J. Radiat. Res., 46, 197–203 (2005) *Regular Paper*

Differences in Effects of Oncogenes on Sensitivity to Anticancer Drugs

Tomoyuki HAMAMOTO[1], Keiji SUZUKI[2], Hitoshi SASAKI[1], Masataka ICHIKAWA[1], Seiji KODAMA[2] and Masami WATANABE[3]*

Oncogene/SHOK cells/Chemosensitivity/IC_{50}.

Methods to predict the responsiveness of a particular tumor to a particular anticancer drug are desirable not only for chemotherapy but also for chemoradiotherapy. Here, we examined the effects of viral or activated oncogenes on sensitivity to anticancer drugs by using SHOK (Syrian hamster Osaka-Kanazawa) cells and their transfectants. The IC_{50} of each transfectant was compared with that of the pSV2Neo transfected control. Cells transfected with the c-*myc*, v-*mos*, or v-*fgr* gene increased their sensitivity to bleomycin, while those transfected with the H-*ras* gene developed resistance. Resistance to cisplatin was conferred by the introduction of the H-*ras* or c-*cot* gene. In the case of adriamycin, the c-*myc* or c-*cot* transfectant increased sensitivity and the H-*ras* transfectant decreased it. Mitomycin C resistance was observed by the introduction of the K-*ras* gene. Thus, the H-*ras* gene was found to be involved in the development of resistance to three of the four anticancer drugs. In addition, we have for the first time shown that *mos* and *cot* have an effect on sensitivity to three and all of the four anticancer drugs, respectively. These results suggest that the expression of each oncogene would differently affect sensitivity to the four anticancer drugs used in this study, and this property could be a possible marker to predict chemosensitivity.

INTRODUCTION

Radiotherapy, along with surgery and chemotherapy, is one of the three most important treatments for cancer. Some cancers at their early stages are completely cured by radiotherapy alone, whereas in many advanced cases local control rates are low with the therapy.[1)] To improve local tumor control and/or eradication of distant metastases, the combination of radiotherapy and chemotherapy (chemoradiotherapy) is currently used. With this combined modality therapy, improved local control and/or increased survival has been shown in malignant lymphoma and many types of carcinoma, such as head and neck, nasopharynx, lung, esophagus, stomach, rectum, prostate, cervix, breast, anus, and bladder.[2,3)] On the other hand, because anticancer drugs have a narrow therapeutic index and a great potential for causing harmful side effects,[4)] the introduction of chemotherapy potentially increases both the spectrum and magnitude of normal-tissue toxicities compared with radiotherapy alone.[3)] Therefore, if patients who are resistant to chemotherapy could be identified before treatment, this would prevent unnecessary toxicity.[3)] Thus, methods to enable accurate prediction of the responsiveness of a particular tumor to a particular anticancer drug are desirable for not only chemotherapy but also chemoradiotherapy.

We conducted an investigation of the intracellular factors that might be useful to predict chemosensitivity, beginning with oncogenes. Oncogenes have been related to malignant progression and poor prognosis in several types of human tumors.[5–8)] The effect of expression of an oncogene on drug sensitivity has been demonstrated by previous reports, using the following: mouse fibroblast cell line NIH3T3,[9–13)] mink lung epithelial cell line,[14)] Friend murine erythroleukemia cell line,[15)] human cell line of teratocarcinoma[16)] and small cell lung carcinoma.[17)] In studies using NIH3T3 cells, Sklar[9)] showed that the activated H-*ras*, v-K-*ras*, or N-*ras* decreased sensitivity to cisplatin (CDDP). The same results were obtained by Isonishi *et al.*[10)] with activated H-*ras*. Peters *et al.*[11)] also observed decreased sensitivity to CDDP and adriamycin (ADR) in H-*ras* transformed lines. However, in activated H-*ras* transfected cells, Toffoli *et al.*[12)] reported no change in sensitivity to CDDP or ADR; further, Niimi *et al.*[13)] and Gao *et al.*[18)] presented findings of no change or, rather, increased sensitivity to ADR and increased sensitivi-

*Corresponding author: Phone: 0724-51-2391,
Fax: 0724-51-2628,
E-mail: nabe@rri.kyoto-u.ac.jp
[1]Department of Hospital Pharmacy, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501; [2]Division of Radiation Biology, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan. [3]Radiation Life Science, Kyoto University Research Reactor Insutitute, 2-1010 Asashiro-nishi, Kumatori, Sennan-gun 590-0494, Japan.

ties to CDDP. Thus, even in the case that the same parent cells are used, the results are not always the same, and the contribution of oncogene activation to chemosensitivity is currently not well established.

For purposes of our study, we selected Syrian hamster Osaka-Kanazawa(SHOK) cells, a clonal cell line derived from Syrian/golden hamster embryo cells, because this is a stable cell line, suitable for the introduction of foreign genes.[19,20] In a previous study using SHOK cells, we reported the effects of mutated or viral oncogenes on sensitivity to γ-rays, ultraviolet light(UV), and heat shock.[20] In this study, we examined the effects of various oncogenes on sensitivity to several anticancer drugs, which are often used in chemoradiotherapy.

