

Expression of the Multidrug Resistance Gene in Human Tumors

Rungsa KIM

Department of Surgery, Research Institute for Nuclear Medicine and Biology, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan
(Director: Prof. Tetsuya TOGE)

ABSTRACT

The expression of MDR1 gene was investigated in human solid tumors with respect to adriamycin resistance. Forty fresh human surgical specimens were analyzed by RNA dot blot assay for their expression of the human MDR1 gene and by immunohistological staining using a monoclonal antibody against P-glycoprotein (MDR1 gene product). The MDR1 mRNA level was increased in 11 cases of 40 cancer patients, including three rectal cancers, two breast cancers, two gastric cancers, one colon cancer, one renal cell carcinoma, one gall bladder cancer and one malignant lymphoma of stomach. However, considerable variation of the MDR1 mRNA level was noted among cancers of a specific type. Immunohistochemical studies with the monoclonal antibody were shown to be positive in 18 tumors. In all tumors tested, the MDR1 mRNA level and the immunohistochemical analysis showed a significant correlation. However, two of five tumors which resisted adriamycin treatment were found to be negative in MDR1 transcript, but positive in immunohistological analysis. These results indicate that immunohistochemical analysis would be more sensitive for detecting P-glycoprotein-expression, and that resistance to adriamycin, being multifactorial, can be associated at least, in part with the increased amount of MDR1 gene product.

Key words: *Multidrug resistance gene (MDR1), P-glycoprotein, Adriamycin resistance, Human tumors*

Multidrug resistance (MDR) of tumor cells is a major problem in cancer chemotherapy^{9,16}. Multidrug resistant cells display cross-resistance to a broad range of anticancer agents, such as anthracyclines, vinca alkaloids, etoposide and actinomycin D. The MDR phenotype is correlated with overexpression of the MDR1 gene which encodes for the transmembrane protein, termed P-glycoprotein, with increased drug efflux of anticancer agents from resistant cells^{2,25,30}.

The anthracycline antibiotic, adriamycin, has a broad spectrum anticancer activity against various tumor cells, such as: breast cancer, hepatocellular carcinoma, renal cell carcinoma, gall bladder cancer, esophageal cancer, gastric cancer, and malignant lymphoma⁷. Adriamycin-resistant variants of tumor cells which are selected in vitro demonstrate cross-resistance to vinca alkaloids and actinomycin D. However, the relationship between adriamycin resistance and multidrug resistance in cells derived from human tumors is little known. It is reported that resistance to adriamycin in tumors occurs by several different mechanisms including: decreased accumulation of drugs in cells²⁰, increased intracellular glutathione²⁴, and overexpression of P-glycoprotein^{2,4,6}. The aim of this study was to in-

vestigate whether or not the overexpression of MDR1 mRNA and/or P-glycoprotein would be useful for predicting natural resistance to adriamycin in human solid tumors prior to initial chemotherapy.

MATERIALS AND METHODS

Cell lines

KB C-2 cell of human epidermoid carcinoma, a drug resistant cell line selected by colchicine, and its parental cell line, KB 3-1-2 were used as positive and negative standards for the quantification of MDR1 transcript levels. The cell lines were grown as monolayer culture at 37°C in 5% CO₂ in α -MEM (minimum essential medium) (GIBCO Labo.) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μ g/ml) (Meiji Co., Japan).

Tumor specimens and RNA extraction

Tumor specimens and some of the adjacent normal tissues were obtained at surgery from 40 cancer patients treated between September 1988 and March 1989 (Table 1). All specimens were frozen immediately and stored at -70°C until use. Total cellular RNA was extracted by the guanidium isothiocyanate homogenization of tissues followed by centrifugation through cesium chloride²³.

Table 1. Expression of MDR1 mRNA and P-glycoprotein in 40 cancer patients

Cancer type	No. of patients	No. of positive cases of MDR1 mRNA (%)	No. of positive cases of MRK16 (%)
Esophageal ca.	5	0 (0)	0 (0)
Gastric ca.	12	2 (16.7)	3 (25.0)
Colon ca.	2	1 (50.0)	1 (50.0)
Rectal ca.	4	3 (75.0)	3 (75.0)
Breast ca.	6	2 (33.3)	5 (83.3)
Hepatocellular ca.	2	0 (0)	1 (50.0)
Renal cell ca.	2	1 (50.0)	1 (50.0)
Gall bladder ca.	2	1 (50.0)	2 (100)
Others	5	1 (20.0)	2 (40.0)

Others: Metastatic liver ca., metastatic renal ca., two malignant lymphomas of stomach, and malignant schwannoma.

