——RAPID COMMUNICATION——

Jpn. J. Cancer Res. (Gann)
79, 301–304 ; March, 1988

LEVELS OF GLUTATHIONE S TRANSFERASE π mRNA IN HUMAN LUNG CANCER CELL LINES CORRELATE WITH THE RESISTANCE TO CISPLATIN AND CARBOPLATIN

Kazuhiko NAKAGAWA,[*1] Jun YOKOTA,[*2] Makio WADA,[*3] Yasutsuna SASAKI,[*1] Yasuhiro FUJIWARA,[*1] Masaharu SAKAI,[*5] Masami MURAMATSU,[*5] Takeo TERASAKI,[*4] Youko TSUNOKAWA,[*3] Masaaki TERADA[*1, *3] and Nagahiro SAIJO[*1]

*[1]*Pharmacology Division,* *[2]*Section of Studies on Metastasis,* *[3]*Genetics Division and* *[4]*Pathology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, and* *[5]*Department of Biochemistry, The University of Tokyo, Faculty of Medicine, Hongo, Bunkyo-ku, Tokyo 113*

The amounts of mRNA for glutathione S transferase π (GST π) were significantly lower in 3 human small cell lung cancer (SCLC) cell lines than in 3 non small cell lung cancer (NSCLC) cell lines. The sensitivities of the 3 SCLC cell lines to cisplatin and carboplatin were much higher than those of the 3 NSCLC cell lines. These results indicate that low levels of GST π mRNA expression in SCLC cell lines inversely correlate to high sensitivity to cisplatin and carboplatin, and further suggest that GST π may play an important role in intracellular inactivation of these drugs.

Key words: Glutathione S transferase π — Drug resistance — Small cell lung cancer — Cisplatin

The clinical response to chemotherapeutic agents is quite different between small cell lung cancer (SCLC) and non SCLC (NSCLC); SCLC is more sensitive to anticancer agents than NSCLC. Cisplatin is one of the most active chemotherapeutic agents for lung cancer and carboplatin is a promising drug for treatment of SCLC. We previously reported that the IC_{50}s of cisplatin and carboplatin for all the SCLC cell lines tested were significantly lower than those for NSCLC cell lines. These results were consistent with the clinical observation that the response rates to cisplatin and carboplatin were much higher in patients with SCLC than in those with NSCLC.[1, 2]

There are several reports on the mechanisms of resistance to anticancer drugs in tumor cells.[3–8] The overexpression of P-glycoprotein encoded by *mdr* gene has been identified as one of the factors involved in development of drug resistance.[4] It has also been suggested that the high levels of glutathione (GSH) in tumor cells might be associated with drug resistance to alkylating agents and cross-linking agents, including cisplatin and carboplatin.[5, 6] Furthermore, it has recently been reported that glutathione S transferase (GST) activity was elevated in human cell lines resistant to cisplatin with or without the elevation of nonprotein sulfhydryl content, suggesting that GST plays an important role in the development of resistance to cisplatin.[7] However, no information is available on the mechanisms underlying the difference of sensitivity to cisplatin and carboplatin in SCLC and NSCLC cells.

Availability of cloned human gene probes of GST π, which is the placental form of GST, and of *mdr1* has enabled us to examine directly the correlation between the levels of expression of GST π and *mdr1* genes and the sensitivities to cisplatin and carboplatin in human lung cancer cell lines. We report here that SCLC cell lines with high sensitivity to these drugs contained much smaller amounts of mRNA for the GST π gene, but that the amounts of *mdr1* mRNA were not correlated with the sensitivity to cisplatin and carboplatin in the lung cancer cell lines tested.

