Jpn. J. Cancer Res. 82, 593–598, May 1991

Establishment of Drug Resistance in Human Gastric and Colon Carcinoma Xenograft Lines

Tetsuya Satta,[1] Ken-ichi Isobe,[2,3] Masaji Yamauchi,[1] Izumi Nakashima,[2] Seiji Akiyama,[1] Katsuki Itou,[1] Tadashi Watanabe[1] and Hiroshi Takagi[1]

[1]*Department of Surgery II and* [2]*Department of Immunology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466*

We established multidrug-resistant human gastric and colon xenograft lines by means of intratumoral injections of four agents, doxorubicin (DXR), cisplatin (CDDP), 5-fluorouracil (5-FU) and mitomycin C (MMC), into subcutaneous SC1NU and SW480 tumors once a week or less. Such intermittent drug exposure is commonly used in clinical chemotherapeutic protocols. All xenograft lines acquired resistance to the injected drugs as evaluated by *in vivo* drug-resistance tests. Many of the drug-resistant lines showed various patterns of cross resistance to other drugs. In order to analyze the mechanism of resistance *in vivo*, we investigated the expression of drug resistance gene, which has been extensively studied *in vitro*. We used four complementary DNAs (cDNAs) for multidrug resistance (MDR1), glutathione *S*-transferase-π (GST-π), thymidylate synthase (TS) and dehydrofolate reductase (DHFR), as probes. We observed GST-π, DHFR and TS mRNA expression at various levels, but MDR1 mRNA expression was found only in SW480/DXR by the method of poly (A^+) RNA selection. Four resistant SW480 lines had higher TS mRNA expressions. Six resistant lines had stronger GST-π mRNA expression. Five resistant lines had higher DHFR mRNA expression. Drug resistance genes related to the treated drug were also expressed in this *in vivo* model; MDR1 in SW480/DXR, GST-π in SW480/CDDP and in SC1NU/CDDP and TS in SW480/5-FU. In contrast to *in vitro* resistant lines which have been reported as models of drug resistance, the expression of drug resistance genes *in vivo* was not always correlated to the acquisition of cross resistance. These resistant xenograft lines and the methods developed to induce drug resistance *in vivo* should be useful for studies on the mechanism of drug resistance in the clinical setting.

Key words: Drug resistance — Xenograft line — GST-π — DHFR — MDR1

The development of resistance to chemotherapy is a major problem in the treatment of cancer. Tumors which are initially responsive to chemotherapy can develop resistance during treatment with cytotoxic agents. Clinically, this is characterized by short periods of remission and failure to respond to subsequent therapy. For many drugs, the mechanism of drug resistance is unknown, and it may depend on the origin of the cells, the degree of resistance, and the method by which resistant clones were selected. A number of investigators have selected sublines of murine and human tumors which were resistant to various drugs *in vitro* by repeated exposure of the target cells to a sublethal concentration of drugs.[1] Many *in vitro* drug resistance mechanisms have been elucidated: multidrug resistance to DXR,[4] actinomycin D and vinca alkaloids,[2-6] GST-π to CDDP[7,8] and DXR,[9,10] DHFR to methotrexate,[11,12] TS to 5-FU,[13-16] and so on. But, there are only a few studies of the development of resistance during *in vivo* treatment.[1,17-22] Repeated treatments at high doses *in vivo* mimic the clinical situation better and may prove more applicable for investigation of the mechanisms of resistance.[1] In the present study, therefore, we have investigated the development of resistance to high doses of DXR, CDDP, 5-FU and MMC in human gastric and colon carcinoma lines, growing as xenografts in nude mice. These four agents are commonly administered to treat progressive gastrointestinal cancers in Japan. We also examined the expression of genes related to drug resistance, and tried to identify the mechanisms of acquisition of *in vivo* drug resistance.

[3] To whom correspondence should be addressed.

[4] Abbreviations used are: DXR, doxorubicin; CDDP, cisplatin; 5-FU, 5-fluorouracil; MMC, mitomycin C; MDR, multidrug resistance; GST-π, glutathione *S*-transferase-π; DHFR, dehydrofolate reductase; TS, thymidylate synthase.

MATERIALS AND METHODS

Nude mice KSN(*nu/nu*) male mice, 5–7 weeks old, were purchased from the Shizuoka Laboratory Animal Center (Shizuoka), and maintained in the Institute of Laboratory Animal Research, Nagoya University. The mice were maintained by conventional methods in a specific pathogen-free environment.