RNA dot blot analysis

Nitrocellulose filters were presoaked in $5 \times \text{SSC}$ ($1 \times \text{SSC} = 0.15\text{M NaCl}/15\text{mM sodium citrate}$, pH 7.0). Two μg of total RNA were dissolved in 10mM phosphate buffer (pH 7.0) and denatured at 60°C for 7 min in the presence of formaldehyde. The sample was then spotted onto the nitrocellulose filter. After baking at 80°C in a vacuum oven, the filters were prehybridized for 6–8 hours at 42°C in 50% formamide, $5 \times \text{SSC}$, $5 \times \text{Denhardt's solution}$ ($1 \times \text{Denhardt's solution} = 0.02\%$ Ficoll, 0.02% Polyvinylpyrrolidone, and 0.02% acetylated bovine serum albumin), 50mM sodium phosphate (pH 6.5), and 200 $\mu\text{g}/\text{ml}$ of salmon sperm DNA. The filters were then hybridized with the ^{32}P -labeled Pvu II fragment of pMDR1 overnight at 42°C in 50% formamide, $5 \times \text{SSC}$, $1 \times \text{Denhardt's solution}$, 1% dextran sulfate, 100 $\mu\text{g}/\text{ml}$ of salmon sperm DNA, and 20mM sodium phosphate (pH 6.5). The pMDR1 was labeled by ^{32}P by oligolabelling method¹². After hybridization, the filters were washed twice for 15 min with $2 \times \text{SSC}/0.1\%$ sodium dodecyl sulfate (SDS) and twice for 10 min in $0.1 \times \text{SSC}/0.1\%$ SDS at 65°C . The autoradiograms were exposed for 1–3 days. Hybridization of the blot with rRNA probe was performed similarly.

Immunohistological staining with MRK16 MoAb

Immunoperoxidase detection of P-glycoprotein with MRK16 MoAb was performed using the streptavidin-biotin method¹⁹. Briefly, cryostat sections (4–6 μm) were made on solid tumors. After air drying, they were fixed by immersion in 3.7% formaldehyde for 15 min, washed in PBS, and then MRK16 MoAb were applied (10 $\mu\text{g}/\text{ml}$) for 30 min. After washing, the samples were treated with biotinylated goat antimouse second antibody, re-washed in PBS, and further stained with the peroxidase conjugated streptavidin (Bio Genex Labo.). Control slides were also treated in the same way. Thereafter, the sections were colorated with diaminobenzidine (DAB) for 5 min. After a final wash in PBS, they were counterstained with 3% methylgreen and examined in a light microscope.

Chemosensitivity testing to adriamycin

In vivo and *in vitro* sensitivity tests of tumors

to adriamycin were performed with three different ways as described previously²¹. For the *in vivo* tests, the nude mouse isotope assay (NMIA) and the subrenal capsule assay (SRCA) were used. For an *in vitro* test, the adenosine triphosphate inhibition assay (ATPA) was employed.

RESULTS

MDR1 mRNA expression in tumors

The MDR1 mRNA levels of fresh human solid tumors from 40 cancer patients, relative to the level in the drug resistant KB C-2 cells, are shown

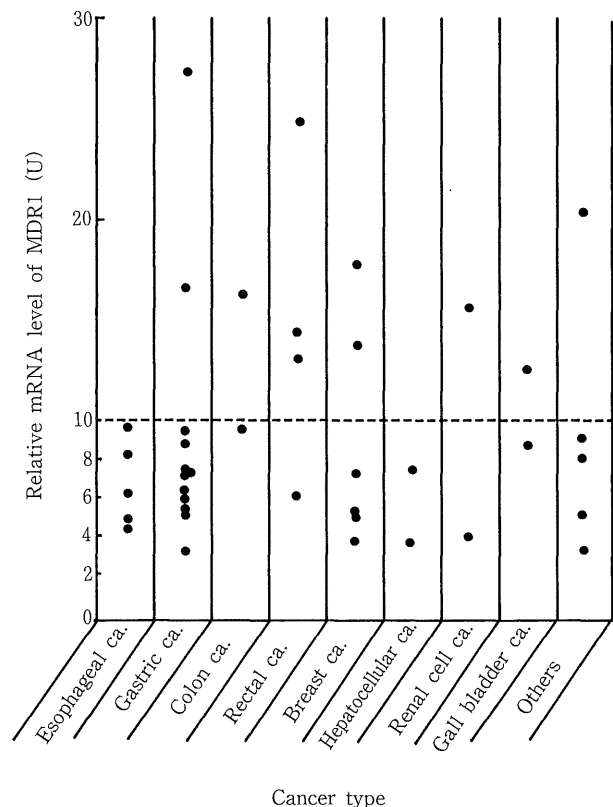


Fig. 1. Distribution of MDR1 mRNA levels in 40 cancer patients.

