Expression of Drug Resistance-related Genes in Head and Neck Squamous Cell Carcinoma and Normal Mucosa

Shitau Hirata,1, 4 Osamu Katoh,3 Tetsuya Oguri,2 Hiromitsu Watanabe1 and Koji Yajin1

1Department of Otorhinolaryngology, 2Second Department of Internal Medicine, Hiroshima University Faculty of Medicine, 3Department of Environment and Mutation, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553

We examined the expression levels of mRNA for multidrug resistance 1 (MDR1), multidrug resistance-associated protein (MRP), human canalicular multispecific organic anion transporter (cMOAT), lung resistance-related protein (LRP), topoisomerase IIα, β (Topo IIα, β) and topoisomerase I (Topo I) genes in human head and neck squamous cell carcinoma (HNSCC) specimens and mucosa (HNM) specimens, to elucidate their roles in relation to the biological characteristics and drug resistance in vivo. Fifty-eight samples (45 head and neck carcinomas and 13 head and neck mucosa) obtained during surgical resection or biopsy from 38 patients were analyzed using the quantitative reverse transcription-polymerase chain reaction (RT-PCR) method. MDR1, MRP, LRP, Topo IIα, Topo IIβ, and Topo I gene transcripts were detected in all the samples tested, but cMOAT mRNA was not detected in them. Comparisons of the expression levels in HNSCC with those in HNM showed that the Topo IIα gene expression level was higher in HNSCC than in HNM (P=0.0298). Moreover, the Topo IIα mRNA level was significantly higher in metastatic lymph node samples of HNSCC than in HNM samples (P=0.0205). There were no significant differences in the six genes’ expression levels between samples exposed to platinum drugs and those not exposed to platinum drugs. These results suggest that it may be effective in anticancer therapy to use topoisomerase-targeting drugs against HNSCC, especially metastatic neck tumors, and that the expression of these genes in HNSCC is not associated with platinum drug exposure.

Key words: DNA topoisomerase II — Drug resistance-related gene — Head and neck squamous cell carcinoma

Chemotherapy has become a very important option for the treatment of head and neck squamous cell carcinomas (HNSCC) in order to preserve the patient’s physical functions. However, some advanced, recurrent or metastatic HNSCCs have multi-drug resistance. Drug resistance may be caused by altered membrane permeability, changes in the host’s drug metabolism and detoxifying pathways, and/or alterations in DNA replication-related enzymes.

A P-glycoprotein encoded by the multidrug resistance 1 (MDR1) gene and a multidrug resistance-associated protein encoded by the multidrug resistance-associated protein (MDR1) gene function as transmembrane drug efflux pumps.1–4 The human canalicular multispecific organic anion transporter (cMOAT) gene is a newly discovered member of the ABC transporter superfamily and has been suggested to participate in platinum drug transport.5 Furthermore, the LRP (lung resistance-related protein) gene, encoding for the human vaults protein, may mediate nucleocytoplasmic and vesicular transport of drugs.6 It is speculated that overexpression of these genes causes cancer cells to become resistant to anticancer drugs.7–9 DNA topoisomerases are nuclear enzymes that play essential roles in DNA replication, transcription, chromosome segregation, and DNA recombination.10 All cells have two major forms of topoisomerases, i.e. type I (Topo I) and type II (Topo II), and Topo I or Topo II induce single or double strand breaks, respectively.10–12 There are two isoforms of Topo II, a 170-kDa isoform (Topo IIα) and a 180-kDa isoform (Topo IIβ), which may have different functions due to the difference in their expression during cell-cycle progression10, 13 and their differential distribution.14 Topoisomerases have been shown to be targets for clinically important anticancer agents.10, 15 It was also reported that Topo IIα or Topo I gene expression increased markedly in malignant tumors.16–21 Furthermore, it has recently been demonstrated that MDR1 and MRP play important roles in resistance to Topo II inhibitors such as etoposide or adriamycin.8, 22, 23

Several studies have examined drug resistance in clinical specimens of the lung,17–19, 24–27 kidney or bladder,28, 29 breast30, 31 and ovaries16, 32, 33 using immunohistochemistry or molecular biological methods. However, it remains to be elucidated whether these factors are related to the drug resistance of head and neck carcinomas. In order to clarify the roles of these drug resistance-related genes in the char-
acusteristics of tumors, we analyzed the expression levels of mRNA for MDR1, MRP, cMOAT, LRP, Topo IIα, Topo IIβ and Topo I in HNSCC specimens in comparison with those in head and neck mucosa (HNM) specimens.