Tumor SC1NU was established directly as a xenograft in KSN(*nu/nu*) mice from the metastatic paragastric

lymph nodes of a 35-year-old female patient in May, 1982 in Nagoya University Hospital. This tumor was diagnosed as poorly differentiated adenocarcinoma with areas of signet-ring cell carcinoma.[23] The human colon carcinoma xenograft line, SW480, was transplanted from the cell line SW480 which was donated by Memorial Sloan Kettering Cancer Center, New York. KB and KB-CH^r-24 were donated by Dr. Kawano, Oita Medical College, Japan. KB-CH^r-24 was maintained in 500 ng/ml of colchicine and showed about 20-fold greater resistance to DXR than the parental KB. CC2NU was established in our University from colon carcinoma in 1988.

Chemicals DXR, 5-FU and MMC were obtained from Kyowa Hakko, Tokyo. CDDP was purchased from Nippon Kayaku, Tokyo.

Development of drug resistance In order to develop drug resistance, drugs were directly injected into the subcutaneous tumor twice a week. The initial dosages were 0.5 mg/kg of DXR, 0.5 mg/kg of CDDP, 5 mg/kg of 5-FU and 0.5 mg/kg of MMC. After three weeks, we injected dosages equal to twice the initial dosages. The passages were performed 6–8 weeks after inoculation. The amounts of agents in the second passage were 4–8 times the initial dosages. Finally, the dosages of drugs after the third passage were increased up to 10 times the initial dosages, and injection was done once a week or less. It took about three months and two passages to reach the maximum dose. We kept the resistant lines *in vivo* by injection of the maximum dose of drugs once a week or less. At nine months after the start of administration at the maximum dose, we started these analyses.

Tumor growth rate The tumor growth was followed by measuring the diameters of tumors three times a week with calipers. The tumor weight was calculated by using the formula $(a^2 \times b)/2$ where b was the largest diameter and a was the diameter perpendicular to b.[1, 17] Growth curves were plotted and the time taken for the tumor to double in volume was obtained. This measurement was done without injection of drugs.

***In vivo* drug resistance test** The tumor (40–60 mg) was transplanted subcutaneously into 4–6 KSN nude mice. An antitumor agent (DXR, CDDP, 5-FU or MMC) was injected directly into the tumor at day 0, 4, and 7 after inoculation of the tumor. The amounts of the injection were 5 mg/kg for DXR, CDDP, MMC and 50 mg/kg for 5-FU. The efficacy trial was done as described below. Tumor weights were calculated by the method described in connection with tumor growth rate. In order to assess antitumor activity, percent growth in drug-treated tumors was calculated by using the formula; (T/C$\times$100), on days 7, 14 and 21 after the inoculation. T and C are median tumor burden for the treatment group and the control group, respectively. We calculated tumor growth delay (T$-$C in days), where T and C are the median times required to reach a predetermined size (1000 mg) for the treatment group and the control group tumors, respectively.[1, 17, 24]

Northern blot analysis Total cellular RNA was extracted from samples which were frozen at -80°C. For MDR1 mRNA detection, poly (A^+) RNA was selected through an oligo (dT) cellulose column. The total RNA (20 μg) and poly (A^+) RNA (10 μg) were electrophoresed on a 3-(N-morpholino)propanesulfonic acid buffer (MOPS) formaldehyde agarose gel and transferred to a Hybond N nylon filter (Amersham International). The detection of MDR1, GST-π, TS and DHFR mRNAs was carried out by northern blot hybridization using cDNA as a probe. A pMDR1 cDNA was donated by Dr. Kuwano, Oita Medical College, a GST-π cDNA was provided by Dr. Muramatsu,[25] Tokyo University, and a TS cDNA was donated by Dr. Seno.[14]

RESULTS

***In vivo* drug resistance test** Fig. 1 shows growth curves of the treated and control groups of SW480 and SW480/CDDP. Control groups showed almost equal growth rates, but the CDDP-treated group of SW480/CDDP grew faster than that of SW480. We obtained the growth curves of all the other resistant lines (data not shown). From these figures we calculated T/C and T$-$C values. Table I shows the calculated values of T/C and T$-$C of parent and resistant lines to the drugs used to