Quantification of MDR1 expression was measured as relative to the expression of the multidrug-resistant KB C-2 cell line, which was arbitrarily assigned a value of 100U for intensity of 2 μg total RNA (dashed line, MDR1 expression of KB 3-1-2).

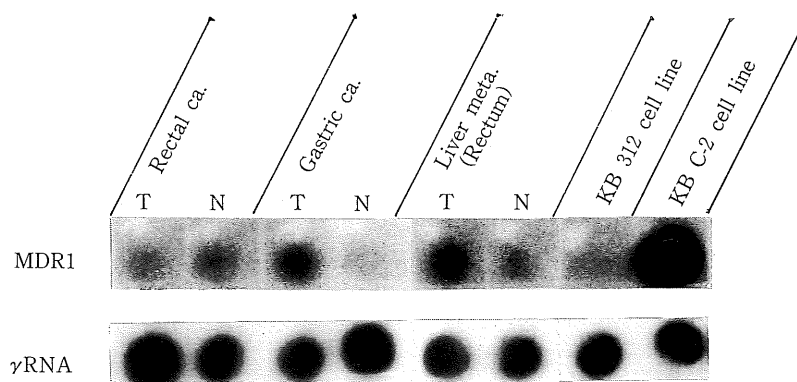


Fig. 2. RNA dot blot analysis of MDR1 gene expression. The filters containing 2 μ g per dot of total RNA from tumor and adjacent normal tissues were hybridized with the following 32 P-labeled probes: MDR1 and rRNA. (T; tumor, N; normal tissue, KB 3-1-2; drug sensitive KB cell line, KB C-2; multidrug resistant KB subline).

in Fig. 1. KB C-2 cells were 42 times more resistant to adriamycin and 100 times more resistant to vinblastine as compared with KB 3-1-2 cells. The MDR1 gene was amplified about 7-fold in KB C-2²¹ and expressed at a high level (Fig. 2). In some experiments, expression of MDR1 mRNA between tumor and normal tissues were studied (Fig. 2). The signal intensity of 2 μ g of KB C-2 total RNA was assigned as an arbitrary value of 100U. The signal from each tumor was measured by densitometer and quantified against that of KB C-2 RNA. The MDR1 mRNA level of more than 10U, which is approximately the same as that of KB 3-1-2, was designated as a positive for the expression of the gene. The quantity of RNA spotted was calibrated by the amount of rRNA. The expression of MDR1 mRNA was detected in 11 cases of 40 tumors, including three rectal cancers, two breast cancers, two gastric cancers, one colon cancer, one renal cell carcinoma, one gall bladder cancer, and one malignant lymphoma of stomach (Table 1). Typical results of a rectal cancer, a gastric cancer and a liver metastasis of rectal cancer are shown in Fig. 2. Considerable variations in the mRNA level among the tumor group positive for MDR1 expression were observed (Fig. 1).

P-glycoprotein expression assayed with MRK16 MoAb

The surgical specimens from 40 cancer patients, representing 12 tumor types, were surveyed for P-glycoprotein expression by immunohistochemical analysis with MRK16 MoAb. None of them received the anticancer agents. The intensity of MRK16 staining was arbitrarily classified according to three categories: negative, intermediate; less than 50% of the tissues were stained positively, and strong; most of the tissues were stained positively. Multidrug resistant cell line of KB C-2 reacted strongly with MRK16 MoAb, while KB 3-1-2 cells, a parental cell line did weakly (Fig. 3; a, b). It was found that 18 tumors reacted positively with MRK16 MoAb, although some of these tumors

showed low levels of MDR1 mRNA, including one of 3 gastric cancers, three of 5 breast cancers, one hepatocellular carcinoma, one of 2 gall bladder cancers, and one malignant lymphoma of stomach (Table 1). The correlation between MDR1 mRNA level and intensity of MRK16 staining was statistically significant (Fig. 4).