MATERIALS AND METHODS

Patients and samples Fifty-eight samples (45 head and neck squamous cell carcinomas and 13 head and neck mucosa) from 38 patients admitted to Hiroshima University Hospital between April 1997 and August 1998 were studied. Fresh specimens of HNSCC and HNM were obtained during surgical resection or biopsy after informed consent had been obtained. The tissues were frozen in liquid nitrogen and stored at −80°C until analyzed.

Reverse transcription-polymerase chain reaction (RT-PCR) Total cellular RNA was extracted using the guani-dinium isothiocyanate-phenol method, and cDNA was synthesized using a random hexamer (Amersham, Buckinghamshire, UK) with Superscript RNase H-reverse transcriptase (GIBCO-BRL, Bethesda, MD), as described previously.24) PCR amplification The reverse-transcribed cDNA from each sample was subjected to PCR amplification using primers based on the MDR1, MRP, cMOAT, LRP, Topo IIα, Topo IIβ, Topo I, and β-actin (internal control) gene sequences. After pre-denaturation at 94°C for 5 min, the cDNA was added to 5 µl of PCR mixture, comprising 1 µl of 10× PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KC1), 1 µl of 15 mM MgCl2, 2 µl of distilled water, 0.2 µl of 20 mM dNTPs (Takara, Tokyo), 0.2 µl of 50 µM forward primer, 0.2 µl of 50 µM backward primer, and 0.4 µl (0.2 U) of Taq polymerase (Promega, Madison, WI). The sequences of the primers used were as follows: MDR1 forward 5′-CTAATAAGAAAGAGATCACT-3′ and reverse 5′-GGCTAGAAGACATGAAAAAACA-3′-8); MRP forward 5′-TGGGACTGGAATGTCACG-3′ and reverse 5′-AGGAATATGCCCGACTTC-3′24); cMOAT forward 5′-CTAATCAGCCTACTCCTGC-3′ and reverse 5′-CTGAGCAGTGAGCGCC-3′24); LRP forward 5′-TTCTGATTGGTGACGC-3′ and reverse 5′-ACTTCTCCTCCTGGACCA-3′27); Topo IIα forward 5′-ACCATTGCGTCCACTTG-3′ and reverse 5′-TCCATATGCTTACGATAACAA-3′, and reverse primer as above, LRP forward primer as above, and reverse primer 5′-TCCACCTTTGGCCGATGCG-3′. We used the PCR products for Topo I and β-actin probes with the following sequences: Topo IIα forward 5′-AACGATTAGCAGCGCTTG-3′, and reverse primer as above, LRP forward primer as above, and reverse primer 5′-TCACTACCTTGGACATGCG-3′. We used the PCR products for MDR1 and LRP probes with the following sequences: MDR1 forward primer 5′-AACGTTAGTACCAAAAGAGGCCTATG-3′, and reverse primer as above, LRP forward primer as above, and reverse primer 5′-TCCACTTTGGCCGATGCG-3′. We used the PCR products for Topo IIα and β-actin probes with the following sequences: Topo IIα forward 5′-GAATTCGCGCATGATAACAA-3′, and reverse primer 5′-GTCTTGTGTTACGATGCG-3′, β-actin forward primer, as above, and reverse primer 5′-AATGGTGATGGACGATGCG-3′ as described previously.24) The radioactivity level was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). The PCR products of MRP, cMOAT, Topo IIα and Topo IIβ described above were used as cDNA probes, and we designed and synthesized PCR products for the MDR1 and LRP probes with the following sequences: MDR1 forward primer 5′-AACGATTAGCAGCGCTTG-3′, and reverse primer as above, LRP forward primer as above, and reverse primer 5′-TCACTACCTTGGCCGATGCG-3′. We used the PCR products for Topo I and β-actin probes with the following sequences: Topo I forward primer 5′-GAATTCGCGCATGATAACAA-3′, and reverse primer 5′-GTCTTGTGTTACGATGCG-3′, β-actin forward primer, as above, and reverse primer 5′-AATGGTGATGGACGATGCG-3′ as described previously.24) The radioactivity associated with gene expression in each sample was expressed as the yield of the target gene relative to that of the β-actin gene.