MATERIALS AND METHODS

Anticancer drugs

Bleomycin (BLM) and CDDP were kindly supplied by Nippon Kayaku Co., Ltd. (Tokyo, Japan). ADR and mitomycin C (MMC) were provided by Kyowa Hakko Kogyo Co., Ltd.(Tokyo, Japan).

Cell line and culture

Characteristics of cells used in this study are shown in Table 1.

The establishment of the SHOK cell line and the transfectants of these cells containing various oncogenes has been reported elsewhere.[19,20] These transfectants, excepting for SHOK (*neo*), SHOK (*myc*), and SHOK (*erbB*), were originated from *foci* formed after the introduction of mutated cellular and viral oncogenes. In the case of SHOK (*neo*), SHOK (*myc*), and SHOK (*erbB*), transfectants were established in a selection medium containing geneticin. Because SHOK (*myc*) and SHOK (*erbB*) cells did not give rise to foci, their expression of an introduced gene was confirmed by RNA blot analysis.[20]

Cells were cultured in Eagle's MEM (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (Biosciences PTY Ltd., Australia) and 20mM HEPES at 37°C in a humidified atmosphere with 5% CO_2.

Drug sensitivity assay

Inhibitory effects of anticancer drugs on growth of SHOK cells and their transfectants were determined by applying the WST-1 assay[21,22] using a "Cell Counting Kit" (Dojindo Laboratories, Kumamoto, Japan). Cells were inoculated onto 96-well microtiter plates at a concentration of 1×10^3 cells/well in 100 μl of medium. Following a 24-h incubation, BLM, CDDP, ADR, or MMC was added in 100 μl of medium with nine concentrations obtained by two-fold serial dilution of the drug. All drug concentrations were tested in triplicate wells. Before dilution with medium, ADR and MMC were dissolved in water and CDDP was dissolved in dimethylsulfoxide (DMSO, Sigma Chemical Co., St.Louis, MO, USA). BLM was dissolved directly in medium. The final concentration of DMSO in CDDP-treated cells did not exceed 0.5%, a concentration which has no inhibitory effect on cell growth. After drug exposure for 48 h, a mixture(20 μl) of 4-[3-(4-indophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate(WST-1) and 1-methoxy-5-methyl-phenazinium methylsulfate(1-methoxy PMS) in 20 mM HEPES buffer(pH 7.4) was added to each well(final concentration: WST-1, 0.5 mM; 1-methoxy PMS, 0.02 mM). The cells were incubated for an additional 2 h, then the reaction was stopped by adding 20 μl of 0.1N HCl (special reagent grade, Wako Pure Chemical Industries Ltd., Osaka, Japan) to each well. The plates were read on a microplate reader (MPR-A4, TOSOH Corp., Japan), using a test wave length of 450 nm and a reference wavelength of 600 nm. The concentration of drug necessary to inhibit the growth of each individual cell line by 50%(IC_{50}) was determined by plotting the logarithm

Table 1. Characteristics of cells used in this study.

cells	Transfected gene	Origins of oncogene	Population doubling time(h)	Maximal cell density ($\times 10^5$ cells/cm^2)
SHOK	none		11.5	2.04
SHOK(*neo*)	*neo*		15.4	0.973
SHOK(*myc*)	c-*myc*	mouse plasmacytoma(MOPC315)	12.7	0.687
SHOK(H-*ras*)	c-H-*ras*	T24 bladder carcinoma	17.3	1.56
SHOK(K-*ras*)	c-K-*ras*	Hut-14 tumorigenic human fibroblast	11.5	1.27
SHOK(N-*ras*)	c-N-*ras*	HL-60 acute promyelocytic leukemia	10.3	2.47
SHOK(*cot*)	c-*cot*	TCO4 human thyroid carcinoma cell line	10.3	2.14
SHOK(*mos*)	v-*mos*	Molomey murine sarcoma virus	13.0	2.03
SHOK(*erbB*)	v-*erbB*	avian erythroblastosis virus	13.4	1.12
SHOK(*fgr*)	v-*fgr*	Gardner-Rasheed feline sarcoma virus	16.6	1.30

of drug concentration versus the percent of treated cells that survived.

Statistical analysis

Data were first analyzed by F-test for equality of variance. If the test revealed equality of variance, a Student's *t*-test was used to evaluate the statistical significance of differences between cell lines. In the case of inequality of variance, a Welch's *t*-test was used. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The survival curves of SHOK cells and their transfectants for BLM, CDDP, ADR and MMC are shown in Fig. 1 and Fig. 2. Each drug dose-dependently inhibited proliferations of cells used in this study in the range of each concentration under our exposing condition. But there was a slight difference in patterns of inhibition of SHOK cells, for example, among the drugs. BLM showed biphasic growth inhibitory effect, which inhibited the cell growth moderately at lower concentrations, but more strongly at concentrations higher than about 200 μg/ml. In contrast, CDDP, ADR and MMC inhibited the cell growth strongly at lower concentrations.