The 6 lung cancer cell lines used in this study were 3 SCLC cell lines (Lu-134-B-S, Lu-135 and Lu-139), 2 adenocarcinoma cell lines (PC-7 and PC-9), and one large cell carcinoma cell line (PC-13).[9] All the cell lines were cultured in RPMI1640 medium containing 10% fetal calf serum (FCS) at 37° under a humidified atmosphere of 5% CO_2. The cells

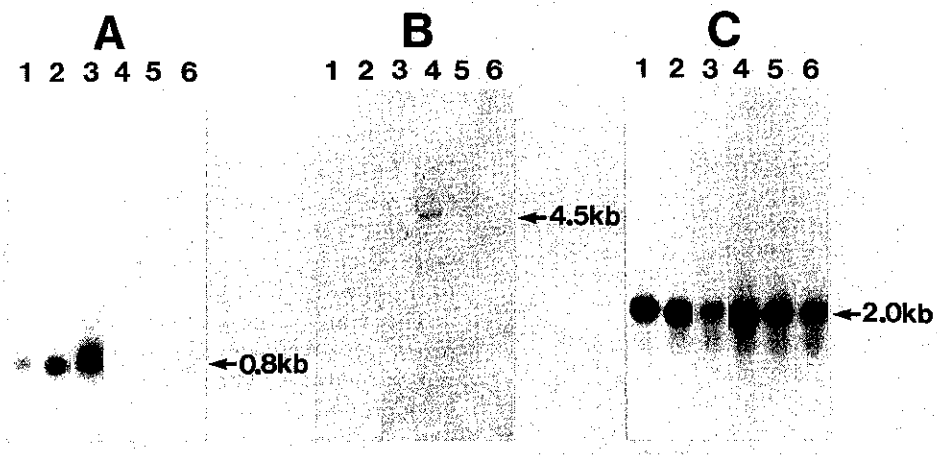

Fig. 1. Expression of GST π gene and *mdr1* gene in human lung cancer cells. Northern blot hybridization analysis on poly(A)$^+$ RNA from 6 human lung cancer cell lines was carried out with human GST π cDNA clone (A), *mdr1* gene (B) or β-actin gene (C) as a probe. After washing out *mdr1* probe, the same filter was rehybridized with β-actin gene: lane 1, PC-7; lane 2, PC-9; lane 3, PC-13; lane 4, Lu-134-B-S; lane 5, Lu-135; lane 6, Lu-139. The size of mRNA in each column was calculated from the locations of 28S and 18S ribosomal RNA.

were harvested at the late logarithmic growth stage and washed with cold phosphate-buffered saline. Total RNA was prepared by the guanidium isothiocyanate-cesium chloride method. Poly(A)$^+$ RNA was obtained by passing total RNA through an oligo(dT) cellulose column.[10] Approximately 2 μg of poly (A)$^+$ RNA was electrophoresed and transferred to nitrocellulose filters. By screening a human placenta cDNA library with rat GST-P cDNA clone as a probe,[11] one GST π cDNA clone termed GSTPi2 was obtained.[12] The GST π gene is a human counterpart of the rat GST-P gene. The 0.7 kb *Eco*RI fragment of GSTPi2 clone and the 0.8 kb *Pvu*II fragment of pMDR1 plasmid DNA[13] were used as specific probes for the GST π gene and *mdr1* gene, respectively. These probes were labeled with [α-^{32}P]dCTP to a specific activity of 2×10^9 cpm/μg DNA using the Multi-priming DNA Labeling System (Amersham Japan). Hybridization was performed for 48 hr under stringent conditions (5$\times$SSC, 50% formamide, 42°). After hybridization, the filters were washed 3 times in 0.1$\times$SSC containing 0.1% SDS at 65° for 15 min. The filter was exposed to film at $-70°$ for 16–20 hr.

Northern blot hybridization analysis on poly(A)$^+$ RNA from 6 lung cancer cell lines with GSTPi2 as a probe showed a single band of 0.8 kb in all the lines (Fig. 1A). The intensity of the band varied in each cell line. In poly(A)$^+$ RNA from the 3 SCLC cell lines only faint bands were visible after 20 hr exposure to the film. In contrast, the levels of GST π mRNA in the 3 NSCLC cell lines were significantly higher than those in the 3 SCLC cell lines. Northern blot hybridization analysis with *mdr* cDNA as a probe showed a faint band at 4.5 kb in poly(A)$^+$ RNA from only one SCLC cell line, Lu-134-B-S (Fig. 1B). The expression of *mdr1* gene in other cell lines was too low to be detected by a one-week exposure. It was reported that normal lung tissue has an intermediate level of *mdr1* mRNA, the same as the colon, liver, jejunum, and rectum.[14]

To confirm that the same amount of intact poly(A)$^+$ RNA was loaded on each lane, the same filter was used for hybridization with β-actin gene probe after washing out the *mdr1* probe (Fig. 1C).