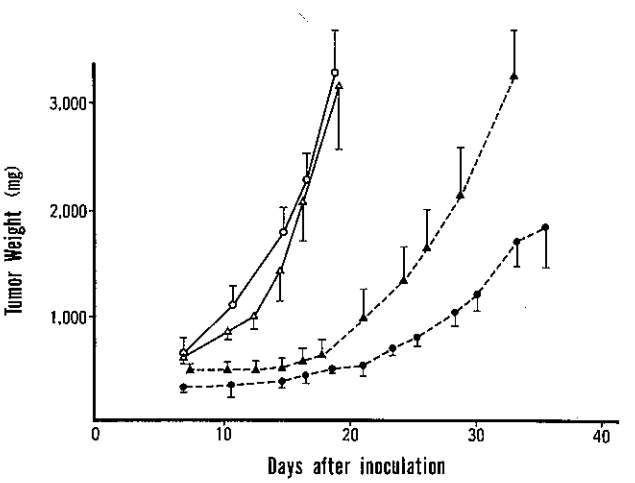

Fig. 1. Tumor growth curve. The tumor growth was measured as described in "Materials and Methods." Drug was injected directly into the subcutaneous tumor on dyas 0, 4, and 7 (about 0.25 ml/mouse). The dosage of CDDP was 0.5 mg/kg. ○; SW480, ●; CDDP-treated SW480, △; SW480/CDDP, ▲; CDDP-treated SW480/CDDP. The bars show the standard deviation.

Table I. *In vivo* Resistance Test of Parents and Resistant Lines

Line	Drug Dosage[a] (mg/kg)	T/C (%)[b] on day			Tumor growth delay (days)[c] (T−C)
		7	14	21	
SW480	DXR	37±5	16±4	9±3	25
SW480/DXR	5	83±15	94±15	53±13	12
SW480	CDDP	46±11	19±10	12±7	15
SW480/CDDP	5	77±12	36±7	23±8	8
SW480	5-FU	51±7	10±2	6±2	25
SW480/5-FU	50	72±6	48±10	25±7	13
SW480	MMC	40±6	7±1.5	1±0.5	ND
SW480/MMC	5	37±7	34±8	5±0.5	15
SC1NU	DXR	13±3	1	0.1	ND
SC1NU/DXR	5	86±14	13±2.1	3±1.5	ND
SC1NU	CDDP	5±1	2±0.5	1±0.5	28
SC1NU/CDDP	5	133±25	15±0.7	6±0.2	26
SC1NU	5-FU	4±1	1	0.3	33
SC1NU/5-FU	50	87±14	29±6	12±3	17
SC1NU	MMC	3±0.5	0.2	0.1	ND
SC1NU/MMC	5	34±1.5	6±2	1±0.5	ND

Mean±SE. ND: not determined.
a) Drug was injected directly into the subcutaneous tumor on days 0, 4, and 7 (about 0.25 ml/mouse). The dosages per injection are listed.
b) T, Median tumor burden for the treatment group; C, median tumor burden for the control group. T/C (%) is percent growth in drug-treated tumor.
c) Tumor growth delay (T−C in days) where T and C are the median times required for the treatment group and the control group tumors, respectively, to reach a predetermined size (1000 mg).

establish resistance. In all resistant lines the T/C value on day 7, 14 or 21 was higher than that of the parental lines with the sole exception of T/C on day 7 in SW480/MMC. All the SC1NU sublines had much higher T/C values than the parental lines. Although some of the SW-480 sublines showed no great difference in T/C values, the growth delays were almost half those of the parental SW480 for DXR, CDDP and 5-FU. From these experiments we confirmed the establishment of resistance in these treated xenograft lines.

Collateral sensitivity SW480/DXR had acquired 6-fold-increased resistance to DXR (T/C value of SW480/DXR divided by that of SW480), 1.3-fold to CDDP and 3-fold to 5-FU. SW480/CDDP had 2- to 3-fold-increased resistance to all drugs. SW480/5-FU had moderate resistance to 5-FU and little to DXR, but it did not show resistance to CDDP and MMC. SW480/MMC had acquired 5-fold-increased resistance to 5-FU and MMC, and 1.5- to 2.5-fold to DXR and CDDP. SC1NU/DXR had 13-fold -increased resistance to DXR, 2- to 4-fold to CDDP and 5-FU, and 10-fold to MMC. SC1NU/CDDP had 7-fold-increased resistance to CDDP, 6-fold to 5-FU and 8-fold to MMC, but showed no increase of resistance to DXR. SC1NU/5-FU had 10- to 30-fold-increased resistance to DXR, CDDP, 5-FU and MMC. SC1NU/MMC had high resistance to all the drugs. Most of the resistant lines had acquired cross resistance to various degrees, with some exceptions (Table II).