To express the differences in chemosensitivity of each cell line observed in Fig. 1 and Fig. 2 numerically, we determined IC_{50}. Table 2 summarizes the IC_{50}s of the anticancer drugs for all the cell lines. SHOK (*neo*), transfected with pSV2Neo plasmid only, showed no significant differences in IC_{50}s compared with those of SHOK cells to BLM, CDDP, or MMC, though the cells were more resistant to ADR. Therefore, to consider the effect of the introduction of a foreign gene, the IC_{50}s of each transfectant were compared with that of SHOK (*neo*). Differences in the effect of pSV2Neo plasmid on sensitivity to anticancer drugs were reported by Gao *et al.*,[18] who found no change in sensitivity to BLM and ADR, but more did find higher resistance to CDDP and higher sensitivity to MMC, based on a comparison with parental NIH3T3 cells. Discrepancies between their results and ours may be due to differences in the characteristics of each parental cell.

In the case of BLM, SHOK (*myc*) cells were most sensitive, and SHOK (*fgr*) cells and SHOK (*mos*) cells were significantly more sensitive than SHOK (*neo*) cells, the IC_{50}s value of which reduced to 4.1%, 42.9%, and 64.4% of SHOK (*neo*) cells, respectively. The IC_{50}s of SHOK cells

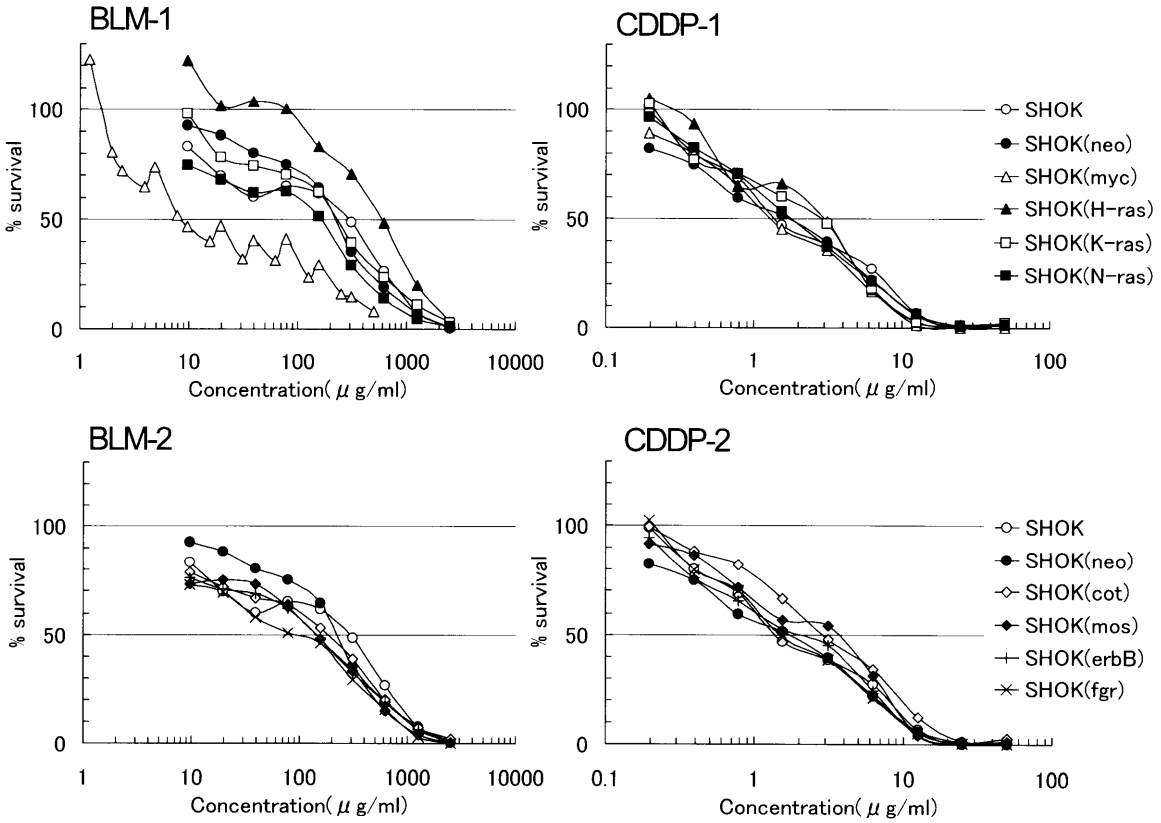

Fig. 1. Survival curves for SHOK cells and their *neo* transfectant and oncogene transfectants following exposure to BLM and CDDP. Comparison of survival curves for SHOK cells and *neo* transfectant with those for *myc*, H-*ras*, K-*ras*, and N-*ras* transfectants (BLM-1 and CDDP-1), or those for *cot*, *mos*, *erbB*, and *fgr* transfectants (BLM-2 and CDDP-2) was made. Each point represents the mean of the % survivals obtained at each concentration. Error bars have been omitted for clarity.

