also observed at 5000 mg/kg/day. Based on these findings, Sitarek et al. (1994) concluded that 1-butanol had adverse effects on the morphological development of fetuses in rats. However, we did not confirm their findings. We have demonstrated here that prenatal 1-butanol has no adverse effect on the morphological development of rat offspring. There are some differences between Sitarek's study and the present study in experimental conditions, such as duration of administration and rat strain used in the experiments. Sitarek et al. (1994) administered 1-butanol to female rats for 8 weeks before mating and throughout the mating and pregnancy period and found fetal anomalies, such as hydrocephaly and dilation of the cerebral ventricles and the renal pelvis. On the other hand, we gave 1-butanol to female rats during the whole period of pregnancy and did not detect fetuses with these anomalies. Administration during the premating and mating period is thought to be excluded from the susceptible period for induction of morphological anomalies such as hydrocephaly/dilation of the cerebral ventricles and dilation of the renal pelvis, because rat fetuses are susceptible to induction of these anomalies during mid and late pregnancy (Wood and Hoar, 1972; Kameyama, 1985). The strain difference of rats used in the experiments may explain the discrepancy in the findings regarding fetal anomalies between the studies. In Sitarek's study (1994), ImP: DAK rats obtained from their own breeding colony were used. No detailed information on this strain of rats was available (Sitarek et al., 1994). In their study, dilation of the lateral ventricle and/or third ventricle of the brain was observed in 2% of fetuses (one of the 12 litters) in the control group. In their another study using Imp: DAK rats, extension of the lateral ventricle and/or third ventricle of the brain was observed in 11.7% of fetuses (8 of the 17 litters) in the control group (Sitarek et al., 1996). However, these anomalies were not found in the control group of their studies using Wistar rats (Baranski et al., 1982), Imp: Lodz rats (Sitarek, 1999, 2001) and Imp: WIST rats (Sitarek and Sapota, 2003). The incidences of dilation of the cerebral ventricles in Imp: DAK rats are thought to be higher than those in the background control data of other strains of rats. The fetal incidence of hydrocephaly/dilation of cerebral ventricles in the control rats of reproductive studies conducted between 1986 and 1993 in 63 research institutes is reported to be 0–0.09% and 0–0.26%, respectively (Nakatsuka et al., 1997). In Crj: CD (SD) IGS rats which were used in the present study, the incidence of dilation of the lateral ventricles of the brain in 19 studies conducted during 1998–2000 is reported to be 0–0.06% in fetuses and 0–0.44% in litters (Barnett et al., 2000). Thus, hydrocephaly/dilation of the cerebral ventricle is not commonly observed in fetuses of common strains of rats.

The difference in terminology used for classification of structural anomalies in fetuses may also explain the discrepancy in the findings regarding fetal anomalies between the studies. Sitarek et al. (1996) stated that minor abnormalities, such as enlarged lateral ventricle and/or third ventricle, are quite frequent in rat fetuses and without having the dose-dependent relationship should not be taken alone as evidence of tested chemical fetotoxicity. However, the Fourth Berlin Workshop on Terminology in Developmental Toxicity noted that changes affecting brain ventricles are more likely to be classified as malformations and classification should be based on the historical control incidences, the nature of the organ affected and the severity (Solecki et al., 2003). In Sitarek's study (1994), dilation of the subarachnoid space was observed in fetuses of rats given 1-butanol at 300 mg/kg/day and higher. This anomaly was also found in fetuses in Imp: DAK rats given *N*-cyclohexyl-2-benzothiazolesulfenamide (Sitarek et al., 1996) and Imp: Lodz rats given *N*-methylmorpholine (Sitarek, 1999). No information on the definition of this anomaly was available in their reports. We are unaware of this anomaly in other literature (Kameyama et al., 1980; Morita et al., 1987; Nakatsuka et al., 1997; Horimoto et al., 1998; Barnett et al., 2000; Solecki et al., 2003).

In conclusion, the administration of 1-butanol to pregnant rats throughout pregnancy had adverse effects on maternal rats and embryonic/fetal growth but had no adverse effects on fetal morphological development even at a maternally toxic dose. The data indicate that 1-butanol induces developmental toxicity only at maternally toxic doses in rats. Based on the significant decreases in maternal body weight gain and fetal weight at 5.0%, it is concluded that the NOAELs of 1-butanol for both dams and fetuses are 1454 mg/kg/day (1.0% in drinking water) in rats.

Acknowledgment

This study was performed in 2003 through 2004 at the Ina Research, Inc. (Ina, Japan) and supported by the Ministry of Health, Labour and Welfare, Japan.

References

Baranski, B., Stetkiewicz, I., Trzcinka-Ochocka, M., Sitarek, K., Szymczak, W., 1982. Teratogenicity, fetal toxicity and tissue concentration of cadmium administered to female rats during organogenesis. Journal of Applied Toxicology 2, 255–259.

Barnett Jr., J.F., Lewis, D., Tappen, A., Hoberman, A.M., Christian, M.S., 2000. Reproductive indices, fetal gross, visceral and skeletal alterations, sexual maturation, passive avoidance and water maze data, a comparison of results in CD(SD)IGS rats and CD(SD) rats. In: Matsuzawa, T., Inoue, H. (Eds.), Biological Reference Data on CD (SD)IGS Rats-2000. CD(SD)IGS Study Group, c/o Charles River Japan Inc., Yokohama, Japan.

Chahoud, I., Buschmann, J., Clark, R., Druga, A., Falke, H., Faqi, A., Hansen, E., Heinrich-Hirsch, B., Helleig, J., Lingk, W., Parkinson,

M., Paumgartten, F.J.R., Pefil, R., Platzek, T., Scialli, A.R., Seed, J., Stahlmann, R., Ulbrich, B., Wu, X., Yasuda, M., Younes, M., Solecki, R., 1999. Classification terms in developmental toxicology: need for harmonization. Report of the second workshop on the terminology in developmental toxicology Berlin, 27–28 August 1998. Reproductive Toxicology 13, 77–82.

Dawson, A.B., 1926. A note on the staining of the skeleton of cleared specimens with arizarin red-S. Stain Technology 1, 123–124.

Dunnett, C.W., 1955. A multiple comparison procedure for comparing several treatments with control. Journal of American Statistical Association 50, 1096–1121.

Horimoto, M., Ariyuki, F., Daidohji, S., Fujii, T., Fukunishi, K., Hanada, S., Ikegami, S., Ishii, H., Inoue, T., Iwase, T., Matsuura, M., Matsuzawa, T., Nishi, N., Ohkubo, Y., Sanbuissho, A., Sekiya, K., Tani, M., Taniguchi, H., Yokomoto, Y., Yoshida, J., Takahashi, M., Yasuda, M., 1998. Terminology of developmental abnormalities in common laboratory mammals (Japanese version 1). Congenital Anomalies 38, 153–237 (Japanese).

IPCS/WHO (International Programme on Chemical Safety/World Health Organization), 1987. Environmental Health Criteria 65. Butanols: Four Isomers: 1-Butanol, 2-Butanol, *tert*-Butanol, Isobutanol. WHO, Geneva.

Kameyama, Y., 1985. Comparative developmental pathology of the central nervous system. In: Marois, M. (Ed.), Prevention of Physical and Mental Congenital Defects. Part A: The Scope of the Problem. Alan R. Liss, New York.

Kameyama, Y., Tanimura, T., Yasuda, M. (Eds.), 1980. Spontaneous malformations in laboratory animals-photographic atlas and reference data. Congenital Anomalies 20, 25–106 (Japanese).

Kimmel, C.A., Wilson, G.J., 1973. Skeletal deviations in rats: Malformations or variations? Teratology 8, 309–316.

MHW, Japan (Ministry of Health and Welfare, Japan), 1997a. Guidelines for Toxicity Studies of Drugs.

MHW, Japan (Ministry of Health and Welfare, Japan), 1997b. The GLP Standards for Non-clinical Safety Studies on Drugs, MHW Ordinance no. 21.