MDR1 mRNA expression, P-glycoprotein expression and resistance to adriamycin in chemosensitivity testing

Among the 40 tumors studied above, the individual chemosensitivity tests were performed on 30 tumor samples. The resistance to adriamycin was observed in 23 of 25 cases (92.0%) tested by NMIA, 23 of 25 cases (92.0%) by ATPA, and 13 of 17 cases (76.5%) by SRCA, respectively. Of these sensitivity negative cases, MDR1 gene expression at the level of mRNA was observed in 30.4% for NMIA, 30.4% for ATPA, and 38.5% for SRCA, respectively. P-glycoprotein expression was detected in 34.8% by NMIA, 46.2% by SRCA, and 36.4% by ATPA, respectively (Table 2). There was no significant correlation between MDR1 mRNA expression, P-glycoprotein expression and resistance to adriamycin in each assay.

MDR1 mRNA expression, P-glycoprotein expression and clinical response to adriamycin

Clinical response was evaluated according to criteria of the Japanese Society for Cancer Therapy²², retrospectively. Among 40 cancer patients tested, five patients showed no clinical response to adriamycin treatment. The relationship between MDR1 mRNA expression, P-glycoprotein expression and the clinical response to adriamycin in these 5 patients is summarized in Table 3. Among them, One patient (No.1) was observed in MDR1 mRNA expression. Three (No.1–3) were stained positively with MRK16 MoAb (Fig. 3; No.1, No.3), whereas the MDR1 mRNA expression could not be observed in two patients (No.2,3). Although the MDR1 mRNA and P-glycoprotein expression were not detected in the other two patients