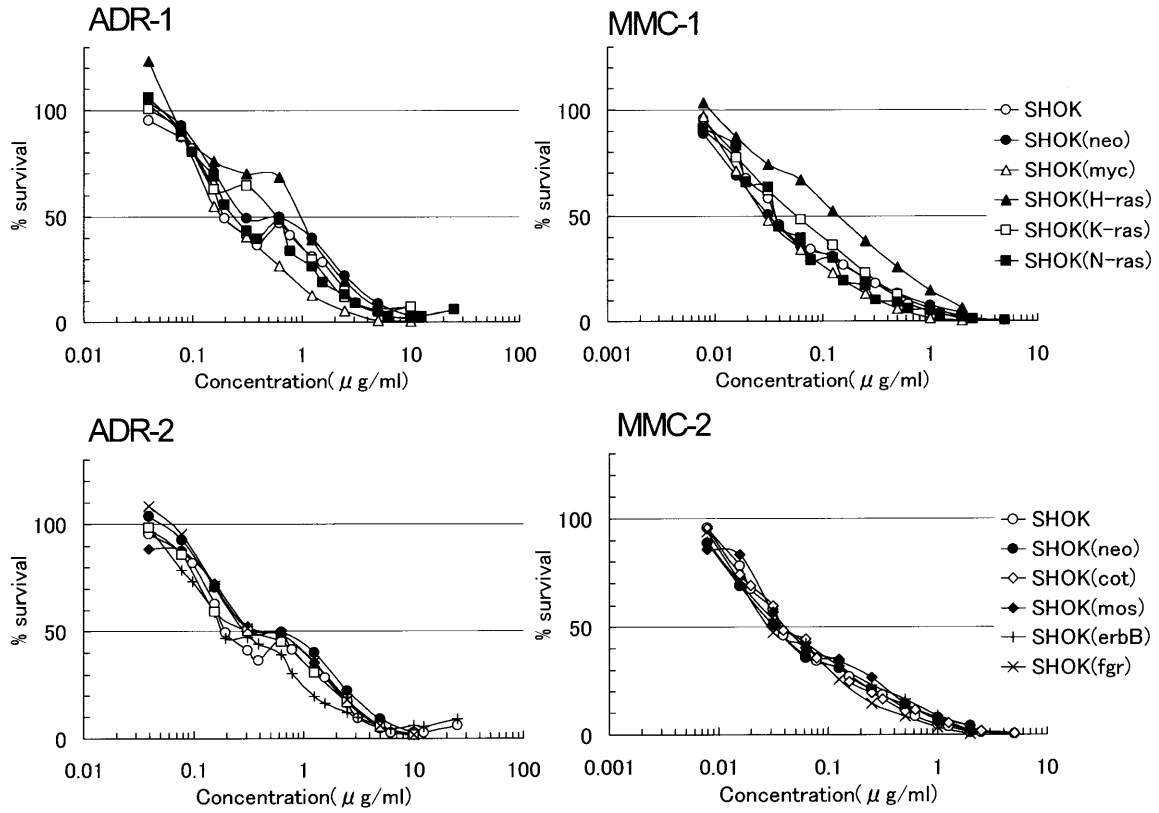

Fig. 2. Survival curves for SHOK cells and their neo gene transfectant and oncogene transfectants following exposure to ADR and MMC. Comparison of survival curves for SHOK cells and *neo* transfectant with those for *myc*, H-*ras*, K-*ras*, and N-*ras* transfectants (ADR-1 and MMC-1), or those for *cot*, *mos*, *erbB*, and *fgr* transfectants (ADR-2 and MMC-2) was made. Each point represents the mean of the % survivals obtained at each concentration. Error bars have been omitted for clarity.

containing the N-*ras* and *erbB* genes also reduced, but not significantly. In contrast, SHOK (H-*ras*) cells were 2.69 times more resistant than SHOK (*neo*) cells. The opposite results were reported by Gao *et al.*,[18] who demonstrated, using NIH3T3 cell lines, that the v-H-*ras* or c-K-*ras* (Val-12) transfectant increased, and that the v-*erbB* transfectant decreased, sensitivity to BLM. Le-Ruppert *et al.*[16] reported that N-*ras* activation had no effect on sensitivity to BLM, based on an investigation using human ovarian teratocarcinoma sublines. BLM uptake is known to be limited by the plasma membrane, and cell electropermeabilization study has demonstrated that its cytotoxicity is closely related to cell permeabilization and to direct internalization of BLM into the cytosol.[23] Therefore, an elevation in BLM influx may be responsible for the sensitization of our five cell lines. In addition to membrane alterations, mechanisms reported to influence BLM cytotoxicity include metabolic inactivation of BLM by a cytosolic hydrolase and elevated DNA repair activity.[24,25] Further investigation is needed to elucidate which of these mechanisms makes the most significant contribution to BLM resistance of SHOK (H-*ras*) cells.