Statistical analysis The statistical significance of differences between the expression levels of each mRNA in tissue samples was analyzed with the Mann-Whitney U-test. All the gene expression levels were skewed toward higher expression, and were subjected to logarithmic transformation so that they approximated more closely to a normal distribution. The statistical calculations and tests were performed using Stat View J 4.11 software (Abacus, CA) and followed by a final incubation at 72°C for 7 min. In order to determine the optimal number of amplification cycles, the accuracy of the quantitative PCR procedure was tested in a titration experiment as described previously.24) The optimal number of cycles for MDR1, MRP, cMOAT and Topo I was 24, for LRP, 28 and for Topo IIα and Topo IIβ, 25. The PCR products were 243, 293, 275, 285, 588, 583 and 247 base pairs (bp) long, corresponding to MDR1, MRP, cMOAT, LRP, Topo IIα and Topo IIβ and Topo I cDNA respectively. We used the β-actin gene as an internal control. The sequences of its primers were: forward 5′-AAGAGGAGCCATCCTACCT-3′ and reverse 5′-TACATGCTGGGCTTGTA-3′. The PCR conditions were as follows: denaturation (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 1 min) for 20 amplification cycles, followed by a final incubation at 72°C for 7 min. The PCR products were 218 bp long. We found that expression levels of β-actin gene from all samples were similar as evaluated by ethidium bromide staining, and therefore, we concluded that the quality of harvested RNA from our samples was acceptable for molecular analysis as described previously.24) Quantitative analysis of PCR products and analysis of mRNA expression The PCR products were electrophoresed with 2% (w/v) agarose gels, transferred to nylon membranes (Hybond N; Amersham) and subjected to hybridization analysis with 32P-labeled cDNA probes using procedures described previously.24) Each filter was washed and the radioactivity level was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). The PCR products of MRP, cMOAT, Topo IIα and Topo IIβ described above were used as cDNA probes, and we designed and synthesized PCR products for the MDR1 and LRP probes with the following sequences: MDR1 forward primer 5′-AACGATTAGCAGCGCTTG-3′, and reverse primer as above, LRP forward primer as above, and reverse primer 5′-TCACTACCTTGGCCGATGCG-3′. We used the PCR products for Topo I and β-actin probes with the following sequences: Topo I forward primer 5′-GAATTCGCGCATGATAACAA-3′, and reverse primer 5′-GTCTTGTGTTACGATGCG-3′, β-actin forward primer, as above, and reverse primer 5′-AATGGTGATGGACGATGCG-3′ as described previously.24) The radioactivity associated with gene expression in each sample was expressed as the yield of the target gene relative to that of the β-actin gene.
RESULTS

Patients’ characteristics  We analyzed 58 samples (45 HNSCC and 13 HNM) from 38 patients using the RT-PCR methods. The patients’ characteristics are presented in Table I. There were 30 men and 8 women. There were 29 men and 7 women in the HNSCC group, and 9 men and 3 women in the HNM group. They ranged in age from 40 to 88 years old in the HNSCC, and from 36 to 88 years old in the HNM. Twenty-six samples had been exposed to platinum drugs, and 22 samples had been treated with radiation therapy. Thirty-seven samples were obtained from patients with a primary site in HNSCC, and 8 samples were obtained from patients with metastatic cervical lymph nodes in HNSCC. Twenty-nine samples were obtained at diagnostic biopsy, and 29 samples were obtained from surgery.

