resistant				
1	*MAPK6*	X80692	0.862	0.003
2	*GCSH*	M69175	0.793	0.006
3	*G22P1*	M32865	0.727	0.017
4	*USP11*	U44839	0.725	0.027
5	*ACTB*	X00351	0.715	0.020
6	*YWHAZ*	M86400	0.706	0.022
7	*IL-10*	M57627	0.694	0.018
8	*RFC4*	M87339	0.677	0.016
9	*CRLF1*	AF059293	0.644	0.033
10	*RPS6*	M20020	0.619	0.042
11	*EMX1*	X68879	0.618	0.043
12	*TK2*	U77088	0.607	0.047
C. Stomach cancer				
Sensitive				
1	*TEAD4*	U63824	0.803	0.001
2	*NR2C2*	U10990	0.713	0.001
3	*CSF1*	M37435	0.711	0.004
4	*RAB28*	X94703	0.695	0.008
5	*CBR3*	Ab004854	0.683	0.007
6	*NFYC*	Z74792	0.639	0.019
7	*PGF*	X54936	0.627	0.022
8	*ERG*	M21535	0.620	0.005
9	*MLLT1*	L04285	0.613	0.015
10	*FOS*	K00650	0.599	0.014
11	*TNFAIP3*	M59465	0.584	0.011
12	*CNR2*	X74328	0.581	0.009
13	*DRPLA*	D31840	0.577	0.024
14	*PSMB5*	D29011	0.572	0.026
15	*SLC6A8*	L31409	0.570	0.017
16	*SERPINB10*	U35459	0.570	0.013
17	*VAT1*	U18009	0.570	0.009
18	*TJP1*	L14837	0.562	0.029
19	*PELP1*	U88153	0.545	0.035
20	*C1QBP*	L04636	0.545	0.024
21	*CDK10*	L33264	0.543	0.045
22	*SERPINA6*	J02943	0.542	0.025
23	*ACTB*	X00351	0.538	0.021
24	*SFRP4*	AF026692	0.538	0.018
25	*EMX1*	X68879	0.535	0.018
26	*ACTB*	X00351	0.529	0.024
27	*RPS9*	U14971	0.528	0.043
28	*AMD1*	M21154	0.522	0.038
29	*RPL26*	X69392	0.522	0.038
30	*HNRPF*	L28010	0.520	0.047
31	*PTMS*	M24398	0.502	0.040
32	*STK12*	AF008552	0.498	0.050
33	*NR2F6*	X12794	0.491	0.046
34	*GBE1*	L07956	0.470	0.049
Resistant				
1	*PSMD8*	D38047	0.747	0.002
2	*LAMP2*	J04183	0.677	0.002
3	*CTSD*	M11233	0.651	0.006
4	*ADORA2B*	M97759	0.645	0.005
5	*ANXA4*	M19383	0.639	0.008
6	*PTPRK*	Z70660	0.638	0.003
7	*RAD23A*	D21235	0.622	0.010
8	*SDHA*	D30648	0.613	0.015
9	*PET112L*	AF026851	0.598	0.024
10	*DAD1*	D15057	0.593	0.025
11	*HSPB1*	X54079	0.588	0.013
12	*PSMA6*	X61972	0.586	0.036
13	*KDELR1*	X55885	0.584	0.028
14	*B2M*	AB021288	0.581	0.023
15	*M6PR*	M16985	0.579	0.038
16	*GCLC*	M90656	0.576	0.015
17	*SPTBN1*	M96803	0.557	0.038
18	*PACE*	X17094	0.547	0.019
19	*RPL24*	M94314	0.539	0.017
20	*SPINT2*	U78095	0.538	0.039
21	*STX4A*	U07158	0.534	0.027
22	*SIAT8B*	U33551	0.532	0.028
23	*CTSK*	U13665	0.529	0.029
24	*DCI*	L24774	0.525	0.044
25	*MEL*	X56741	0.525	0.045
26	*PITPNB*	D30037	0.523	0.038
27	*YY1*	M76541	0.512	0.043
28	*RAB1*	M28209	0.495	0.037
29	*UBE2L6*	AF031141	0.492	0.045
30	*PSMB7*	D38048	0.484	0.049

NOTE: Column 2 shows the name of the gene according to HUGO database. Column 4 shows Pearson correlation coefficient between chemosensitivity to drugs and gene expression. "Sensitive" indicates candidate genes sensitive to each drug. "Resistant" indicates genes resistant to each drug.