BLM, like ionizing radiation, efficiently generates double-strand DNA breaks and is therefore regarded as radiomimetic. We previously reported that SHOK (*mos*) cells, SHOK (*cot*) cells, and SHOK (N-*ras*) cells developed resistance to γ-rays,[20] which dose not coincide with the data of BLM in this study. Because BLM molecules, unlike radiation, need to bind to DNA in order to exhibit their cytotoxic activity,[26] one or more of the above-mentioned mechanisms that could influence BLM cytotoxicity may contribute to this difference.

In the case of CDDP, no cells were more sensitive than SHOK (*neo*) cells. SHOK (*cot*) cells and SHOK (H-*ras*) cells were 1.81 and 1.75 times significantly more resistant than SHOK (*neo*) cells, respectively. The IC_{50} value of SHOK cells containing the K-*ras* and *mos* genes was elevated to 1.69 and 1.92 times that of SHOK (*neo*) cells, respectively, though not significantly. This is the first report showing CDDP resistance as a result of *cot* transformation. The effect of transformation by the oncogenes used in this study on sensitivity to CDDP has previously been reported by several groups. Sklar[9] reported that NIH3T3 cells transformed by mutated H-*ras*, K-*ras* or N-*ras* were 4.5- to 8.5-fold more resistant to CDDP, and that transformation of these cells by v-*mos* also conferred 2-fold CDDP resistance. With regard to H-*ras*, K-*ras*, and *mos*, our results resemble those of

Table 2. Sensitivity of SHOK cells and their transfectants to anticancer drugs

Cells	IC_{50} (μg/ml)			
	BLM	CDDP	ADR	MMC
SHOK	277.8 ±56.1	1.60±0.26	0.248±0.029*	0.0360±0.0032
SHOK(*neo*)	219.4 ±14.0	1.59±0.18	0.469±0.091	0.0323±0.0032
SHOK(*myc*)	9.08± 1.60**#	1.44±0.24	0.199±0.029*	0.0302±0.0048
SHOK(H-*ras*)	591.0 ±64.9*#	2.78±0.37*	0.939±0.082*	0.1398±0.0415#
SHOK(K-*ras*)	214.4 ±58.1	2.68±0.60	0.588±0.066	0.0600±0.0042**
SHOK(N-*ras*)	146.8 ±31.8	1.86±0.36	0.315±0.065	0.0371±0.0050
SHOK(*cot*)	180.4 ±29.3	2.87±0.16**	0.248±0.040*	0.0414±0.0033
SHOK(*mos*)	141.2 ± 3.0*#	3.05±0.72	0.464±0.049	0.0407±0.0028
SHOK(*erbB*)	150.9 ±21.1	2.01±0.11	0.340±0.036	0.0405±0.0076
SHOK(*fgr*)	94.1 ±21.8**	1.66±0.45	0.469±0.071	0.0324±0.0022

Each value represents the mean ± SEM of 3–5 separate experiments.
Significantly different from SHOK(*neo*), *$p < 0.05$, **$p < 0.01$ by Student's or Welch's *t*-test.
Welch's *t*-test was used.

Sklar. Resistance to CDDP in H-*ras* transfectants has also been reported by other groups.[10,11] However, no change,[12] or rather increase[11,13,18] in sensitivity to CDDP was observed in H-*ras* transformed NIH3T3 cells. In a study by Gao *et al.*,[18] c-K-*ras*(Val-12)-transfected NIH3T3 cells were shown to have increased sensitivity to CDDP. Le-Ruppert *et al.*,[16] by using cells from human ovarian teratocarcinoma sublines, reported that N-*ras* activation increased sensitivity to CDDP. With regard to *myc*, CDDP resistance was observed in c-*myc* transfected NIH3T3 cells.[13] Sklar *et al.*,[15] using Friend murine erythroleukemia cells, demonstrated that increased expression of c-*myc* induces increased CDDP resistance. Further, Van Waardenburg *et al.*[17] reported that down-regulation of endogenously expressed c-*myc* in a CDDP-resistant human small cell lung carcinoma subline resulted in increased sensitivity to CDDP. In contrast, Gao *et al.*[18] found no change in sensitivity to CDDP in v-*myc*-transfected NIH3T3 cells, which is in agreement with our data. In v-*erbB*-transfected NIH3T3 cells, Gao *et al.* found increased sensitivity to CDDP, which does not coincide with our data. It has been reported that the major mechanisms appearing to contribute to CDDP resistance include impairment of CDDP accumulation, elevated levels of methallothioneins (MTs) or glutathione (GST), and enhanced DNA repair.[10,27] Isonishi *et al.*[10] demonstrated that CDDP resistance in mutant c-H-*ras* overexpressed NIH3T3 cells was associated with an impairment of cellular CDDP accumulation, and with an increase in MT content but without change in GST content. Their results may be a clue to identifying which mechanism contributes to CDDP resistance of SHOK (H-*ras*) cells.