Tumor growth rate Although the drug-resistant tumors grew continuously in nude mice, some drug-resistant lines grew more slowly than the original tumors. The drug-resistant lines SW480/CDDP, SW480/5-FU and SW480/MMC had almost the same doubling time (days) as the original SW480 line. However, SW480/DXR and all the drug-resistant lines of SC1NU (SC1NU/DXR, SC1NU/CDDP, SC1NU/5-FU and SC1NU/MMC) grew more slowly than the original SW480 or SC1NU line (Table III).

Northern blot analysis Parental strains (SC1NU and SW480) had no detectable MDR1 mRNA expression. All the resistant xenograft lines had no detectable MDR1 mRNA expression by normal northern blot analysis using total RNA. But poly (A^+) RNA selection enabled us to detect MDR1 mRNA in SW480/DXR. On the other hand, SC1NU and SW480 had moderate levels of GST-π mRNA expression. We found that some drug-resistant xenograft lines (SW480/DXR, SW480/CDDP, SW480/5-FU, SW480/MMC, SC1NU/CDDP and

Table II. *In vivo* Collateral Sensitivity

	T/C[b] (%) on day 14			
	DXR[a]	CDDP[a]	5-FU[a]	MMC[a]
SW480	16±4	19±10	10±2	7±1.5
SW480/DXR	94±15	24±8	28±8	NT
SW480/CDDP	30±5	36±7	29±4	25±5
SW480/5-FU	29±1.5	20±7	48±10	2±1
SW480/MMC	39±10	29±5	46±11	34±8
SC1NU	1	2±0.5	1	0.2
SC1NU/DXR	13±2.1	4±1	4±1.5	2±0.5
SC1NU/CDDP	1±0.2	15±0.7	6.5±3.6	1.6±0.7
SC1NU/5-FU	21±8	21±4	29±6	2±2
SC1NU/MMC	12±4.7	8.6±4.5	30±25	6±2

Mean±SE. NT: not tested.
a) Drug was injected directly into the subcutaneous tumor on days 0, 4, and 7 (about 0.25 ml/mouse). The dosages per injection are listed.
b) T, Median tumor burden for the treatment group; C, median tumor burden for the control group. T/C (%) is percent growth in drug-treated tumor.

Table III. *In vivo* Growth Rate

SW480	4.6±0.14
SW480/DXR	9.3±1.3
SW480/CDDP	5.3±0.5
SW480/5-FU	5.7±0.6
SW480/MMC	5.2±0.46
SC1NU	2.9±0.32
SC1NU/DXR	4.2±0.32
SC1NU/CDDP	4.5±0.27
SC1NU/5-FU	4.3±0.1
SC1NU/MMC	4.7±0.12

Mean±SE. The tumor growth was measured as doubling time (days) by the method described in "Materials and Methods."