Morita, H., Ariyuki, F., Inomata, N., Nishimura, K., Hasegawa, Y., Miyamoto, M., Watanabe, T., 1987. Spontaneous malformations in laboratory animals: frequency of external, internal and skeletal malformations in rats, rabbits and mice. Congenital Anomalies 27, 147–206.

Nakatsuka, T., Horimoto, M., Ito, M., Matsubara, Y., Akaike, M., Ariyuki, F., 1997. Japan Pharmaceutical Manufacturers Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Congenital Anomalies 37, 47–138.

Nelson, B.K., Brightwell, W.S., Khan, A., Burg, J.R., Goad, P.T., 1989a. Lack of selective developmental toxicity of three butanol isomers administered by inhalation to rats. Fundamental and Applied Toxicology 12, 469–479.

Nelson, B.K., Brightwell, W.S., Robertson, S.K., Khan, A., Krieg Jr., E.F., Massari, V.J., Burg, 1989b. Behavioral teratology investigation of 1-butanol in rats. Neurotoxicology and Teratology 11, 313–315.

Nishimura, K., 1974. A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. Congenital Amomalies 14, 23–40 (Japanese).

Sitarek, K., 1999. Maternal and fetal toxicity of *N*-methylmorpholine by oral administration in rats. Teratogenesis, Carcinogenesis, and Mutagenesis 19, 369–376.

Sitarek, K., 2001. Embryolethal and teratogenic effects of carbendazim in rats. Teratogenesis, Carcinogenesis, and Mutagenesis 21, 335–340.

Sitarek, K., Berlinska, B., Baranski, B., 1994. Assessment of the effect of *n*-butanol given to female rats in drinking water on fertility and prenatal development of their offspring. International Journal of Occupational Medicine and Environmental Health 7, 365–370.

Sitarek, K., Berlinska, B., Baranski, B., 1996. Effect of oral Sulfenamide TS administration on prenatal development in rats. Teratogenesis Carcinogenesis and Mutagenesis 16, 1–6.

Sitarek, K., Sapota, A., 2003. Maternal-fetal distribution and prenatal toxicity of 2,2,4-trimethyl-1,2-dihydroquinoline in the rat. Birth Defects Research, Part B 68, 375–382.

Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods, seventh ed. Iowa State University Press.

Solecki, R., Bergmann, B., Bürgin, H., Buschmann, J., Clark, R., Druga, A., Van Duijnhoven, E.A.J., Duverger, M., Edwards, J., Freudenberger, H., Guittin, P., Hakaite, P., Heinrich-Hirsch, B., Hellwig, J., Hofmann, T., Hübel, U., Khalil, S., Klaus, A., Kudicke, S., Lingk W., Meredith, T., Moxon, M., Müller, S., Paul, M., Paumgartten, F., Röhrdanz, E., Pfeil, R., Rauch-Ernst, M., Seed, J., Spezia, F., Vickers, C., Woelffel, B., Chahoud, I., 2003. Harmonization of rat fetal external and visceral terminology and classification: Report of the Fourth Workshop on the Terminology in Developmental Toxicology, Berlin, 18–20 April 2002. Reproductive Toxicology, 17, 625–637.

Wilson, J.G., 1973. Methods for administering agents and detecting malformations in experimental animals. In: Wilson, J.G., Warkany, J. (Eds.), Teratology: Principles and Techniques. The University of Chicago Press, Chicago, pp. 262–277.

Wood, D.C., Hoar, R.M., 1972. Apparently hydronephrosis as a normal aspect of renal development in late gestation of rats: the effect of methyl salicylate. Teratology 6, 191–196.

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT•

Reproductive Toxicology 20 (2005) 21–30

Reproductive Toxicology

www.elsevier.com/locate/reprotox

In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) affects tooth development in rhesus monkeys

Iku Yasuda [a], Mineo Yasuda [b,*], Hiroshi Sumida [c], Hideshi Tsusaki [d], Akihiro Arima [d], Toshio Ihara [d], Shunichiro Kubota [e], Kazuo Asaoka [f], Kazuhiro Tsuga [a], Yasumasa Akagawa [a]

[a] *Department of Advanced Prosthodontics, Division of Cervico-Gnathostomatology, Programs for Applied Biomedicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8553, Japan*
[b] *Department of Clinical Engineering, Faculty of Health Sciences, Hiroshima International University, Hiroshima 724-0695, Japan*
[c] *Department of Clinical Radiology, Faculty of Health Sciences, Hiroshima International University, Hiroshima 724-0695, Japan*
[d] *Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories Ltd., Kagoshima 891-1394, Japan*
[e] *Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan*
[f] *Primate Research Institute, Kyoto University, Aichi 484-8506, Japan*

Received 26 October 2004; received in revised form 3 December 2004; accepted 20 December 2004
Available online 18 March 2005

Abstract

We thought to validate the current tolerable daily intake (TDI) value for dioxin (4 pg/kg) in Japan. Pregnant rhesus monkeys received an initial dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 0, 30, or 300 ng/kg subcutaneously) on day 20 of gestation; the dams received additional injection of 5% of the initial dose every 30 days until day 90 after delivery. The teeth of stillborn, postnatally dead, and surviving offspring (now approximately 4 years old) were evaluated. None of the offspring in the 0 and 30 ng/kg groups ($n = 17$ and 15, respectively) had tooth abnormalities, whereas 10 of 17 in the 300 ng/kg had them. These findings suggest the lowest-observed adverse-effect level (LOAEL) for TCDD in the rhesus monkey is between 30 and 300 ng/kg, and probably is close to that for rodents (86 ng/kg) on which the current TDI was based. It is reasonable to conclude that the current TDI needs no immediate modification.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Dioxin; TCDD; Tooth; Rhesus monkey; Primate; TDI; Developmental toxicity; LOAEL

1. Introduction

Dioxins are ubiquitous environmental pollutants. Although contamination levels are decreasing [1], the adverse effects of dioxins, especially their reproductive and developmental toxicities, still attract much public concern, and regulatory agencies worldwide are seeking to define a reasonable permissible intake level. In Japan, the current tolerable daily intake (TDI) of dioxin and dioxin-related compounds has been set at 4 pg toxic equivalent (TEQ)/kg/day [2]. This value was calculated from the lowest-observed adverse-effect level (LOAEL) in experimental animals, mostly rodents. A single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring [3]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI of 4 pg/kg was established. However, great differences between the biological half-life of TCDD in humans and rodents have called into question the validity of this calculation. To obtain a more reliable LOAEL for dioxins, in 1999 we initiated a long-term developmental toxicity study in rhesus monkeys.

* Corresponding author. Tel.: +81 829 36 0224; fax: +81 829 36 0224.
E-mail address: mineoyas@ck9.so-net.ne.jp (M. Yasuda).

0890-6238/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.reprotox.2004.12.016

In rodents, the teeth are known targets of the developmental toxicity of dioxin; in utero and lactational TCDD exposure affects incisor and molar development in rats [4]. Tooth abnormalities also occurred among human populations accidentally exposed to dioxins [5] or polychlorinated biphenyls (PCBs) and polychlormated dibenzofurans (PCDF) [6,7]. During our monkey experiment, some offspring were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate offspring exposed to TCDD in utero and while nursing. Macroscopic observation revealed tooth abnormalities in the offspring from mothers exposed to a relatively high dose of TCDD (300 ng/kg on day 20 of gestation and 15 ng/kg every 30 days during pregnancy) [8]. This finding prompted us to examine surviving offspring radiographically, and we found that tooth abnormalities occurred at a high frequency in the high-dose group. These offspring are still alive and growing, and various studies are in progress. This report describes the dental findings obtained as of April 2004.