Expression levels of the seven genes  

MDR1, MRP, LRP, Topo IIα, Topo IIβ, and Topo I gene transcripts were detected in all the samples tested, but cMOAT gene transcript was not detected in any of them. Representative autoradiographs of RT-PCR for the 7 genes, and for β-actin gene in HNSCC and HNM samples are shown in Fig. 1. There was considerable variation among tumors and normal mucous membrane tissues in the expression levels of the six genes. The median and range expression levels of the six genes except cMOAT gene are summarized in Table II. The expression levels of Topo IIα mRNA in HNSCC were significantly higher than those in HNM (P=0.0298, Table II). On the other hand, the differences in the expression levels for the other five genes in HNSCC and HNM were not significant (Table II). Then, we compared the levels of expression of Topo IIα mRNA among the metastatic lymph node samples, primary carcinoma samples and normal mucosal samples. The median expression levels of Topo IIα were 0.625 (0.121–2.542) in the metastatic lymph node samples (N=8), 0.351 (0.011–2.848) in the primary carcinoma samples (N=37) and 0.136 (0.006–0.694) in the normal mucosal samples.
Drug Resistance-related Gene in Head and Neck Carcinoma

Table III. Median Values of Topo IIα Gene Expression in Normal Mucosa, Metastatic Lymph Nodes and Primary Site

|       | Muα | Lyβ | Prα |
|-------|-----|-----|-----|
| N     | 13  | 8   | 37  |
|       | 0.136a | 0.625 | 0.351 |
|       | (0.006–0.694)a | (0.121–2.542) | (0.011–2.848) |

Mu vs. Ly, \( P=0.025 \); Ly vs. Pr, \( P=0.13 \) (NS); Mu vs. Pr, \( P=0.0616 \) (NS).

a) Mucosa samples.

b) Metastatic lymph node samples.

c) Primary carcinoma samples.

d) Medians.

e) Ranges.

P-values <0.05 were considered to be significant.

NS, not significant.

HNM (N=13) (Table III). The expression levels in the metastatic lymph nodes were significantly higher than those in the HNM (P=0.0205), and the expression levels in the primary carcinoma samples were relatively higher than those in the normal mucosal samples (P=0.0616) (Table III). In contrast, there was no difference between the expression levels in the metastatic lymph node samples and those of the primary carcinoma samples (P=0.1300) (Table III). The differences in the expression levels for the other five genes among the metastatic lymph node samples, primary carcinoma samples and normal mucosal samples were not significant (data not shown). We also compared the expression levels of these six genes between samples exposed to platinum drugs and those not exposed to platinum drugs. There were, however, no differences between the expression levels of these six genes in the previously treated and non-treated groups in both HNSCC and HNM (data not shown).

DISCUSSION

Topoisomerases are the targets of several anticancer drugs, but there has been no report on topoisomerases in head and neck carcinomas. This paper is the first, as far as we know, to provide detailed data about the steady-state levels of mRNA for Topo IIα, Topo IIβ and Topo I in head and neck clinical carcinoma specimens based on molecular-biological methods. Our data showed that the level of Topo IIα expression in HNSCC was significantly higher than that in HNM, and furthermore, that the level of Topo IIα expression in metastatic lymph node samples was significantly higher than that in HNM, while the expression level in the primary carcinoma samples was relatively higher than that in HNM, whereas no such difference was observed for Topo IIβ and Topo I.