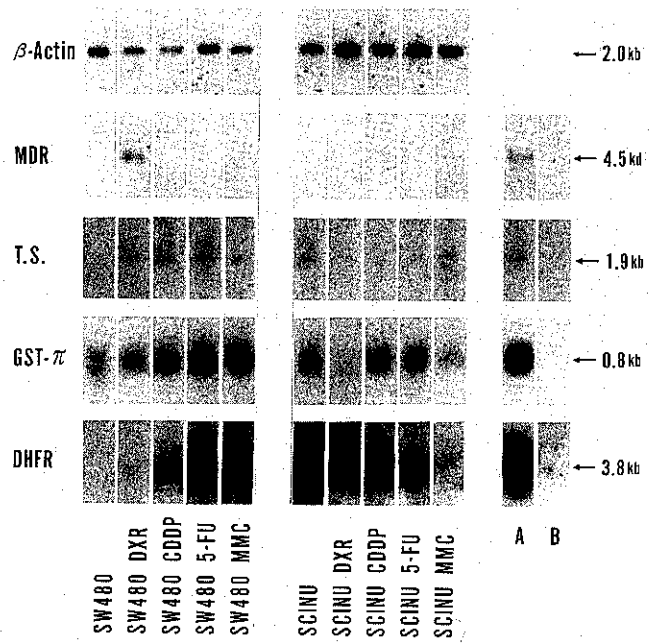

Fig. 2. Northern blot analysis of parents and resistant lines. A: KB-CH^r-24 was used as the positive control of MDR1, being 20-fold more resistant than KB (donated by Dr. Kuwano, Oita Medical College). CC2NU was used as the positive control of TS, GST-π and DHFR. It was established in our University from colon carcinoma in 1988. B: As a negative control of MDR1, TS, GST-π and DHFR, KB was used. It is a renal carcinoma cell line which was donated by Dr. Kuwano, Oita Medical College. For the detection of MDR, poly (A^+) RNA selection was used while total RNA was used, in the cases of TS, GST-π and DHFR.

SC1NU/5-FU) had stronger GST-π mRNA expression than that of the parental lines. But SC1NU/DXR and SC1NU/MMC had weaker GST-π mRNA expression than the parental lines. Although parental SW480 had almost no detectable level of TS mRNA expression, SW480/DXR and SW480/MMC had acquired low-grade TS mRNA expression. SW480/CDDP and SW480/5-FU had higher levels of TS mRNA expression. SC1NU/MMC had the same level of TS mRNA expression as SC1NU. SC1NU/CDDP and SC1NU/5-FU had less TS mRNA expression than SC1NU, while SC1NU/DXR had almost lost the TS mRNA expression. Five sublines (SW480/CDDP, SW480/5-FU, SW480/MMC, SC1NU/DXR, SC1NU/CDDP) had higher DHFR mRNA expression, and three other sublines (SW480/DXR, SC1NU/5-FU and SC1NU/MMC) had decreased DHFR mRNA expression (Fig. 2).

DISCUSSION

In the present study, we investigated the development of resistance to four commonly used antitumor agents (DXR, CDDP, 5-FU and MMC) at high doses in chemosensitive human stomach and colon carcinoma xenograft lines. All four drugs are normally given as single agents or as components of multi-agent therapy regimens to progressive cancer patients. For the establishment of resistance *in vivo*, we first tried intraperitoneal drug administration. But we could not detect any tumor growth difference between the treated and control groups, and the mice died of drug toxicity. Therefore, we employed a direct injection method into subcutaneous tumors at a high drug concentration. By this method, we could obtain drug-resistant lines and the treated mice

all remained alive. We obtained *in vivo* growth curves to calculate the percent growth in drug-treated tumor (T/C) and the tumor growth delay (T−C), as reported by Mattern *et al.*[1,17] We also used the MTT assay,[26,27] directly transferring the tumor cells from *in vivo* to *in vitro* culture. The results showed the same tendencies as the *in vivo* resistance assay (data not shown). But we think the *in vivo* resistance assay is more reliable in this case. The drug-resistant lines displayed different degrees of cross resistance, and even loss of resistance in some sublines. Similar results were reported by Mattern *et al.*[1,17] In order to elucidate the mechanism of this variety of cross resistance, we checked the expression of genes which were thought to be related to drug resistance. MDR1 gene was shown to be expressed in cell lines resistant to DXR, actinomycin-D and vinca alkaloids. In this study we could detect MDR1 mRNA expression only in SW480/DXR but not SC1NU/DXR by northern blot hybridization. We used verapamil and cyclosporin A to reverse the DXR resistance. Cell line SW480/DXR (which was derived from xenograft SW480/DXR, and was about 10-fold more resistant to DXR than parental SW480) reverted to a state sensitive to DXR in the presence of 10 μM verapamil or 0.8 μM cyclosporin A. It had also been reported that the DXR resistance was not induced by MDR1 gene expression alone.[9,28,29]