2. Materials and methods

2.1. Animals

Colony bred adult female rhesus monkeys (age, 3–10 years; weight, 4–7 kg) were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). Details of breeding conditions are given elsewhere [9]. Briefly, the animals were housed in stainless-steel cages (68 cm × 70 cm × 77 cm), and received approximately 144 g of solid diet (Harlan Tekland, Harlan Sprague Dawley Inc., Indianapolis, IN, USA) daily. The rooms were maintained at 26 ± 2 °C and 50 ± 10% relative humidity and on a 12-h light cycle (lights on, 06:00–18:00 h). Female monkeys were allowed to cohabit with males on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period (day 13 of the menstrual cycle) was designated as day 0 of gestation (GD 0). On GD 18 or 19, pregnancy was confirmed by ultrasonography (SSD-2000, Aloka Co. Ltd., Tokyo, Japan) of animals anesthetized by an intramuscular injection of 5% ketamine hydrochloride (5–10 mg/kg, Sigma–Aldrich Corporation, St. Louis, MO, USA). Pregnant monkeys were divided into three groups, each consisting of approximately 20 animals. During gestation, all dams were observed for general condition at least once daily and they were weighed once every 20 days.

The dams were allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PND 0). Delivered offspring were examined macroscopically, and allowed to cohabit with their mothers for approximately 1 year. The offspring were weighed once every 10 days until PND 90, once every 20 days until PND 150, and once every 30 days thereafter. The animals were reared in the monkey facility of Shin Nippon Biomedical Laboratories Ltd. (SNBL, Kagoshima, Japan) and were treated humanely according to the guidelines of animal experiments for SNBL. Animal excreta and carcasses were handled with extreme care, and all waste was burned in an incinerator equipped with an afterburner held at >800 °C.

2.2. Chemicals and administration

TCDD (lot number 110899, purity >98% as determined by gas chromatography, Wellington Laboratories Inc., Guelph, Ontario, Canada) was dissolved in a mixture of toluene/dimethylsulfoxide (DMSO; 1:2, v/v) at a concentration of 300 ng/ml. The solution was prepared by Kanto Kagaku Co. Ltd. (Tokyo, Japan) and final concentrations were confirmed by gas chromatography. Confirmed pregnant female monkeys received TCDD subcutaneously into the back region on GD 20 at an initial dose of 30 or 300 ng/kg. This route was selected to avoid uncertainty of absorption by oral administration. The dosing volume was 0.1 ml/kg for the lower-dose group and 1 ml/kg for the higher-dose group. Controls received the vehicle in a volume of 1 ml/kg. To maintain the desired body burden, dams received 5% of the initial dose (i.e., 1.5 or 15 ng/kg) every 30 days during pregnancy and lactation until PND 90. For the maintenance dosing, a TCDD solution at a concentration of 30 ng/ml was prepared, and animals in the lower-dose group received 0.05 ml/kg in each injection whereas those in the higher-dose group received 0.5 ml/kg. The total dose administered to the higher-dose group was 405 ng/kg (300 + 15 × 7 for dams with gestation length less than 170 days) or 420 ng/kg (300 + 15 × 8 for dams with gestation length 170 days or more) and that to the lower-dose group was 40.5 or 42 ng/kg. The lower-dose level was set at about one-third of the LOAEL body burden in rodents (86 ng/kg) and the higher one at about three times the LOAEL. The maintenance-dosing schedule was set according to the assumption that the biological half-life of TCDD in rhesus monkeys is approximately 1 year [10].

2.3. Macroscopic observation

Stillborn fetuses and offspring that died by PND 100 were necropsied, and the upper and lower jaws were dissected for detailed observation. Macroscopic observation was made under a dissecting microscope (SZX12, Olympus Corporation, Tokyo, Japan). Photographs were taken using a digital camera (C-4040, Olympus). Surviving offspring were anesthetized by intramuscular injection of ketamine at 10 mg/kg into the thigh before intraoral examination, and photographs were taken using an intraoral digital camera (Crystal Cam II, GC Co. Ltd., Tokyo, Japan).

2.4. Radiographic observation

Conventional intraoral radiographs were taken using a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co.

Table 1
Pregnancy outcome and postnatal mortality

Dose of TCDD	No. of dams	No. of abortions	No. of stillbirths	No. of live births	No. of early postnatal deaths[a]	Gestation length (days)	Birth weight (g)
Control	23	2	3	18	1	161.8 ± 7.8	426.1 ± 58.6
30 ng/kg	20	0	5	15	0	163.8 ± 5.9	426.8 ± 56.9
300 ng/kg	20	1	3	16	2	164.9 ± 9.7	402.7 ± 62.1
300 ng/kg[b]	9	5	1	3	0	165.0 ± 3.0	466.0 ± 87.1

[a] Death by PND 100.
[b] Additional group.

Ltd., Kyoto, Japan) with a charge-coupled device (CCD; Gendex Visualix, Dentsply International Inc., York, PA).

2.5. *Statistical analysis*

All the data were analyzed using JMP5.1.1J (SAS Institute Japan, Tokyo, Japan). Analysis of variance was used to compare measurement data, such as length of gestation and body weight. The incidence of tooth abnormalities was compared by using Fischer's exact probability test. A statistically significant difference was confirmed at $P < 0.05$.

3. Results

3.1. *Pregnancy outcomes*

TCDD administration apparently had no effect on maternal health. Pregnancy outcomes are summarized in Table 1. Abortions, stillbirths, and early postnatal deaths occurred at fairly high frequencies in the TCDD-treated groups as well as the control group. The prenatal and early postnatal mortality rate of the offspring was higher in the 300 ng/kg group (41%) than in the control group (26%), but the difference was not statistically significant ($P > 0.1$). In an attempt to increase the number of surviving offspring in the 300 ng/kg group, we added nine dams to the group approximately 2 years after the initiation of the experiment. However, only two surviving offspring were added due to a high incidence of abortions. There were no significant differences in the average length of gestation and average birth weight among the three groups.

3.2. *Dental findings*

3.2.1. *Dentition in normal rhesus monkeys*

The number and types of teeth of the rhesus monkey are similar to those of humans. The number of deciduous and permanent teeth is 20 and 32, respectively. Fig. 1 illustrates outlines of these teeth and the code for designation of each tooth used in Tables 2 and 3. Neonatal monkeys usually have no erupted teeth. The central incisors erupt during the first postnatal month. The approximate ages of eruption of deciduous teeth are 0.5 month for the central incisors, 1 month for the lateral incisors, 2 months for the canines, 2.5 months for the first molars, 5 months for the second molars in the lower jaw, and 7 months for the second molars in the upper jaw. Those of permanent teeth are 2.5 years for the central incisors, 2.7 years for the lateral incisors, 3.5 years for the canines and the first premolars, 2.7 years for the second premolars, 1.5 years for the first molars, 3.5 years for the second molars, and 5.5 years for the third molars [11]. During this study, we found that there were fairly large inter-individual variations for the age of eruption of teeth.

3.2.2. *Dental findings in stillborn offspring and those that died postnatally*

The incidences of tooth abnormalities are given in Table 4. During the early stage of this study, some carcasses from stillbirths and postnatal deaths were discarded inadvertently; therefore, the numbers of specimens in Table 4 are smaller than the total numbers of stillbirths and live births in Table 1. Stillborn fetuses from the control group had no erupted teeth in either the upper or lower jaw (Fig. 2A and E). However, conventional radiographs clearly revealed the presence of 20

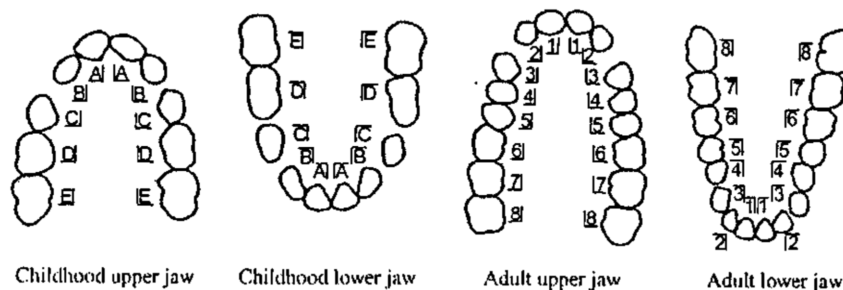

Fig. 1. Diagram illustrating outlines of rhesus teeth seen from the occlusal plane and the code for designation used in Tables 2 and 3. (A–E) Deciduous teeth: (A) central incisor, (B) lateral incisor, (C) canine, (D) first molar, (E) second molar. (1–8) Permanent teeth: (1) central incisor, (2) lateral incisor, (3) canine, (4) first premolar, (5) second premolar, (6) first molar, (7) second molar, (8) third molar. (⌟) Upper right, (⌞) upper left, (⌝) lower right, (⌜) lower left.