A higher expression level of Topo IIα in tumors compared with normal tissues has been reported in lung cancer, ovarian cancer and breast cancer. High expression levels of the α isoform were seen in rapidly proliferating cells, in contrast to the β isoform, which did not vary in resting cells or through the cell cycle. Moreover, Giaccone et al. reported that higher expression levels of Topo IIα were associated with higher expression of Ki-67, a cell proliferation marker, whereas no correlation was found between expressions of Ki-67 and Topo IIβ or Topo I in non-small cell lung cancer. Furthermore, it was demonstrated that increased Topo II gene expression showed a strong positive correlation with cell sensitivity to Topo II inhibitors in lung cancer cell lines. In fact, small cell lung cancer (SCLC) is more sensitive to Topo II inhibitors than non-small cell lung cancer (NSCLC) in clinical situations, and Syahruddin et al. demonstrated that the expression levels of the Topo IIα gene in SCLC were significantly higher than those in NSCLC. Thus, it appears that Topo II inhibitors can be selective for tumors with higher Topo IIα expression, assuming that Topo IIα is the principal target enzyme. On the other hand, Yamazaki et al. reported that the sensitivity of lung cancer cell lines to Topo II inhibitors can not be explained by the Topo IIα content levels or Topo II catalytic activity. Sandri et al. indicated that Topo IIβ may play a significant role as a target for anti-tumor therapy, because the Topo IIβ protein is more widely expressed than the Topo IIα protein in breast cancer cells. Further studies are required to determine whether the level of Topo IIα or Topo IIβ expression can be good markers of sensitivity to Topo II inhibitors. Furthermore, our findings suggest that although Topo II inhibitors have not been used in head and neck cancer chemotherapy, the higher expression of Topo IIα in HNSCC than in HNM appears to be linked to cell proliferation in tumor specimens, especially metastatic lymph node specimens, and therefore it may be worth considering the use of topoisomerase-targeting anti-tumor drugs against HNSCC.

We also found in this study that the MDR1, MRP and LRP genes were expressed in HNSCC and HNM to varying degrees. There have been several reports on the expression of these three proteins or genes in several normal tissues and cancer specimens. However, there are only a few reports on the expression of P-gp, MRP, and LRP in head and neck carcinomas. Schneider et al. speculated that the genes associated with oncogenic development may also activate P-gp concomitantly in human cancers. Duensing et al. showed that P-gp is a marker...
for tumor progression in renal cell carcinomas. Therefore, we attempted to clarify the biological characteristics of the MDR1, MRP and LRP genes in HNSCC and HNM. There were no significant differences in these three genes between HNSCC and HNM, or between metastatic lymph node samples and HNM. Our results suggest that MDR1, MRP and LRP are not related to oncogenic activation in HNSCC.

Furthermore, previous reports demonstrated that MDR1 and MRP play important roles in resistance to Topo II inhibitors such as etoposide or Adriamycin.6, 22, 23 We did not detect any differences between the steady-state levels of MDR1 or MRP mRNA in HNSCC and HNM, suggesting that it may be worth considering the use of topoisomerase-targeting antitumor drugs against HNSCC.

Platinum drugs are frequently used in the chemotherapy of head and neck carcinomas. It has been suggested that MRP, cMOAT and LRP expressions correlate with resistance to Topo II inhibitors such as etoposide or Adriamycin.5, 44, 45 We did not detect any differences between the steady-state levels of samples exposed to platinum drugs and those not exposed to platinum drugs, and we found no differences among the steady-state levels of MRP and LRP mRNA expression levels between samples exposed to platinum drugs and those not exposed to platinum drugs; further, we did not detect cMOAT gene expression (data not shown). These results suggest that the expressions are not associated with platinum drug exposure in HNSCC, although the relationship between baseline expression levels and response to platinum drugs remains to be elucidated. We did not analyze factors such as thymidylate synthase46 or dihydropyrimidine dehydrogenase47 that are supposed to be associated with 5-fluorouracil (5-FU) sensitivity. Activities or expression levels of these enzymes may influence the effectiveness of 5-FU or the prognosis of HNSCC patients. Further studies will be required to analyze whether the activities or the expression levels of these genes are associated with 5-FU sensitivity before and after drug administration.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (10877265) and the Satake Foundation. We are grateful to Drs. T. Takahashi (Second Department of Internal Medicine, Hiroshima University), K. Kuramoto (Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University), S. Kimura, N. Miyake and K. Hayashi (Department of Otorhinolaryngology, Hiroshima University) for their practical suggestions during the study, and to Ms. Y. Ide (Department of Otorhinolaryngology, Hiroshima University) for technical assistance.