It has been reported that GST-π elevation indicates increased cellular detoxication potential.[2,7–10,27,30,31] We found that all SW480 resistant lines, SC1NU/CDDP and SC1NU/5-FU, had high levels of GST-π mRNA expression. But we could not find any relationship to the drug resistance. TS plays a central role in DNA biosynthesis, since it represents the sole source of *de novo* thymidine, and the availability of thymidine is rate-limiting in DNA synthesis. TS has been shown to be amplified in response to a variety of chemotherapeutic agents, especially 5-FU.[15–17] All our resistant sublines had acquired 5-FU resistance. TS mRNA expression was amplified in only four SW480 sublines, being correlated with *in vivo* resistance to 5-FU. Four other SC1NU resistant sublines had equivalent or decreased TS mRNA expression. This discrepancy between 5-FU resistance and TS mRNA expression may be due to the slower tumor growth of SC1NU resistant lines than that of SC1NU, as shown by Seno *et al.*[14] We could not detect any relation between the DHFR mRNA expression and the drug resistance. Finally, these resistant xenograft lines had acquired various patterns and degrees of resistance. Each resistant line showed induction of specific mRNA only when it was stimulated by the drug which was used for establishment of that line (MDR1 in SW480/DXR, GST-π in SW480/CDDP and SC1NU/CDDP, and TS in SW480/5-FU). We could induce drug resistant genes as above. We found no clear pattern of cross resistance. It seems that resistance *in vivo* is much more complex than *in vitro*. This complexity should offer opportunities to obtain insight into the problem of drug resistance in the clinic.

(Received August 17, 1990/Accepted February 6, 1991)

REFERENCES

1) Mattern, J., Bak, M., Jr., Hoevor, K. H. and Volm, M. Development of drug resistance in a human epidermoid lung carcinoma xenograft line. *Br. J. Cancer,* **58,** 30–33 (1988).
2) Fujo, A. T., Ueda, K. and Pastan, I. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. USA,* **84,** 265–269 (1987).
3) Turuo, T. Mechanisms of multidrug resistance and implications for therapy. *Jpn. J. Cancer Res.,* **79,** 285–296 (1988).
4) Goldstein, L. J., Galski, H. and Pastan, I. Expression of a multidrug resistance gene in human cancers. *J. Natl. Cancer Inst.,* **81,** 116–124 (1989).
5) Bridget, T. H., Richard, D. H. and Angela, S. B. Identification of differential drug responses and mechanism of resistance in vincristine-resistant cell lines developed either by exposure to the drug or to fractionated radiation. *Cancer Treat. Rev.,* **11,** 73–79 (1984).
6) Marcel, O., Borje, S. A. and Kenneth, B. M. Drug sensitivity and cross-resistance of the 4′-(9-acridinylamino)methanesulfon-m-anisidide-resistant subline of HL-60 human leukemia. *Cancer Res.,* **46,** 3330–3333 (1986).
7) Nakagawa, K., Yokota, J., Wada, M., Sasaki, Y., Fujiwara, Y., Sakai, M., Muramatsu, M., Terasaki, T., Tsunokawa, Y., Terada, M. and Saijo, N. Levels of glutathione *S* transferase π mRNA in human lung cancer cell lines correlate with the resistance to cisplatin and carboplatin. *Jpn. J. Cancer Res.,* **79,** 301–304 (1988).
8) Teicher, B. A., Holden, S. A., Kelley, M. J., Shea, T. C., Cucchi, C. A., Rosowsky, A., Henner, W. D. and Frei, V. E. Characterization of a human squamous carcinoma cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res.,* **47,** 388–393 (1987).
9) Batist, G., Tulpule, A. and Cowan, K. H. Over expression of a novel anionic glutathione transferase in multidrug resistant human breast cancer cells. *J. Biol. Chem.,* **261,** 15544–15549 (1986).
10) Wang, Y., Teicher, B. A., Shea, T. C., Holden, S. A., Rosbe, K. W., Al-Achi, A. and Henner, W. D. Cross-resistance and glutathione-S-transferase-π levels among four human melanoma cell lines selected for alkylating agent resistance. *Cancer Res.,* **49,** 6185–6192 (1989).