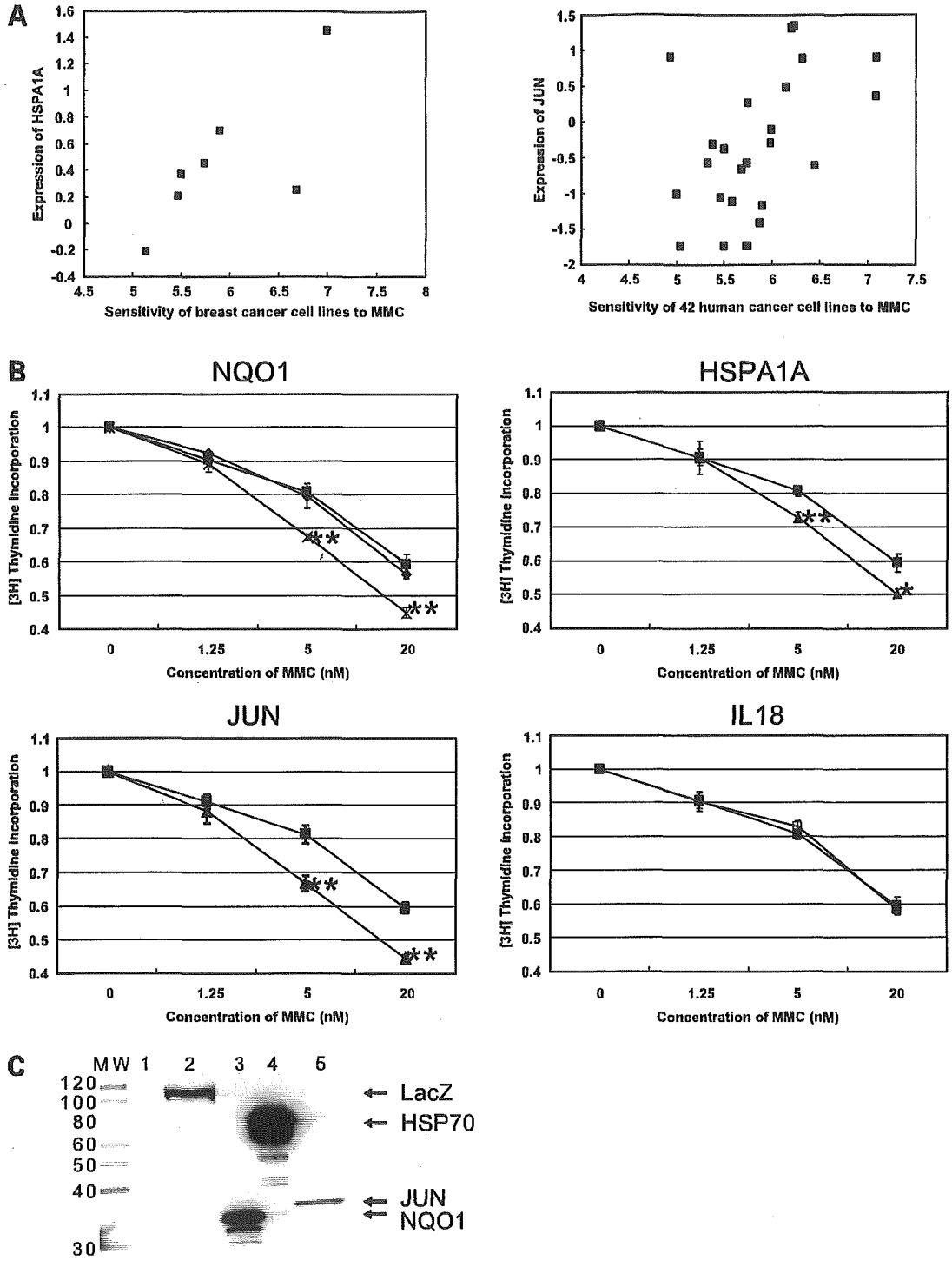

Figure 3. Relationships between MMC sensitivity and expression of HSPA1A in breast cancer cell lines (**A**, *left*) or JUN in 42 cell lines (**A**, *right*). Each symbol indicates one cell line. *X axis*, MMC sensitivity; *Y axis*, expression of HSPA1A or JUN. Pearson correlation coefficients between MMC sensitivity and expression of HSPA1A and JUN were 0.75 ($P = 0.05$) and 0.473 ($P = 0.015$), respectively. **B**, growth inhibition curves by MMC in mock (■), LacZ (◆), NQO1 ( ), HSPA1A (▲), JUN ( ), or IL-18 (□) transfected HT1080 cells. This growth inhibition by MMC was enhanced in HT1080 cells transfected with NQO1, HSPA1A, and JUN. *, $P < 0.002$; **, $P < 0.0001$, *t* test against mock-transfected cells. **C**, expressions of genes were certified by immunoblotting with anti-*myc* antibody: *myc*-tagged LacZ (*lane 2*), NQO1 (*lane 3*), 70-kDa heat shock protein (*HSP70*; *lane 4*), and JUN (*lane 5*).