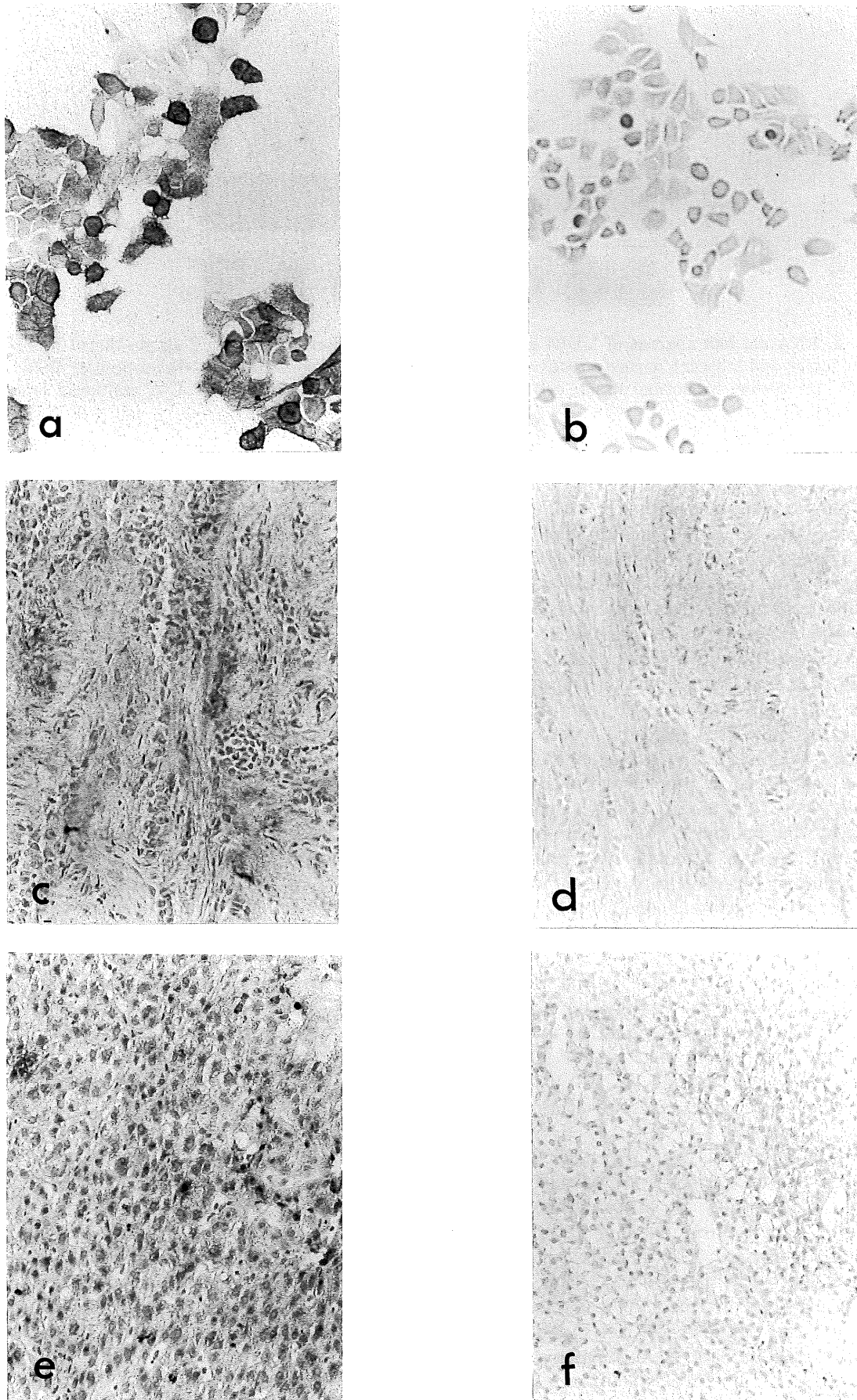


Fig. 3. Immunohistochemical staining of P-glycoprotein on the KB cell lines and untreated human tumors by MRK16 MoAb. a; KB C-2 cells, b; KB 3-1-2 cells, c; breast cancer, d; negative control, e; hepatocellular carcinoma, f; negative control, original magnification $\times 500$

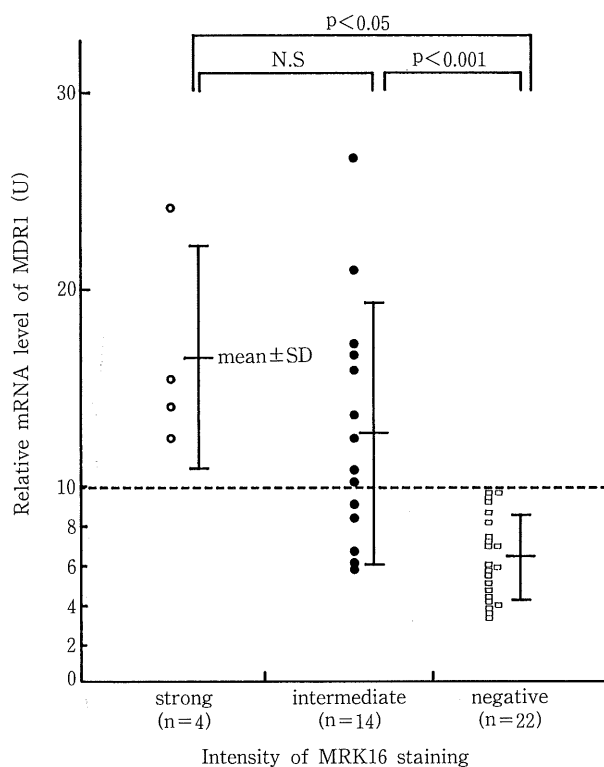


Fig. 4. Correlation between MDR1 mRNA level and intensity of MRK16 staining. The intensity of MRK16 staining is classified as follows: negative, intermediate; less than 50% of the tissues were stained positively, strong; most of the tissues were stained positively.

(No.4,5), these failed to respond to adriamycin treatment.

DISCUSSION

The resistance to anthracyclines such as adriamycin presents a major problem for the successful treatment of many human cancers. This is particularly important clinically, because in addition to the development of anthracycline resistance, patients often simultaneously develop resistance to other classes of anticancer agents (multiple drug resistance; MDR). Multidrug resistance has been studied extensively in rodent and human tumor cell lines^{8,27}. A number of MDR cell lines have been isolated to show a good correlation between the level of MDR1 gene amplification, the increased MDR1 mRNA expression, and the degree of MDR¹⁰. However, exceptions have been reported⁴. Overexpression without gene amplification can also be observed^{4,15,28}. Overexpression of P-glycoprotein without MDR1 gene amplification has been reported in MDR human sarcoma cells²⁸, and human breast cancer cells¹⁵. In the previous study²¹, in order to predict natural resistance to adriamycin, we investigated 50 fresh human surgical specimens using Southern blot analysis, and found that there was no amplification of the MDR1 gene in any of the tumors tested. Five of these cancer patients showed no response to adriamycin clinically. Our data suggested that MDR1 gene amplification is rarely seen among clinical samples with natural