Table 2
Tooth abnormalities detected in stillbirths and early postnatal deaths in the 300 ng/kg group

Offspring no.	Sex	Death categories	Age[a]	Abnormal findings
34[b]	♂	Abortion	GD 128	–
37[c]	♂	Stillbirth	GD 164	$\underline{A\vert AD}$ $\overline{A\vert A}$ precocious eruption, dysplasia $\underline{\vert B}$ missing
40[c]	♀	Early postnatal death	PND 26	$\underline{\vert D}$ precocious eruption, incomplete calcification $\underline{A\vert A}$ missing
43[d]	♂	Stillbirth	GD 176	–
57[d]	♂	Early postnatal death	PND 1	$\underline{BA\vert ABD}$ $\overline{A\vert A}$ precocious eruption, incomplete calcification
103[d]	♀	Stillbirth	GD 173	–

(–) No abnormalities were detected.
[a] GD: gestation days; PND: postnatal days.
[b] Total dose of TCDD administered to the dams: 345 ng/kg.
[c] Total dose of TCDD administered to the dams: 360 ng/kg.
[d] Total dose of TCDD administered to the dams: 375 ng/kg.

Table 3
Tooth abnormalities detected in surviving offspring in the 300 ng/kg group

Offspring no.	Sex	Age[a] at observation	Abnormal findings
31[b]	♀	941, 1041, 1122, 1430	$\underline{542\vert 24}$ missing, $\underline{\vert 5}$ cone-shaped, $\overline{5\vert}$ maldirected
33[c]	♂	960, 1060, 1449	–
35[c]	♀	936, 1036, 1425	–
39[b]	♂	921, 1021, 1102, 1410	$\underline{542\vert 245}$ missing
42[c]	♀	926, 1026, 1415	$\underline{5\vert 5}$ missing, $\underline{\vert 4}$ cone-shaped
44[c]	♂	926, 1026, 1415	$\overline{54\vert 45}$ maldirected
60[c]	♂	899, 999, 1080, 1388	$\underline{542\vert 245}$ $\overline{5\vert 5}$ missing
66[b]	♂	849, 949, 1030, 1338	$\underline{52\vert 2}$ $\overline{1\vert 1}$ missing, $\underline{\vert 45}$ cone-shaped, maldirected, $\overline{54\vert 45}$ maldirected
102[c]	♂	177, 278	–
106[c]	♀	198, 299, 380, 688	$\underline{A\vert A}$ $\underline{4\vert 24}$ missing
109[c]	♀	189, 290, 679	–

(–) No abnormalities were detected.
[a] Postnatal days.
[b] Total dose of TCDD administered to the dams: 420 ng/kg.
[c] Total dose of TCDD administered to the dams: 405 ng/kg.

well-formed deciduous teeth (Fig. 3A and E). Each tooth could be identified by its characteristic shape and size.

Dental examination of the dead offspring revealed tooth abnormalities only in the 300 ng/kg group. Three of the five animals had tooth abnormalities such as precocious eruption, dysplasia, incomplete calcification, and missing teeth. Although the incidence of tooth abnormalities in the 300 ng/kg group was high (60%), it did not differ significantly from the control incidence (0%; $P > 0.1$), perhaps because of the small sample size. Descriptions of offspring with tooth abnormalities follow, and representative macroscopic photographs and conventional radiographs are shown in Figs. 2 and 3, respectively. Abnormal findings are summarized in Table 2.

Offspring No. 37 was stillborn on GD 164. The deciduous upper central incisors and left first molar had erupted precociously (Fig. 2B). The erupted teeth were irregular in shapes and apparently were destroyed. X-ray examination revealed incomplete calcification in the erupted teeth, and the deciduous upper left lateral incisor was missing (Fig. 3B). The deciduous lower central incisors also had erupted precociously; these teeth were dark brown (Fig. 2F) and their

Table 4
Incidence of tooth abnormalities

Group	Stillbirths and early postnatal deaths			Surviving offspring		
	No. of offspring	No. of offspring with tooth abnormalities (%)		No. of offspring	No. of offspring with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	13	0	(0)
300 ng/kg	5	3	(60)	8	6	(75)[b]
300 ng/kg[a]	1	0	(0)	3	1	(33)

[a] Additional group.
[b] Significantly different from the control group ($P < 0.01$).

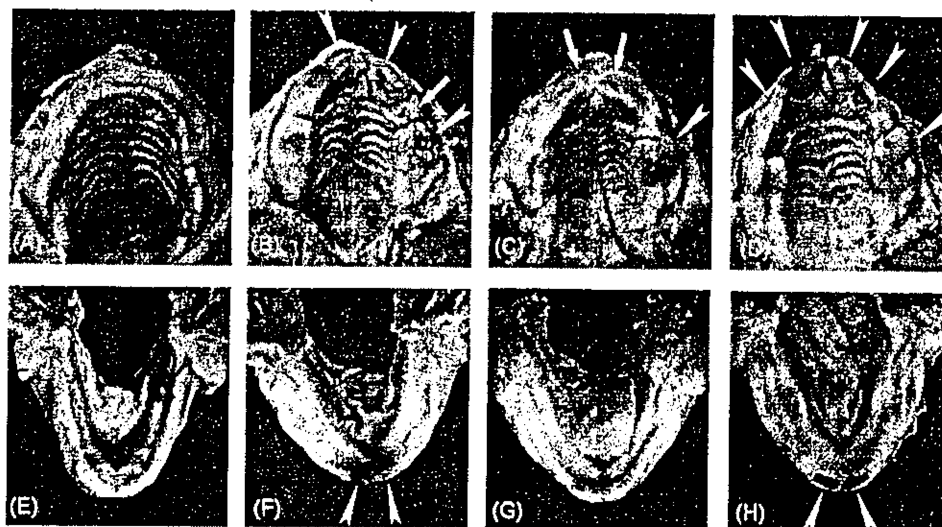

Fig. 2. Macroscopic photographs of jaws from a control offspring (A and E) and from offspring in the 300 ng/kg group with tooth abnormalities (B–D, F–H), which were stillborn or died early postnatally. Upper jaws (A–D) and lower jaws (E–H). Arrowheads indicate precocious eruption. Arrows point the location of missing teeth detected by X-ray. Offspring numbers and ages: (A and E) No. 10, GD 146; (B and F) No. 37, GD 164; (C and G) No. 40, PND 26; (D and H) No. 57, PND 1.

calcification seemed slightly retarded (Fig. 3F) as compared with that of a control animal stillborn at an earlier gestational age (Fig. 3E, No. 10: stillborn on GD 146).

Offspring No. 40 died postnatally on PND 26. The deciduous upper left first molar had erupted precociously (Fig. 2C). The four cusps were discernible macroscopically but were unclear in the radiograph (Fig. 3C), indicating retarded calcification of the tooth. X-ray examination revealed that both the deciduous upper central incisors were missing. A slight deviation of the anterior nasal septum to the left was noted (Fig. 3C). The lower teeth were still in the gum, and no abnormality was detected radiographically (Fig. 3G).

Offspring No. 57 died when a neonate, on PND 1. The bilateral deciduous upper central and lateral incisors, upper left first molar (Fig. 2D), and bilateral lower central incisors (Fig. 2H) had erupted precociously. The lower incisors were dark brown. X-ray examination revealed retarded calcification in these precociously erupted teeth (Fig. 3D and H).