In the case of ADR, SHOK (*myc*) cells and SHOK (*cot*) cells were significantly more sensitive than SHOK (*neo*) cells; their IC_{50} value reduced to 42.4% and 52.9% of SHOK (*neo*) cells, respectively. In contrast, SHOK (H-*ras*) cells were 2.0 times more resistant than SHOK (*neo*) cells. This is the first report showing increased ADR sensitivity as a result of *cot* transformation. In *myc*-transformed NIH3T3 cells, increased sensitivity to ADR, which is consistent with our results, has been previously observed,[20] though resistance to ADR has also been observed.[13] In agreement with our observations, resistance to ADR has been reported in *H-ras* transformed NIH3T3 cells,[11] as well as Mink lung epithelial cells.[14] However, no change[13] or increase[18] in sensitivity to ADR has been observed in H-*ras*-transformed NIH3T3 cells. In N-*ras*-transformed NIH3T3 cells, Gao *et al.*[18] showed increased sensitivity to ADR. A correlation has been reported between intracellular ADR accumulation and sensitivity to ADR in NIH3T3 cells transfected with several oncogenes.[28] Because ADR efflux did not differ among the group including the *ras*-family transfectants and the vector-transfected control,[28] Gao *et al.*[18] postulated that changes in ADR influx might be responsible for the changes in ADR sensitivity in those transfectants. Decreased ADR accumulation is known to be related to an overexpression of P-glycoprotein. Peters *et al.*[11] observed that expression of P-glycoprotein was not different between NIH3T3 cells and their H-*ras* transfectant, although the ADR accumulation in the H-*ras* transfectant was significantly lower. The same non-P-glycoprotein-related resistance to ADR, which is associated with a lower accumulation of ADR, was also observed in other H-*ras* transformed lines.[14] Although we did not examine ADR accumulation and the P-glycoprotein expression of our cell lines, a similar mechanism may contribute to the ADR resistance of SHOK (H-*ras*) cells.

In the case of MMC, as well as that of CDDP, no cells were more sensitive than SHOK (*neo*) cells. SHOK (K-*ras*) cells were 1.86 times significantly more resistant than SHOK (*neo*) cells. The IC_{50} value of SHOK (H-*ras*) cells was elevated to as much as 4.33 times that of SHOK (*neo*) cells, though elevation itself was not significant. In H-*ras*-transformed NIH3T3 cells, no change[12] or increase[29] in sensitivity to MMC was reported. MMC is bioreductively activated by a number of oxidoreductases, and produces lethal adducts with DNA. In those enzyme systems, decrease in DT-diaphorase (DTD) activity has been known to be associated mainly with MMC resistance.[30] Further investigation is needed to ascertain whether decrease in DTD activity contributes to MMC resistance of SHOK (K-*ras*) and SHOK (H-*ras*) cells.

Some of our data in the present study did not agree with the previously published data, especially with that of Gao *et al.*[18] using NIH3T3 cells, although their results are not always the same as other studies using the same parent cells. Studies using NIH3T3 cells have raised some problems requiring clarification, such as the heterogeneity of their chromosome constitution and their difference in cell cycle distribution.[20] The former has been relevant to clonal heterogeneity of radiation response, along with the malignancy and growth properties of cells, while the latter has been associated with the sensitivity of cells.[20] Because we previously confirmed that there is no alteration in either modal karyotype or cell cycle distribution among SHOK cells and their transfectants[20] whereas Gao *et al.*.[18] did not referred to these points, we speculate that some differences in chemosensitivity of transfectants between NIH3T3 cells and SHOK cells may come from these differences in characteristics of the parent cells.

Our results suggest that the expression of each oncogene would differently affect sensitivity to the four anticancer drugs used in this study. Our previous report, using SHOK cells, showed that each of *mos*, *cot*, and N-*ras* transfectants developed resistance to γ-rays.[20] In the present study, *mos* tansfectant and *cot* transfectant increased sensitivity to BLM and ADR, respectively. Therefore, if a clinical tumor tissue specimen is revealed to express *mos* or *cot* oncogene, we might have to choose BLM or ADR, respectively, instead of radiotherapy. Further, SHOK (H-*ras*) presented resistance significantly for BLM, CDDP, and ADR, and without significance but with about four times higher IC_{50} value necessary for MMC. We also reported findings of SHOK (H-*ras*) resistance to UV and heat shock, and an absence of resistance to γ-rays.[20] Therefore, if a specimen shows H-*ras* expression, radiotherapy might have to be chosen rather than these drugs and hyperthermia. Investigation of sensitivity to additional anticancer drugs having different mechanisms of action than those investigated herein would further the understanding of the drug resistance profile of SHOK (H-*ras*) cells.

The expression of *mos* or *cot* in human neoplasia has not been reported, until recently that of *mos* in lung carcinoma and astrocytic tumors,[31–33] as well as overexpression and mutation of *cot* in Hodgikin's disease, nasopharyngeal carcinoma, gastric and colonic adenocarinomas, and breast cancer,[34–36] has been shown. In this study we for the first time shown that effect of *mos* on sensitivity to three (except CDDP) of the four anticancer drugs, and effect of *cot* on that to all of the four anticancer drugs. Therefore, our results, along with our previous results of γ-rays and heat shock, would be useful information on the selection of the most suitable modality to treat tumors that express *mos* or *cot*.