Table 2. Correlation between MDR1 mRNA expression, P-glycoprotein expression and resistance to adriamycin assayed with three chemosensitivity tests

Assays	+/P	-/P	+/N	-/N	Expression of MDR1 mRNA or P-glycoprotein /N (%)
NMIA	1 [1]	1 [1]	7 [8]	16 [15]	7/23 (30.4) 8/23 (34.8)
SRCA	0 [1]	4 [3]	5 [6]	8 [7]	5/13 (38.5) 6/13 (46.2)
ATPA	0 [1]	2 [2]	7 [8]	16 [14]	7/23 (30.4) 8/22 (36.4)

[] : assayed with MRK16 MoAb
 NMIA : nude mouse isotope assay
 SRCA : subrenal capsule assay

ATPA : adenosine triphosphate inhibition assay
 P, N : sensitivity positive or negative
 +, - : expression of MDR1 mRNA or P-glycoprotein

Table 3. Correlation between MDR1 mRNA expression, P-glycoprotein expression and clinical response to adriamycin in 5 cancer patients

Patient no.	Cancer type	Response*	MDR1 mRNA expression	P-glycoprotein expression
1	Breast ca.	NC	+	+
2	Breast ca.	NC	-	+
3	Hepatocellular ca.	PD	-	+
4	Metastatic liver ca. (breast)	PD	-	-
5	Hepatocellular ca.	PD	-	-

* Clinical response: NC (no change), PD (progressive disease)

resistance to adriamycin. These results suggest that the level of P-glycoprotein is transcriptionally or translationally regulated⁶, and in clinical application, MDR1 mRNA level was more significant than MDR1 gene amplification. Thus, overexpression of P-glycoprotein has been proposed as a major cause of multidrug resistance in clinical cases^{3,6,26,31}.

In this study, the level of MDR1 mRNA and P-glycoprotein was measured in 40 human solid tumor samples, and compared to that of drug resistant KB C-2 and its parental cells. Some of these results were also compared with those of chemosensitivity tests and clinical response to adriamycin of the corresponding patients. There was no correlation between MDR1 mRNA expression, P-glycoprotein expression and natural resistance to adriamycin in the chemosensitivity tests. This might suggest that there is another mechanism of adriamycin resistance apart from the case of P-glycoprotein overexpression. The discrepancy between the expression of MDR1 mRNA and P-glycoprotein could be mainly due to the low sensitivity of dot blot analysis to detect the expression of MDR1 mRNA because of variable amounts of stromal cells in cancer tissues.

Clinically, five patients displayed no response to adriamycin. One of 2 breast cancers in which MDR1 mRNA level was not detected, showed P-glycoprotein expressing cancer cells histologically. Moreover, one of 2 hepatocellular carcinoma showed a low level of MDR1 mRNA, while it had P-glycoprotein expression detected by MRK16 MoAb. These results indicate that the natural resistance to adriamycin is associated at least in part with the increased expression of MDR1 gene, and the expression of P-glycoprotein rather than MDR1 mRNA level show better clinical relevance to natural resistance to adriamycin in these tumors. Furthermore, we observed that two patients, whose tumors had extremely low expression of MDR1 mRNA and P-glycoprotein, were refractory to adriamycin treatment. This evidence suggests that there might be other mechanisms responsible for adriamycin resistance, such as an increase in intracellular levels of glutathione¹, a rapid onset of DNA repair³², or a decrease in topoisomerase II activity¹³.

The MDR1 gene expression has been reported in various normal human tissues; high or intermediate levels in adrenal gland, kidney, lung, liver, jejunum, colon and rectum, and low in many other organs¹⁴. However, within the group of cancers with high or intermediate MDR1 mRNA levels, it was reported that there were considerable variations of MDR1 mRNA levels in these tumors¹⁷. Our data also showed that there was considerable variation from cancer to cancer with high or intermediate MDR1 mRNA levels. These variations in MDR1 mRNA expression may be due to several factors, such as tumor heterogeneity, tissue origin,

histological type, and resistant subpopulations in cancer tissues. Therefore, to investigate the existence of heterogeneous expression of MDR1 gene, immunohistochemical studies of tumor specimens may be useful in distinguishing the differential expression of various cell subpopulations.