3.2.3. Dental findings in surviving offspring

The incidences of tooth abnormalities in the surviving offspring are given in Table 4. The incidence in the 300 ng/kg group (total, 70%) was significantly higher than that in the control group (0%; $P<0.01$). Representative macroscopic

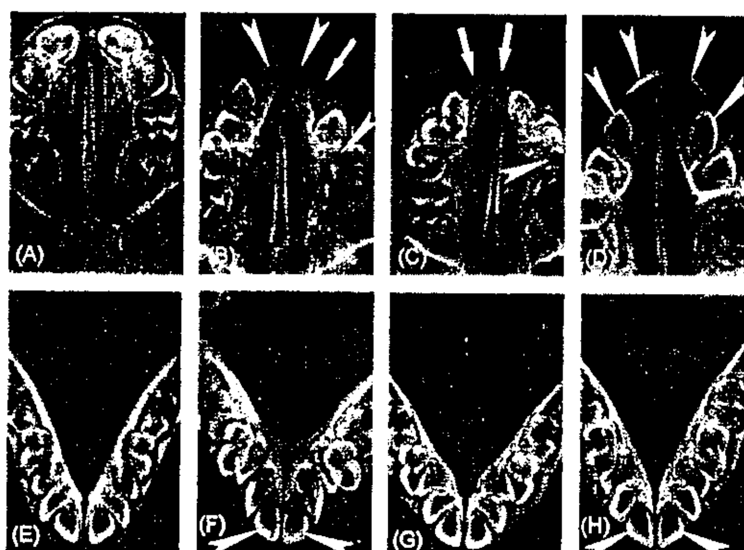

Fig. 3. Conventional radiographs of jaws shown in Fig. 2. Radiographs are arranged corresponding to Fig. 2. Upper jaws (A–D) and lower jaws (E–H). Arrowheads and arrows indicate precocious eruption and missing teeth, respectively. Offspring numbers: (A and E) No. 10; (B and F) No. 37; (C and G) No. 40; (D and H) No. 57.

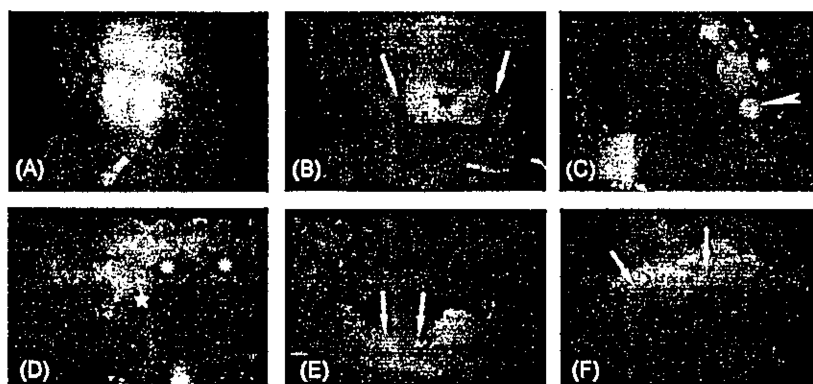

Fig. 4. Macroscopic photographs of surviving offspring in the control group (A) and 300 ng/kg group with tooth abnormalities (B–F). Arrows: missing; arrowhead: cone-shaped; star: maldirected; asterisks: remaining deciduous teeth. Offspring numbers and ages: (A) No. 1, PND 1438; (B and C) No. 31, PND 1430; (D) No. 44, PND 1415; (E) No. 66; PND 1338; (F) No. 106, PND 688. (A) Upper and lower incisors; (B) upper incisors; (C) upper left molars; (D) lower right molars; (E) lower incisors; (F) upper incisors.

and radiographic photographs are shown in Figs. 4 and 5, respectively. Abnormal findings are summarized in Table 3.

3.2.3.1. Offspring observed between approximately PND 800 and PND 1400. In the vehicle-treated group, offspring were at the stage of losing the deciduous teeth during the period of PND 800–PND 1400. In the majority of animals, the permanent central and lateral incisors and the first molars had erupted. By conventional radiography, all the permanent teeth except for the third molars were detectable. Descriptions of a control animal and the monkeys from the 300 ng/kg group with tooth abnormalities follow.

Offspring No. 1 is a control animal. Fig. 4A shows the central upper and lower jaws on PND 1438. The permanent central incisors had erupted. Fig. 5A is a radiograph of the anterior upper jaw taken on PND 1049. The midline is approximated by the left border of the picture. The deciduous central incisor had been lost and the permanent central incisor had erupted. The deciduous lateral incisor still remained but was being pushed up by the growing permanent incisor. The permanent canine was discernible deep to the long root of the deciduous canine on the distal side of the root of the permanent lateral incisor. The canine could be easily identified by the pointed shape of the crown. Fig. 5B shows the upper left molars radiographed on PND 1438. The deciduous first molar had been lost and the first premolar had erupted. The crown of the deciduous second molar remained posterior to the permanent first premolar, and it was being pushed up by the perma-

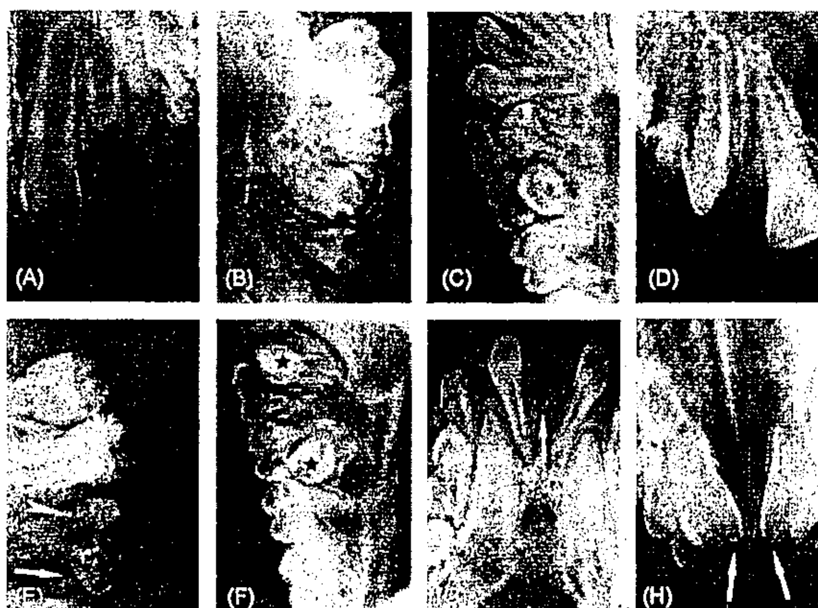

Fig. 5. Conventional radiographs of surviving offspring in the control group (A–C) and 300 ng/kg group with tooth abnormalities (D–H). Arrows: missing; arrowhead: cone-shaped; stars: maldirected. Offspring numbers and ages: (A) No. 1, PND 1049; (B and C) No. 1, PND 1438; (D and E) No. 31, PND 1430; (F) No. 44, PND 1415; (G) No. 66, PND 1338; (H) No. 106, PND 688. (A) Upper left incisors and canine; (B) upper left molars; (C) lower right molars; (D) upper right incisor and canine; (E) upper left molars; (F) lower left molars; (G) lower incisors; (H) upper incisors.

nent second premolar. The permanent first and second molars had erupted, but the third molar could not be seen clearly. Fig. 5C shows the lower right lateral incisor, canine, and molars on PND 1438. The lateral incisor and canine were permanent teeth, but the deciduous first and second molars still remained, being pushed up by the growing permanent first and second premolars. The permanent first molar had erupted.

Offspring No. 31–66 are members of the 300 ng/kg group.

Offspring No. 31 was observed macroscopically on PND 1430. It was found that both the upper permanent lateral incisors were missing (Fig. 4B, arrows). The upper left second premolar had erupted but its crown was small and cone-shaped (Fig. 4C, arrowhead). These defects were confirmed by radiography (Fig. 5D, arrow; Fig. 5E arrowhead). Radiographs taken on PND 1430 showed that the upper left deciduous first molar remained, and no permanent first premolar was found between the roots of the deciduous first molar (Fig. 5E, arrow); therefore, a missing permanent first premolar was diagnosed. Similar findings from the upper right side led to diagnosis of missing first and second premolars on this side. In the lower jaw, the right second premolar had erupted, but its crown was on the lingual side of the alveolar gum. Radiographically, the axis of the second premolar was inclined in a lingual and distal direction.

Offspring No. 39 was examined radiographically on PND 1410. The upper permanent lateral incisors and the first and second premolars were missing bilaterally.