In breast cancer, both *in vitro* studies and clinical studies have shown that amplification of the *HER-2/neu* oncogene may play a role in predicting sensitivity to anticancer drugs.[37] Accumulation of clinical studies could confirm that each oncogene that affects sensitivity to one or several anticancer drugs investigated in this study could be a marker to predict chemosensitivity in a particular human tumor.

REFERENCES

1. Tanaka, M. (2002) Chemoradiotherapy for lung, esophageal, and uterine cervix cancer. Nippon Acta Radiologica **62**: 479–483.
2. Malas, L. and Cox, J. D. (2003) Principles of combining radiation therapy and chemotherapy. In: Radiation Oncology: Rationale, Technique, Results. 8th ed., Eds J. D. Cox and K. Kian Ang, pp.108–121, Mosby, St. Louis.
3. Stewart, F. A. and Bartelink, H. (2002) The combination of radiotherapy and chemotherapy. In: Basic Clinical Radiobiology. 3rd ed., Ed G. G. Steel, pp.217–230, Arnold, London.
4. Calabresi, P. and Chabner, B. A. (2001) Chemotherapy of Neoplastic diseases, In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10th ed., Eds J. G. Hardman and L. E. Limbird, pp.1381–1388, McGraw-Hill, New York.
5. Bos, J. L. (1989) *ras* oncogenes in human cancer: a review. Cancer Res. **49**: 4682–4689.
6. Field, J. K. and Spandidos, D. A. (1990) The role of *ras* and *myc* oncogenes in human solid tumours and their relevance in diagnosis and prognosis(review). Anticancer Res. **10**: 1–22.
7. Spandidos, D. A., Liloglou, T. and Field, J. K. (1993) Prognostic significance of oncogenes and tumor suppressor genes in human malignancy. Stem Cells **11**: 194–198.
8. Duffy, M. J. (1995) Can molecular markers now be used for early diagnosis of malignancy?. Clin. Chem. **41**: 1410–1413.
9. Sklar, M. D. (1988) Increased resistance to *cis*-diamminedichloroplatimum (II) in NIH3T3 cells transformed by *ras* oncogenes. Cancer Res. **48**: 793–797.
10. Isonishi, S., Hom, D. K., Thiebaut, F. B., Mann, S. C., Andrews, P. A., Basu, A., Lazo, J. S., Eastman, A. and Howell, S.B. (1991) Expression of c-Ha-*ras* oncogene in mouse NIH3T3 cells induces resistance to cisplatin. Cancer Res. **51**: 5903–5909.
11. Peters, G. J., Wets, M., Keepers, Y. P. A. M., Oskam, R., Van Ark-Otte, J., Noordhuis, P., Smid, K. and Pinedo, H.M. (1993) Transformation of mouse fibroblasts with the oncogenes H-*ras* or *trk* is associated with pronounced changes in drug sen-