Interestingly, MDR1 gene expression was observed at lower levels in breast and gastric cancers. However, our data showed that the level of MDR1 expression in some breast and gastric cancers were higher than those in the adjacent normal tissues. It has been previously reported that the overexpression of P-glycoprotein RNA was demonstrated in carcinogen-induced, preneoplastic and neoplastic rat liver nodules^{11,29}. Thus, when some normal cells were transformed, the activation of an oncogene and MDR1 RNA expression may occur simultaneously.

In conclusion, we can say that the natural resistance to adriamycin in human solid tumors may be multifactorial. The ultimate clinical significance of P-glycoprotein will require more extensive and controlled studies. Such investigations should compare the levels of P-glycoprotein expression with the disease outcome and with defined chemotherapy in a prospective clinical protocol. Our own data suggest that natural resistance to adriamycin apparently exists in many human solid tumors and that it could be associated, at least in part, with the increased amount of MDR1 gene product.

ACKNOWLEDGEMENTS

The author is grateful to Professor Tetsuya Toge, Professor K. Yokoro and Dr. O. Niwa for their helpful guidance. I would like to also thank Dr. T. Tsuruo (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research) and Dr. S. Akiyama (Institute of Cancer Research, Kagoshima University) for kindly providing monoclonal antibody (MRK16), cells and DNA probes.

(Received May 22, 1990)

(Accepted July 19, 1990)

REFERENCES

1. **Batist, G., Tulpule, A., Shinha, B. K., Katki, A. G., Meyers, C. H. and Cowan, K. H.** 1986. Overexpression of a novel anionic glutathione transferase in multidrug resistant human breast cancer cells. *J. Biol. Chem.* **261**: 15544-15549.
2. **Beck, W. T.** 1987. The cell biology of multidrug resistance. *Biochem. Pharmacol.* **36**: 2879-2887.
3. **Bourhis, J., Goldstein, L. J., Riou, G., Pastan, I., Gottesman, M. M. and Benard, J.** 1989. Expression of a human multidrug resistance gene in ovarian carcinoma. *Cancer Res.* **49**: 5062-5065.
4. **Bradley, G., Juranka, P. F. and Ling, V.** 1988. Mechanism of multidrug resistance. *Biochimica. Biophysica. Acta* **948**: 87-128.
5. **Bradley, G., Naik, M. and Ling, V.** 1989. P-glycoprotein expression in multidrug-resistant human