Offspring No. 42 had an upper left first premolar that was cone-shaped, and the upper second premolars were missing bilaterally according to observation on PND 1415.

Offspring No. 44 had a lower right first premolar that was inclined in a lingual and mesial direction, and it had erupted on the lingual side of the alveolar gum (PND 1415; Fig. 4D, star). Because of this maldirection, the deciduous first molar still remained. Similarly, the lower right second premolar was maldirected, and the deciduous first and second molar also remained (Fig. 4D, asterisks). The lower left first premolar had not erupted, but a radiograph sowed that the first and second premolar were maldirected (Fig. 5F, stars) and the deciduous first and second molars remained.

Offspring No. 60 was evaluated macroscopially and radiographically on PND 1388. These studies indicated that both lateral incisors and both first and second premolars were missing from the upper jaw. In addition, both second premolars were missing from the lower jaw.

Offspring No. 66 had an upper jaw from which the bilateral permanent lateral incisors and the right second premolars were missing, and the left first and second premolars were cone-shaped. In the lower jaw, the permanent central incisors were found to be absent on PND 1338 (Fig. 4E, arrows). The remaining permanent incisors were close to the canines, and there was a wide space between the two incisors (Fig. 5G, arrow), indicating that the incisors were lateral ones. The upper left first and second premolars had been erupted by PND 1338, but were cone-shaped and maldirected. The lower first and second premolars were also maldirected bilaterally.

3.2.3.2. Offspring observed between approximately PND 200 and PND 700. The two surviving offspring that were added to the 300 ng/kg group were approximately PND 200 at the time of their first radiographic examination. They were followed until approximately PND 700. Only one of these animals, *Offspring No. 106*, had obvious tooth abnormalities. This animal had a wide space between the small incisors in the upper jaw; this defect first was observed on PND 198, and was confirmed on PND 688 (Fig. 4F, arrows). Radiographs taken on PND 688 showed the growing permanent central incisors and a wide median gap between the remaining deciduous teeth (Fig. 5H, arrows), indicating that the deciduous central incisors were missing. In addition, the upper bilateral first premolars and the left permanent lateral incisor were missing.

4. Discussion

The results of the present study clearly showed that prenatal and lactational exposure to TCDD with an initial dose of 300 ng/kg and a maintenance dose of 15 ng/kg affected tooth development in rhesus monkeys. The exposure began on GD 20, when the rhesus embryo is at the stage of primitive streak formation, corresponding to Carnegie stage 8 in the human embryo [12], and no tooth germs are present. In humans, the dental lamina, the earliest indication of teeth, appears by the 6th week of development [13]. The human embryo at 6 weeks after fertilization (Carnegie stage 17) corresponds to the rhesus embryo at 5 weeks after fertilization [14]. The human permanent tooth bud first appears around 10 weeks after fertilization, which corresponds to approximately 8 weeks after fertilization in the rhesus.

According to our observations, the 20 deciduous teeth had been well shaped by the time of delivery in the control offspring. Although the last maintenance injection of TCDD was done on PND 90, the offspring was considered to be exposed to TCDD via milk until weaning, approximately 1 year after birth. Even after weaning, TCDD that had accumulated in the various tissues gradually was released into the blood and could have affected developing permanent teeth. Hence, it is reasonable to assume that deciduous as well as permanent teeth were exposed to TCDD throughout the critical period of development and that the observed tooth abnormalities were associated with TCDD exposure.

Unfortunately, some of the carcasses from the stillbirths and postnatal deaths were discarded, and therefore, unavailable for dental examination. However, all the dental abnormalities we identified were noted after the disposal, and we feel that no bias was introduced into the sampling of the specimens. The incidences of tooth abnormalities among stillbirths and early postnatal deaths did not differ significantly between the control (0%) and 300 ng/kg (60%) groups, probably because of the small sample size. However, the difference among the surviving offspring is statistically significant and we, therefore, reasonably consider that

all the observed tooth abnormalities are due to TCDD exposure.

Developmental studies with TCDD in the rhesus monkey have been performed for more than 25 years and by several groups [15–19]. The main finding in these studies was abortions, and tooth abnormalities were not reported. However, one dioxin-related compounds, 3,4,5,3′,4′,5′-hexachlorinated biphenyl (Aloclor 1242) has been reported to affect tooth development in rhesus monkeys [20]. These animals had cystic periodontal lesions around the unerupted molars 13 months after consumption of food containing 400 ppm Aloclor 1242 for 40 days, suggesting that dioxins might affect developing teeth in primates.

TCDD affects tooth development in rodents. A single oral dose of 1 μg/kg to pregnant rats on GD 15 disturbed postnatal molar development in the offspring [21]. Lactational exposure through maternal intraperitoneal administration of TCDD to rats at a dose level of 1000 μg/kg on PND 1 also affected molar development in the offspring [22,23]. In addition, growing incisors in rats were sensitive to continuous exposure to TCDD for 20 weeks beginning from 10 weeks of age [24].

Human epidemiological studies have been conducted to examine possible association between dioxin exposure and tooth abnormalities. In Finland, 102 6–7-year-old children who were breast-fed for an average of 10.5 months were studied. Milk samples were collected when the children were 4 weeks old, and the concentrations of dioxins and furans were determined. The total exposure to dioxins was calculated from the concentrations in milk and the duration of breast-feeding. The frequency and severity of hypomineralization of teeth correlated with the total exposure [25].

Follow-up studies after accidental exposure to dioxins also have indicated that the teeth are targets of developmental toxicity of these toxicants. High frequencies of delayed eruption and missing permanent teeth occurred among children with fetal Yusho or Yuchen (oil disease), which occurred in 1968 in Japan and in 1979 in Taiwan after ingestion of rice oil, contaminated with PCBs and PCDFs [6,7]. In addition, examination of 48 people exposed to dioxins because of the notorious accident in Seveso, Italy, in 1976 revealed a high incidence of developmental defects of enamel and missing permanent teeth [5]. These subjects had been younger than 9.5 years at the time of the accident and were examined for tooth abnormalities 25 years afterward. Plasma collected in 1976 had TCDD concentrations that ranged from 23 to 26,000 ng/kg in serum lipid. Subjects with higher serum-TCDD levels had developmental dental defects more often than those with lower TCDD levels.

In the present study, we found positional differences among teeth as manifestations of the sensitivity to the developmental toxicity of TCDD. Even before eruption, each tooth can be easily identified in light of the position of the canine, which is large and has a characteristically pointed crown. The canines were not affected in any of our monkeys; the vulnerable teeth were the central and lateral incisors, deciduous first molars, and the first and second premolars. In the patients with Yusho, the most frequent missing tooth was the lower premolar, followed by the lower lateral incisor [6]. In humans, the lateral incisor and the second premolar are considered to have a regressing tendency in the process of evolution [26], and these teeth are missing relatively frequently in the general population. This intrinsic regressive tendency might be exacerbated by exogenous toxicants, resulting in positional differences in sensitivity.

It is well known that interactions between the ectoderm covering the first branchial arch and the mesenchyme derived from the neural crest are important in tooth morphogenesis. Several signal molecules and their receptors have been identified [27]. TCDD is a potent modulator of epithelial cell growth and differentiation [28], and most of its toxic effects are mediated by the aryl hydrocarbon receptor (AhR) [29]. For example cleft palate induction by TCDD was completely abolished in AhR knockout mice [30]. In mouse tooth buds, AhR is expressed in secretory odontoblasts and ameloblasts [23], suggesting the pathway via AhR as a mediator of dental toxicity of TCDD. One candidate for the pathway of TCDD action on tooth morphogenesis involves epidermal growth factor (EGF) and the EGF receptor (EGFR). TCDD added to cultured embryonic mouse mandibular molar tooth germs induced depolarization of ameloblasts and disturbed morphogenesis [31]. EGF added to the TCDD-containing medium suppressed the adverse effects of TCDD. The effect of TCDD was less dramatic on tooth germs from EGFR knockout mice [31]. Although no study has assayed expression of EGF or EGFR during tooth morphogenesis in rhesus embryos, these findings suggest that the EGF–EGFR signaling system may work in tooth development in the rhesus monkey as well as the mouse and that disturbance of this system by TCDD results in dysmorphogenesis of rhesus teeth.