sitivity and metabolism. Int. J. Cancer **54**: 450–455.
12. Toffoli, G., Viel, A., Tumiotto, L., Buttazzi,P., Biscontin G. and Boiocchi, M. (1989) Sensitivity pattern of normal and Ha-ras transformed NIH3T3 fibroblasts to antineoplastic drugs. Tumori, **75**: 423–428.
13. Niimi, S., Nakagawa, K., Yokota, J., Tsunokawa, Y., Nishio, K., Terashima, Y., Shibuya, M., Terada, M. and Saijo, N. (1991) Resistance to anticancer drugs in NIH3T3 cells transfected with c-*myc* and/or c-H-*ras* genes. Br. J. Cancer **63**: 237–241.
14. Wishart, G. C., Plumb, J. A., Spandidos, D. A. and Kerr, D. J. (1991) H-*ras* transfection in mink lung epithelial cells may induce "atypical" multidrug resistance. Eur. J. Cancer **27**: 673.
15. Sklar, M.D. and Prochownik, E.V. (1991) Modulation of *cis*-platinum resistance in Friend erythroleukemia cells by c-*myc*. Cancer Res. **51**: 2118–2123.
16. Le-Ruppert, K., Masters, J. R. W., Knuechel, R., Seegers, S., Tainsky, M. A.,Hofstaedter F. and Buettner, R. (1992) The effect of retinoic acid on chemosensitivity of PA-1 human teratocarcinoma cells and its modulation by an activated N-*ras* oncogene. Int. J. Cancer **51**: 646–651.
17. Van Waardenburg, R. C. A. M., Prins, J., Meijer, C., Uges, D. R. A., de Vries, E. G. E. and Mulder, N. H. (1996) Effects of c-*myc* oncogene modulation on drug resistance in human small cell lung carcinoma cell lines. Anticancer Res. **16**: 1963–1970.
18. Gao, X., Asaumi, J., Kawasaki, S., Nishikawa, K., Kuroda, M., Takeda, Y., Hiraki, Y., Ihara M. and Ohnishi, T. (1995) Sensitivity of anticancer drugs in NIH3T3 cells transfected with oncogenes accompanied by pSV2neo vector. Anticancer Res. **15**: 1911–1914.
19. Higashi, T., Sasai, H., Suzuki, F., Miyoshi, J., Ohuchi, T., Takai,S. ,Mori, T. and Kakunaga, T. (1990) Hamster cell line suitable for Transfection assay of transforming genes. Proc. Natl. Acad. Sci. USA **87**: 2409–2413.
20. Suzuki, K., Watanabe M. and Miyoshi, J. (1992) Differences in effects of oncogenes on resistance to γ rays, ultraviolet light, and heat shock. Radiat. Res. **129**: 157–162.
21. Ishiyama, M., Shiga, M., Sasamoto, K., Mizoguchi, M. and He, P. (1993) A new sulfonated tetrazolium salt that produces a highly water- soluble formazan dye. Chem. Pharm. Bull. **41**: 1118–1122.
22. Ishiyama, M., Tominaga, H., Shiga, M., Sasamoto,K., Ohkura, Y., Ueno, K. and Watanabe, M. (1995) Nobel cell proliferation and cytotoxicity assays using a tetrazolium salt that produces a water-soluble formazan dye. *In vitro* toxicol. **8**: 187–190.
23. Poddevin, B., Orlowski, S., Belehradek, J. Jr. and Mir, L. M. (1991) Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culture. Biochem. Pharmacol. **42** (Suppl.): S67–S75.
24. R. T. Dorr (1992) Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. Semin. Oncol. **19**: 3–8.
25. Sikic, B. I. (1986) Biochemical and cellular determinants of bleomycin cytotoxicity. Cancer Surv. **5**: 81–91.
26. Chabner, B. A., Ryan, D. P., Paz-Ares, L., Garcia-Carbonero, R. and Calbresi, P. (2001) Antineoplastic agents, In: Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10th ed., Eds J. G. Hardman and L. E. Limbird, pp.1389–1459, McGraw-Hill, New York.
27. Andrews P. A. and Howell S. B. (1990) Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. Cancer Cells **2**: 35–43.
28. Gao, X. (1995) Studies on sensitivity to adriamycin in NIH3T3 cells transfected with several oncogenes. Jpn. J. Hyperthermic Oncol. **11**: 162–170.
29. Hisawa, T., Tokita, H. and Ike, Y. (1996) Differential chemosensitivitiy in oncogene-transformed cells. J. Exp. Ther. Oncol. **1**: 162–170.
30. Sartorelli, A. C., Hodnick, W. F., Belcourt, M. F., Tomasz, M., Haffty, B., Fischer J. J. and Rockwell, S. (1994) Mitomycin C: a prototype bioreductive agent. Oncol. Res. **6**: 501–508.
31. Gorgoulis, V. G., Zacharatos, P., Mariatos, G., Liloglou, T., Kokotas, S., Kastrinakis, N., Kotsinas, A., Athanasiou, A., Foukas, P., Zoumpourlis, V., Kletsas, D., Ikonomopoulos, J., Asimacopoulos, P. J., Kittas, C. and Field, J. K. (2001) Deregulated expression of c-*mos* in non-small cell lung carcinoma: relationship with *p53* status, genomic instability, and tumor kinetics. Cancer Res. **61**: 538–549.
32. Perunovic, B., Athanasiou, A., Quilty, R. D., Gorgoulis, V. G., Kittas, C. and Love, S. (2002) Expression of mos in astrocytic tumors and its potential role in neoplastic progression. Hum. Pathol. **33**: 703–707.
33. Athanasiou, A., Perunovic, B., Quilty, R. D., Gorgoulis, V. G., Kittas, C. and Love, S. (2003) Expression of mos in ependymal gliomas. Am. J. Clin. Pathol. **120**: 699–705.
34. Eliopoulos, A. G., Davies, C., Blake, S. S., Murray, P., Najafipour, S., Tsichlis, P. N. and Young, L. S. (2002) The oncogenic protein kinase Tpl-s/Cot contributes to Epstein-Barr virus-encoded latent infection membrane protein 1-induced NF-κB signaling downstream of TRAF2. J. Virol. **76**: 4567–4579.
35. Ohara, R., Hirota, S., Onoue, H., Nomura, S., Kitamura, Y. and Toyoshima, K. (1995) Identification of the cells expressing *cot* proto-oncogene mRNA. J. Cell Sci. **108**: 97–103.
36. Sourvinos, G., Tsatsanis, C. and Spandidos, D. A. (1999) Overexpression of the *Tpl-2/Cot* oncogene in human breast cancer. Oncogene **18**: 4968–4973.
37. Dhingra, K. and Hortobagyi, G. N. (1996) Critical evaluation of prognostic factors. Semin. Oncol. **23**: 436–445.

Received on January 5, 2005
1st Revision received on February 3, 2005
Accepted on February 4, 2005