In order to examine the relationship between the sensitivity and the expression of GST π and *mdr1* genes, the IC_{50}s of cisplatin and carboplatin for the SCLC cell lines (Lu-134-B-S, Lu-135 and Lu-139) and the NSCLC cell lines (PC-7, PC-9 and PC-13) were es-

Table I. IC_{50}s of Cisplatin and Carboplatin for SCLC and NSCLC Cell Lines

Cell line		IC_{50} (μg/ml) ± SD[a]	
		Cisplatin	Carboplatin
SCLC	Lu-134-B-S	0.27±0.08	0.80±0.39
	Lu-135	0.25±0.06	0.90±0.71
	Lu-139	0.20±0.11	0.70±0.21
NSCLC[b]	PC-7	1.80±0.55	4.42±1.50
	PC-9	0.95±0.66	4.80±1.23
	PC-13	0.90±0.47	5.42±0.92

a) Sensitivity to the drugs was estimated by colony-forming assay as described in the text. The mean and standard deviation of IC_{50} in three independent experiments are presented.
b) The data were from our previously published results.[1]

timated by means of colony-forming assay as described previously (Table I).[1,2] Briefly, 1 ml of cell suspension in RPMI1640 medium containing 10% FCS and 0.3% agar (cell concentration, $1–3\times10^5$ cells/ml) was plated onto the bottom layer of enriched McCoy's 5A medium containing 0.5% agar in a 35 mm plastic Petri dish. After plating, tumor cells were incubated at 37° in 5% CO_2 in a highly humidified incubator for 12–21 days and the colonies in the Petri dish were counted with an automatic particle counter (Shiraimatsu, Japan).

The ranges of the IC_{50}s of cisplatin and carboplatin for the these SCLC cell lines were 0.20–0.27 μg/ml and 0.70–0.90 μg/ml, respectively, while those for the NSCLC cell lines were previously shown to be 0.90–1.80 μg/ml and 4.8–5.4 μg/ml, respectively (Table I).[1] Thus, the IC_{50}s of these drugs for SCLC cell lines were 3- to 9-fold less than those for NSCLC cell lines. High sensitivity to cisplatin and carboplatin in SCLC cell lines was correlated with low levels of mRNA expression of the GST π gene. These results suggest that the low level of GST π may be responsible for the high sensitivities to the 2 compounds in the SCLC cell lines. However, the amounts of GST π mRNA in the 3 NSCLC cell lines used in this study did not exactly correlate with the resistance to cisplatin and carboplatin. This may be due to the presence of other factors effecting the resistance to those drugs, for example, differences of intracellular concentration of methallothionein, drug uptake and repair of drug-induced DNA damage. On the other hand, the present results clearly showed that the level of *mdr1* mRNA did not correlate with the sensitivity of these lung cancer cell lines to cisplatin and carboplatin, indicating that the P-glycoprotein-mediated efflux system was not in any major way responsible for the difference of sensitivity to these drugs in the cell lines. GSH plays an important role in detoxification of alkylating agents and cross-linking agents and in repair of cellular injury by these drugs. One of the functions of GST is to conjugate these drugs to GSH. Although it is necessary to study further the correlation between GST π mRNA level and the sensitivity to cisplatin and carboplatin in a wide variety of cells, these results suggest that the low levels of GST π in SCLC cells may be an important factor influencing the high sensitivity to cisplatin and carboplatin in SCLC cells.

We thank Igor B. Roninson, Ph.D. (University of Illinois College of Medicine) for providing a DNA probe, pMDR1. We are grateful to Dr. Yukio Shimosato (National Cancer Center Research Institute, Tokyo) and Prof. Yoshihiro Hayata (Tokyo Medical College) for providing lung cancer cell lines. This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, and from the Ministry of Education, Science and Culture, Japan.