11) Alt, F. W., Kellems, R. E., Bertino, J. R. and Schimk, R. T. Selective multiplication of dehydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. *J. Biol. Chem.*, **258**, 1357–1370 (1978).
12) Rodenhuis, S., McGuire, J. J., Nakayanan, R. and Bertino, J. R. Development of an assay system for the detection and classification of methotrexate resistance in fresh human leukemic cells. *Cancer Res.*, **46**, 6513–6519 (1986).
13) Woolley, P. V. and Tew, K. D. "Mechanisms of Drug Resistance in Neoplastic Cells," pp. 127–157 (1988). Academic Press, Inc., San Diego.
14) Takeishi, K., Kaneda, S., Ayusawa, D., Shimizu, K., Goto, O. and Seno, T. Nucleotide sequence of a functional cDNA for thymidilate synthase. *Nucleic Acids Res.*, **13**, 2035–2043 (1985).
15) Scanlon, K. J. and Sabet, M. K. Elevated expression of thymidilate synthase cycle genes in cisplatin-resistant human ovarian carcinoma A 2780 cells. *Proc. Natl. Acad. Sci. USA*, **85**, 650–653 (1988).
16) Sabet, M. K., Rossi, J. J., Lu, Y., Ma, J. X., Chen, J., Miyachi, H. and Scanlon, K. J. Detection of drug resistance in human tumors by *in vitro* enzymatic amplification. *Cancer Res.*, **48**, 5775–5778 (1988).
17) Mattern, J., Bak, J. M. and Volm, M. Occurrence of a multidrug-resistant phenotype in human lung xenografts. *Br. J. Cancer*, **56**, 407–411 (1987).
18) Schabel, F. M., Jr. Nitrosoureas: a review of experimental antitumor activity. *Cancer Treat. Rep.*, **60**, 665–698 (1976).
19) Dano, K. Development of resistance to adriamycin (NSC-123127) in Ehrlich ascites tumor *in vivo*. *Cancer Chemother. Rep.*, Part 1, **56**, 321–326 (1972).
20) Skovsgaard, T. Development of resistance to rubidazone (NSC-164011) in Ehrlich ascites tumour *in vivo*. *Cancer Chemother. Rep. Part 1*, **59**, 301–305 (1975).
21) Mcmillan, T. J., Stephens, T. C., Peacock, J. H. and Steel, G. G. Development of MeCCNU-resistance in clonally derived lines of Lewis lung carcinoma. *Eur. J. Cancer Clin. Oncol.*, **23**, 801–805 (1987).
22) Bence, J., Somfai, R. S. and Tgati, E. Development of some characteristics of a P388 leukemia strain resistant to 1,1; 5,6-dianhydrogalactitol. *Eur. J. Cancer Clin. Oncol.*, **22**, 773–778 (1986).
23) Akiyama, S., Amo, H., Watanabe, T., Matsuyama, M., Sakamoto, J., Imaizumi, M., Ichihashi, H., Kondo, T. and Takagi, H. Characteristics of three human gastric cancer cell lines, NU-GC-2, NU-GC-3 and NU-GC-4. *Jpn. J. Surg.*, **18**, 438–446 (1988).
24) Bissery, M. C., Valeriote, F. A., Chabot, G. G., Crissman, J. D., Yost, C. and Corbett, T. H. Flavone acetic acid (NSC347512)-induced DNA damage in Glasgow osteogenic sarcoma *in vivo*. *Cancer Res.*, **48**, 1279–1285 (1988).
25) Kano, T., Sakai, M. and Muramatsu, M. Structure and expression of a human class pi glutathione-S-transferase messenger RNA. *Cancer Res.*, **47**, 5626–5630 (1987).
26) Twentyman, P. R. and Luscombe, M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *Br. J. Cancer*, **56**, 279–285 (1987).
27) Hida, T., Ueda, R., Takahashi, T., Watanabe, H., Kato, T., Suyama, M., Sugiura, T., Ariyoshi, Y. and Takahashi, T. Chemosensitivity and radiosensitivity of small cell lung cancer cell lines studied by a newly developed 3-(4,5-dimethylthiazol- 2-yl)- 2,5 -diphenyltetrazorium bromide (MTT) hybrid assay. *Cancer Res.*, **49**, 4785–4790 (1989).
28) Harker, W. G., Slade, D. L. and Dalton, W. S. Multidrug resistance in mitozantrone-selected HL-60 leukemia cells in the absence of P-glycoprotein overexpression. *Cancer Res.*, **49**, 4542–4549 (1989).
29) Haber, M., Norris, M. D. and Kavallaris, M. Atypical multidrug resistance in a therapy-induced drug-resistant human leukemia cell line (LALW-2): resistance to Vinca alkaloids independent of P-glycoprotein. *Cancer Res.*, **49**, 5281–5287 (1989).
30) Shea, T. C., Kelley, S. C. and Henner, W. D. Identification of an anionic form of glutathione transferase present in many human tumors and human tumor cell line. *Cancer Res.*, **48**, 527–533 (1988).
31) Dusre, L., Mimnaugh, E. G., Myers, C. E. and Sinha, B. K. Potentiation of doxorubicin cytotoxicity by buthionine sulfoximine in multidrug-resistant human breast tumor cells. *Cancer Res.*, **49**, 511–515 (1989).