In addition to altered epithelial–mesenchymal interaction, excessive apoptosis may be involved in the pathogenesis of tooth defects. TCDD added to organ culture of mouse molar tooth germs did not affect cell proliferation but increased apoptosis in the epithelium, resulting in defective molar [32]. In the process of cleft palate induction in mice by TCDD, excessive apoptosis was observed in the epithelium covering the palatal processes and in the palatal mesenchyme [33]. It is plausible that apoptosis induced by TCDD played a role in induction of tooth defects in the present experiment. Cleft palate was not detected in the present study. Probably the dose levels were too low to induce cleft palate in the rhesus monkey. In the sensitive C57 BL strain of mice, the LOAEL level for induction of cleft palate was reported to be 3000 ng/kg/day by oral administration during the period of organogenesis [34]. Detailed examinations of possible target organs of developmental toxicity of TCDD including the urinary, reproductive, and immune systems are in progress.

Our examination of the surviving offspring until the age of approximately 4 years revealed tooth defects only in the 300 ng/kg group. By macroscopic observation with the digital

camera, we could not detect mineralization defects reported in humans [5,25]. Because the permanent molars are still developing in 4-year-old rhesus monkeys, detailed further observation may reveal some subtle abnormalities, such as enamel defects in the offspring currently diagnosed as normal in the 30 and 300 ng/kg groups. Blood samples taken from pregnant and lactational mothers and milk samples await analyses for TCDD concentrations. Although the dosing schedule in the present study was set to keep the body burden at 30 or 300 ng/kg, the actual maternal body burden should be assessed after the autopsy of the mothers and analyses for TCDD, because the TCDD half-life in rhesus monkeys has shown fairly large inter-individual variations [10]. Plasma samples taken at intervals from the offspring are also waiting for TCDD analyses. Assuming that the actual body burden was not much different from the programmed one, the LOAEL body burden for the developmental toxicity of TCDD in rhesus monkeys is considered to be somewhere between 30 and 300 ng/kg and is probably on the order of 86 ng/kg, the value used to set the current TDI in Japan. In 2002, a panel of experts surveyed various data in the literature and concluded that no urgent change was necessary in the current TDI of 4 pgTEQ/kg/day [35]. The results of our present study support this conclusion. However, we should wait to draw a definite conclusion until the measurement of the actual body burden of the dams and detailed examinations of various organs of the offspring.

Acknowledgements

This study was supported by Health Labour Sciences Research Grants for Research on Chemical Risk from the Ministry of Health Labour and Welfare of Japan. The authors thank Prof. Keiji Tanimoto, Department of Oral and Maxillofacial Radiology, Graduate School of Biomedical Sciences, Hiroshima University for his valuable suggestions for dental radiography.

References

[1] Hays SM, Aylward LL. Dioxin risks in perspective; past, present, and future. Regul Toxicol Pharmacol 2003;37:170–1.

[2] Environmental Health Committee of the Central Environment Council, Living Environment Council and Food Sanitation Investigation Council. Memorandum on tolerable daily intake (TDI) of dioxin and related compounds (Japan). Tokyo: Environmental Agency and Ministry of Health and Welfare, Japan; 1999.

[3] Gray Jr LE, Ostby JS, Kelce WR. A dose–response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in male Long Evans hooded rat offspring. Toxicol Appl Pharmacol 1997;146:11–20.

[4] Mietinen HM, Alaluusua S, Tuomisto J, Viluksela M. Effect of in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on rat molar development. Toxicol Appl Pharmacol 2002;184:57–66.

[5] Alaluusua S, Calderara P, Gerthoux PM, et al. Developmental dental aberrations after the dioxin accident in Seveso. Environ Health Perspect 2004;112:1313–8.

[6] Fukuyama H, Anan Y, Akamine A, Aono M. Alteration in stomatological findings of patients with Yusho (PCB poisoning) in the general examination. Fukuoka Acta Med 1979;70:187–98.

[7] Lan S-J, Yen Y-Y, Ko Y-C, Chen E-R. Growth and development of permanent teeth germ of transplacental Yu-Cheng babies in Taiwan. Bull Environ Contam Toxicol 1989;42:931–4.

[8] Yasuda I, Yasuda M, Sumida H, et al. Effect of in utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on tooth development in rhesus monkeys. Organohalogen Compd 2003;64:431–4.

[9] Ihara T, Oneda S, Yamamoto T, et al. An embryotoxic/teratogenic potential and abortifacient effect study of interferon alafacon-1 (Infergen®) via subcutaneous administration to rhesus monkeys. Cong Anom 1999;39:223–42.

[10] Bowman RE, Schantz SL, Weerasinghe NCA, Gross ML, Barsotti DA. Chronic dietary intake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at 5 or 25 parts per trillion in the monkey: TCDD kinetics and dose–effect estimate of reproductive toxicity. Chemosphere 1989;18:243–52.

[11] Tajima Y, editor. Biological characteristic data of experimental animals. Tokyo: Soft Science; 1989.

[12] Heuser CH, Streeter GL. Development of the macaque embryo. Contrib Embryol Carneg Inst 1941;29:15–55.

[13] Sadler TW. Langman's medical embryology. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2004.

[14] Gibnau AAM, Geijsberts GM. Developmental stages in the rhesus monkey (*Macaca mulatta*). Adv Anat Embryol Cell Biol 1981;68:1–84.

[15] Allen JR, Barsotti DA, Van Miller JP, Abrahamson LJ, Lalich JJ. Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Food Cosmet Toxicol 1977;15:401–10.

[16] Allen JR, Barsotti DA, Lambrecht LK, Van Miller JP. Reproductive effects of halogeneated aromatic hydrocarbons on nonhuman primates. Ann NY Acad Sci 1979;320:419–25.

[17] Barsotti DA, Abrahamson LJ, Allen JR. Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Bull Environ Contam Toxicol 1979;21:463–9.

[18] Schantz SL, Barsotti DA, Allen JR. Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol Appl Pharmacol 1979;48(Pt 2):A180.

[19] McNulty W-P. Fetotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for rhesus macaques (*Macaca mulatta*). Am J Primatol 1984;6:41–7.

[20] McNulty W-P. Toxicity and fetotoxicity of TCDD, TCDF and PCB isomers in rhesus macaques (*Macaca mulatta*). Environ Health Perspect 1985;60:77–88.

[21] Kattainen H, Tuukkanen J, Simanainen U, et al. In utero/lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure impairs molar tooth development in rats. Toxicol Appl Pharmacol 2001;174:216–24.

[22] Lukinmaa P-J, Sahlberg C, Leppäniemi A, et al. Arrest of rat molar tooth development by lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Appl Pharmacol 2001;173:38–47.

[23] Gao Y, Sahlberg C, Kiukkonen A, et al. Lactational exposure of Han/Wistar rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin interferes with enamel maturation and retards dentin mineralization. J Dent Res 2004;83:139–44.

[24] Kiukkonen A, Viluksela M, Sahlberg C, et al. Response of the incisor teeth to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a dioxin-resistant and a dioxin-sensitive rat strain. Toxicol Sci 2002;69:482–9.

[25] Alaluusua S, Lukinmaa P-L, Koskimies M, et al. Developmental dental defects associated with long breast feeding. Eur J Oral Sci 1996;104:493–7.