(Received Oct. 28, 1987/Accepted Jan. 21, 1988).

REFERENCES

1) Takahashi, H., Sasaki, Y., Saijo, N., Sakurai, M., Nakano, H., Nakagawa, K., Hoshi, A., Jett, J. R. and Hong, W. S. *In vitro* colony inhibition of carboplatin against stomach and lung cancer cell lines in comparison with cisplatin. *Cancer Chemother. Pharmacol.*, **19**, 197–200 (1987).
2) Takahashi, H., Saijo, N., Shinkai, T., Eguchi, K., Sasaki, Y., Tamura, T., Sano, T., Nakano, H., Nakagawa, K., Sakurai, M., Hong, W-S., Hoshi, A. and Hayata, Y. Chemosensitivity test for human small cell lung cancer cell lines *in vitro*. *Jpn. J. Clin. Oncol.*, **16**, 253–260 (1986).

3) Zijlsta, J. G., de Vries, E. G. E. and Mulder, N. H. Multifactorial drug resistance in an adriamycin-resistant human small cell lung carcinoma cell line. *Cancer Res.*, **47**, 1780–1784 (1987).

4) Gros, P., Neriah, Y. B., Croop, J. M. and Housman, D. E. Isolation and expression of a complementary DNA that confers multidrug resistance. *Nature*, **323**, 728–731 (1986).

5) Hamilton, T. C., Winter, M. A., Louie, K. G., Batist, G., Behrens, B. C., Tsuruo, T., Grotzinger, K. R., McCoy, W. M., Young, R. C. and Ozols, R. F. Augmentation of adriamycin, melphalan and cisplatin cytotoxicity in drug resistant and sensitive human ovarian carcinoma cell lines by BSO mediated GSH depletion. *Biochem. Pharmacol.*, **34**, 2583–2586 (1985).

6) Arrick, B. A. and Nathan, C. F. Glutathione metabolism as a determinant of therapeutic efficacy; a review. *Cancer Res.*, **44**, 4224–4232 (1984).

7) Teicher, B. A. Holden, S. A. Kelley, M. J. Shea, T. C. Cucchi, C. A. Roswsky, A., Henner, W. D. and Frei, E., III. Characterization of a human squamous carcinoma cell line resistant to cis-diamminedichloroplatinum(II). *Cancer Res.*, **47**, 388–393 (1987).

8) Wolf, C. R., Hayward, I. P., Lawrie, S. S., Buckton, K., McIntyre, M. A., Adams, D. J., Lewis, A. D. Scott, A. R. R. and Smyth, J. F. Cellular heterogeneity and drug resistance in two ovarian adenocarcinoma cell lines derived from a single patient. *Int. J. Cancer*, **39**, 695–702 (1987).

9) Terasaki, T., Shimosato, Y., Nakajima, T., Tsumuraya, M., Morinaga, S., Hirohashi, S., Yamaguchi, K., Kato, K., Ichinose, H. and Nagatsu, T. Change in cell characteristics due to culture conditions in cell lines from human small cell lung cancer. *Jpn. J. Clin. Oncol.*, **16**, 203–212 (1986).

10) Maniatis, T., Gritsch, E. F. and Sambrook, J. "Molecular Cloning; A Laboratory Manual" (1982). Cold Spring Harbor Laboratory, New York.

11) Suguoka, Y., Kano, T., Okuda, A., Sakai, M., Kitiwada, T. and Muramatsu, M. Cloning and the nucleotide sequence of rat glutathione S-transferase P cDNA. *Nucleic Acids Res.*, **13**, 6049–6057 (1985).

12) 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).

13) Roninson, D. E., Chin, J. E., Choi, K., Gross, P., Housman, D. E., Fojo, A., Shen, D. W., Gottesman, M. M. and Pastan, I. Isolation of human *mdr* DNA sequences amplified in multidrug-resistant KB carcinoma cells. *Proc. Natl. Acad. Sci. USA*, **83**, 4538–4542 (1986).

14) Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M. and Pastan, I. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. USA*, **84**, 265–269 (1987).