- ovarian carcinoma cell lines. *Cancer Res.* **49**: 2790-2976.
6. Broxterman, H. J., Pinedo, H. M., Kuiper, C. M., Hoeven, J. J. M., Lange, P., Quak, J. J., Scheper, R. J., Keizer, H. G., Schuurhuis, G. J. and Lankelma, J. 1989. Immunohistochemical detection of P-glycoprotein in human tumor cells with a low degree of drug resistance. *Int. J. Cancer* **43**: 340-343.
 7. Carter, S. K. 1975. Adriamycin - a review. *J. Natl. Cancer Inst.* **55**: 1265-1274.
 8. Chen, C. J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M. and Roninson, I. B. 1986. Internal duplication and homology with bacterial transporter proteins in the *mdr1* (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* **47**: 381-389.
 9. Curt, G. A., Clendeninn, N. J. and Chabner, B. A. 1984. Drug resistance in cancer. *Cancer Treat. Rep.* **68**: 87-99.
 10. Endicott, J. A. and Ling, V. 1989. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu. Rev. Biochem.* **58**: 137-171.
 11. Fairchild, C. R., Percy Ivy, S., Rushmore, T., Lee, G., Koo, P., Goldsmith, M. E., Myers, C. E., Farber, E. and Cowan, K. H. 1987. Carcinogen-induced *mdr* overexpression is associated with xenobiotic resistance in rat liver preneoplastic liver nodules and hepatocellular carcinoma. *Proc. Natl. Acad. Sci.* **84**: 7701-7705.
 12. Feiberg, A. P. and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6-13.
 13. Ferguson, P. J., Fisher, M. H., Stephenson, J., Li, D., Zhou, B. and Cheng, Y. 1988. Combined modalities of resistance in etoposide-resistant human KB cell lines. *Cancer Res.* **48**: 5956-5964.
 14. Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M. and Pastan, I. 1987. Expression of a multidrug resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci.* **84**: 265-269.
 15. Fuqua, S. A. W., Moretti-Rojas, I. M., Schneider, S. L. and McGuire, W. L. 1987. P-glycoprotein expression in human breast cancer cells. *Cancer Res.* **47**: 2013-2016.
 16. Gerlach, J. H., Kartner, N., Bell, D. R. and Ling, V. 1986. Multidrug resistance. *Cancer Surv.* **5**: 24-46.
 17. Goldstein, L. J., Galski, H., Fojo, A., Willingham, M., Lai, S. L., Gazdar, A., Pirker, R., Green, A., Crist W., Brodeur, G. M., Lieber, M., Cossman, J., Gottesman, M. M. and Pastan, I. 1989. Expression of a multidrug resistance gene in human cancers. *J. Natl. Cancer Inst.* **81**: 116-124.
 18. Hamada, H. and Tsuruo, T. 1986. Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistance tumor cells as revealed by monoclonal antibodies. *Proc. Natl. Acad. Sci.* **83**: 7785-7789.
 19. Hsu, S. M., Raine, L. and Fanger, H. 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabelled antibody (PAP) procedure. *J. Histochem. Cytochem.* **29**: 579-580.
 20. Keizer, H. G., Schuurhuis, G. J., Broxterman, H. J., Lankelma, J., Schoonen, W. G. E. J., Rijn, J., Pinedo, H. M. and Joenje, H. 1989. Correlation of multidrug resistance with decreased drug accumulation, altered subcellular drug distribution, and increased P-glycoprotein expression in untreated SW-1573 human lung tumor cells. *Cancer Res.* **49**: 2988-2993.
 21. Kim, R., Saeki, T., Takagami, S., Kirihara, Y., Jinushi, K., Nishiyama, M., Niimoto, M., Hattori, T. and Okada, K. 1990. Prediction of the resistance of human tumors to adriamycin by chemosensitivity tests and DNA analysis of the multidrug resistance gene. *Jpn. J. Surg.* **20**: 192-196.
 22. Koyama, Y. 1986. Criteria of Japanese Society for Cancer Therapy. *Jpn. J. Cancer Chemotherapy* **13**: 391-396 (in Japanese).
 23. Maniatis, T., Fritsch, E. F. and Sambrook J. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
 24. Moscow, J. A., Fairchild, C. R., Madden, M. J., Ranson, D. T., Wieand, H. S., O'Brien, E. E., Poplack, D. G., Cossman, J., Myers, C. E. and Cowan, K. H. 1989. Expression of anionic glutathione-S-transferase and P-glycoprotein genes in human tissues and tumors. *Cancer Res.* **49**: 1422-1428.
 25. Riordan, J. R., Deuchars, K., Kartner, N., Alon, N., Trent, J. and Ling, V. 1985. Amplification of P-glycoprotein genes in multidrug resistance mammalian cell lines. *Nature (London)* **316**: 817-819.
 26. Salmon, S. E., Grogan, T. M., Miller, T., Scheper, R. and Dalton, W. S. 1989. Prediction of doxorubicin resistance in vitro in melanoma, lymphoma, and breast cancer by P-glycoprotein staining. *J. Natl. Cancer Inst.* **81**: 696-701.
 27. Scotto, K. W., Biedler J. L. and Melera, P. W. 1986. Amplification and expression of genes associated with multidrug resistance in mammalian cells. *Science* **232**: 751-755.
 28. Scudder, S. A., Roninson, I. B., Davatellis, G., Fukumoto, M. and Sikic, B. 1987. Increased expression of the *mdr1* gene in multidrug resistant variants of human sarcoma cells. *Proc. Am. Assoc. Clin. Oncol.* **6**: 13-18.
 29. Thorgeirsson, S. S., Huber, B. E., Sorrell, S., Fojo, A., Pastan, I. and Gottesman, M. M. 1987. Expression of the multidrug-resistant gene in hepatocarcinogenesis and regenerating rat liver. *Science* **236**: 1120-1122.
 30. Ueda, K., Clark, D. P., Chen, C., Roninson, I. B., Gottesman, M. M. and Pastan, I. 1987. The human multidrug resistance (*mdr1*) gene. *J. Biol. Chem.* **262**: 505-508.
 31. Volm, M., Efferth, T., Bak, M., Ho, A. D. and Mattern, J. 1989. Detection of the multidrug resistant phenotype in human tumors by monoclonal antibodies and the streptavidin-biotinylated phycoerythrin complex method. *Eur. J. Cancer Clin. Oncol.* **25**: 743-749.
 32. Yin, M. B., Bankusli, I. and Rustum, Y. M. 1989. Mechanism of the in vivo resistance to adriamycin and modulation by calcium channel blockers in mice. *Cancer Res.* **49**: 4729-4733.