[26] Sakai T. Regression of human teeth. J Jpn Assoc Dental Sci 1978;31:978–84.
[27] Thesleff I. Developmental biology and building a tooth. Quintessence Int 2003;34:613–20.
[28] Osborne R, Greenlee WF. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. Toxicol Appl Pharmacol 1985;77:434–43.
[29] Mimura J, Fujii-Kuriyama Y. Regulatory roles of AhR. Environ Sci 2002;9:71–81.
[30] Mimura J, Yamashita K, Nakamura K, et al. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mice lacking the Ah (dioxin) receptor. Genes Cells 1997;2:645–54.
[31] Partanen A-M, Alaluusua S, Miettinen PJ, et al. Epidermal growth factor receptor as a mediator of developmental toxicity of dioxin in mouse embryonic teeth. Lab Invest 1998;78:1473–81.
[32] Partanen A-M, Kiukkonen A, Sahlberg C, et al. Developmental toxicity of dioxin to mouse embryonic teeth in vitro: arrest of tooth morphogenesis involves stimulation of apoptotic program in the dental epithelium. Toxicol Appl Pharmacol 2004;194:24–33.
[33] Takagi TN, Matsui KA, Yamashita K, Ohmori H, Yasuda M. Pathogenesis of cleft palate in mouse embryos exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Teratog Carcinog Mutagen 2000;20:73–86.
[34] Courtney KD, Moore JA. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Appl Pharmacol 1971;20:396–4031.
[35] Working Group for Assessment of Effects of Dioxin on Health. Report of the working group for assessment of effects of dioxin on health. Tokyo: Ministry of Health Labour and Welfare of Japan; 2002.

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

Noriyuki Nakatsu,[1,2] Yoko Yoshida,[1] Kanami Yamazaki,[1] Tomoki Nakamura,[1] Shingo Dan,[1] Yasuhisa Fukui,[3] and Takao Yamori[1]

[1]Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research; [2]Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences; and [3]Laboratory of Biological Chemistry, Department of Applied Biological Chemistry, Faculty of Agricultural and Life Science, University of Tokyo, Tokyo, Japan

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

Received 9/7/04; revised 12/16/04; accepted 1/25/05.

Grant support: Ministry of Education, Culture, Sports, Science and Technology of Japan Aid for Scientific Research on Priority Areas; Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (B) and Exploratory Research; and research grant from the Princess Takamatsu Cancer Research Fund.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Takao Yamori, Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-10-6, Ariake, Koto-ku, Tokyo 135-8550, Japan. Phone: 81-3-3520-0111; Fax: 81-3-3570-0484. E-mail: yamori@ims.u-tokyo.ac.jp

Copyright © 2005 American Association for Cancer Research.

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:

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

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

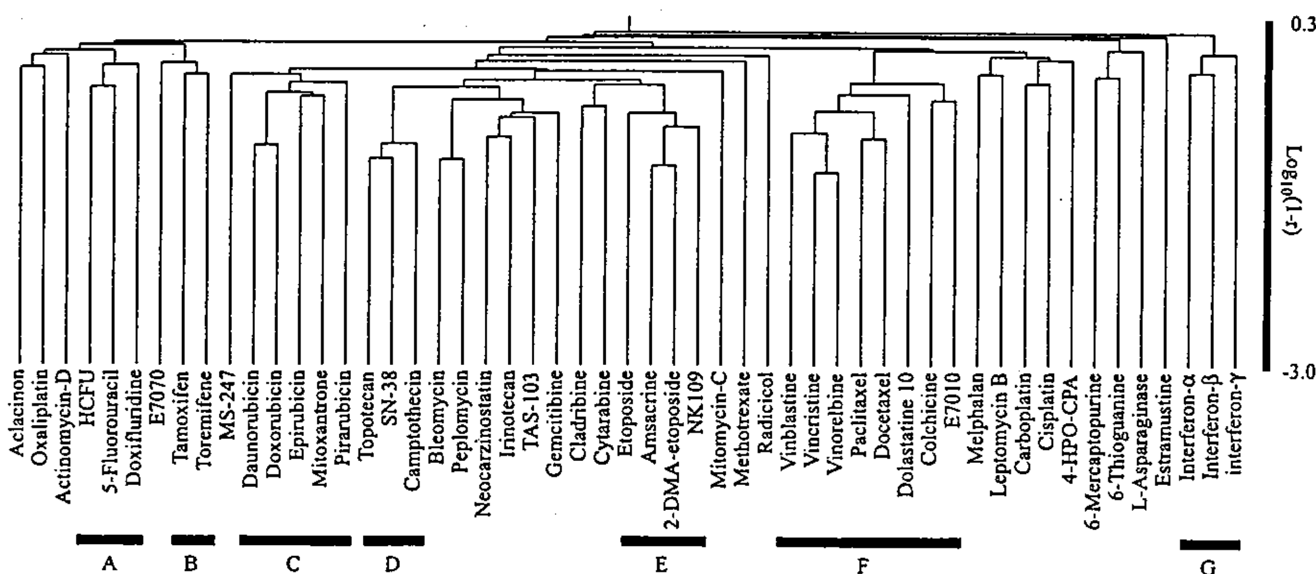

Figure 1. Hierarchical clustering of 53 anticancer drugs based on their activity on 45 human cancer cell lines. Hierarchical clustering method was "average linkage method" using Pearson correlation as distance. Fifty-three drugs were classified into several clusters, each consisting of drugs with similar 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

(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											
		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

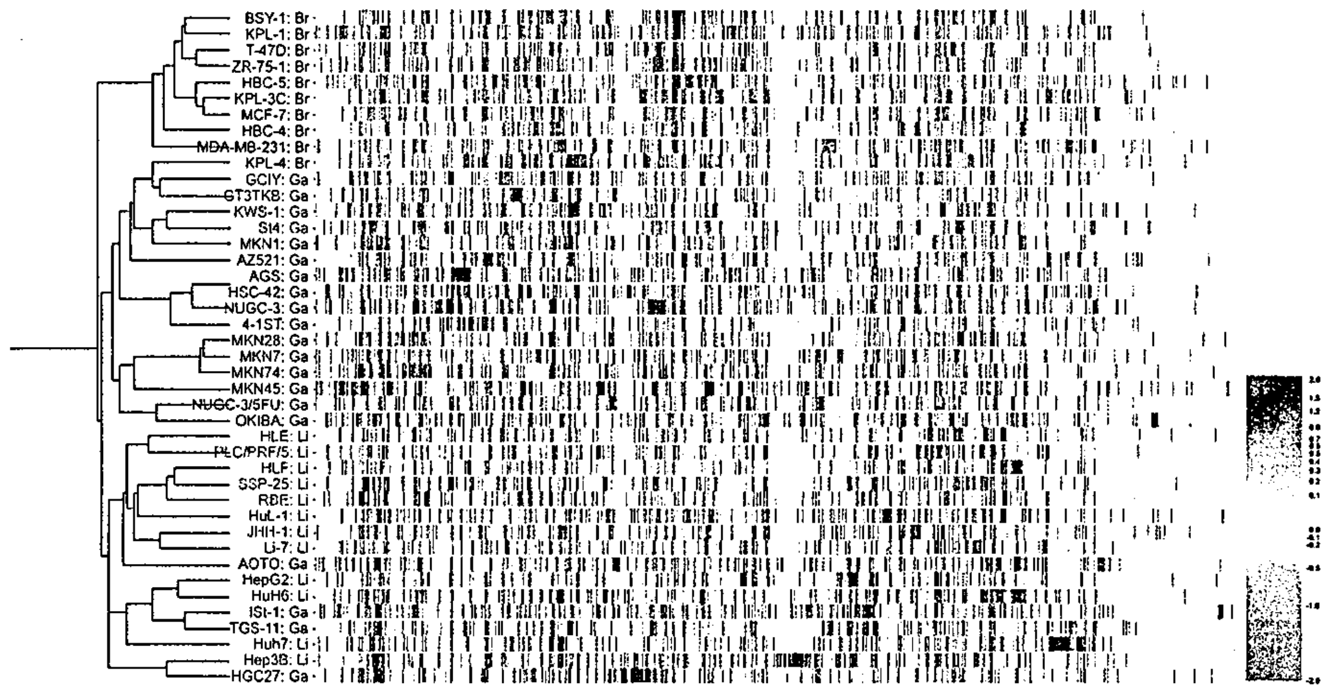

Figure 2. Hierarchical clustering of 42 human cancer cell lines based on their gene expression profiles. Gradient color indicates relative level ($\log_2$ transformed) of gene expression. *Red*, high expression of gene (2.0); *yellow*, normal expression of gene (0.0); *green*, low expression of gene (−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