(Received June 29, 1999/Revised October 25, 1999/Accepted October 27, 1999)

REFERENCES

1) Chen, C., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M. and Roninson, I. B. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell, 47, 381–389 (1986).
2) Gottesman, M. M. and Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu. Rev. Biochem., 62, 385–427 (1993).
3) Germann, U. A. P-Glycoprotein—a mediator of multidrug resistance in tumour cells. Eur. J. Cancer, 32A, 927–944 (1996).
4) Loe, D. W., Deeley, R. G. and Cole, S. P. C. Biology of the multidrug resistance-associated protein, MRP. Eur. J. Cancer, 32A, 945–957 (1996).
5) Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawkami, M., Kagotani, K., Okumura, K., Akiyama, S. and Kuwano, M. A human canalicular multispecific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. Cancer Res., 56, 4124–4129 (1996).
6) Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Schroeijs, A. B., van der Valk, P. and Schepers, R. J. Major vault protein LRP-related multidrug resistance. Eur. J. Cancer, 32A, 979–984 (1996).
7) Zhou, D. C., Zittoun, R. and Marie, J. P. Expression of multidrug resistance-associated protein (MRP) and multidrug resistance (MDR1) genes in acute myeloid leukemia. Leukemia, 9, 1661–1666 (1995).
8) Hasegawa, S., Abe, T., Naito, S., Kotoh, S., Kumazawa, J., Hipfner, D. R., Cole, S. P. C. and Kuwano, M. Expression of multidrug resistance-associated protein (MRP), MDR1, and DNA topoisomerase II in human multidrug-resistant bladder cancer cell lines. Br. J. Cancer, 71, 907–913 (1995).
9) Fairchild, C. R., Ivy, S. P., Rushmore, T., Lee, G., Koo, P., Goldsmith, M. E., Myers, C. E., Farber, E. and Cowan, K. H. Carcinogen-induced mdr overexpression is associated with xenobiotic resistance in rat preneoplastic liver nodules and hepatocellular carcinomas. Proc. Natl. Acad. Sci. USA, 84, 7701–7705 (1987).
10) Nittis, J. L. and Beck, W. T. Antitopoisomerase drug action and resistance. Eur. J. Cancer, 32A, 958–966 (1996).
11) Wang, J. C. DNA topoisomerases: why so many? J. Biol. Chem., 266, 6659–6662 (1991).
12) Drake, F. H., Hofmann, G. A., Bartus, H. F., Matern, M. R., Crooke, S. T. and Mirabelli, C. K. Biochemical and pharmacological properties of p 170 and p 180 forms of topoisomerase II. Biochemistry, 28, 8154–8160 (1989).
Drug Resistance-related Gene in Head and Neck Carcinoma

13) Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K. and Drake, F. H. Proliferation- and cell cycle-dependant differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. *Cell Growth Differ.*, 2, 209–214 (1991).

14) Meyer, K. N., Kjeldsen, E., Straub, T., Knudsen, B. R., Hickson, I. D., Kikuchi, A., Kreipe, H. and Boege, F. Cell cycle-coupled relocation of type I and II topoisomerases and modulation of catalytic enzyme activities. *J. Cell Biol.*, 136, 775–788 (1997).

15) Chen, A. Y. and Liu, L. F. DNA topoisomerases: essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.*, 34, 191–218 (1994).

16) Cornarotti, M., Capranico, G., Bohm, S., Oriana, S., Spatti, G. B., Mariani, L., Ballabio, G. and Zunino, F. Gene expression of DNA topoisomerases I, IIα, IIβ and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Int. J. Cancer*, 67, 479–484 (1996).

17) Giacone, G., van Ark-Otto, J., Scaglotti, G., Capranico, G., van der Valk, P., Rubio, G., Dalesio, O., Lopez, R., Zunio, F., Walboomers, J. and Pinedo, H. M. Differential expression of DNA topoisomerases in non-small cell lung cancer and normal lung. *Biochim. Biophys. Acta*, 1264, 337–346 (1995).

18) Syahruddin, E., Oguri, T., Takahashi, T., Isobe, T., Fujiwara, Y. and Yamakido, M. Differential expression of DNA topoisomerase Iα and IIβ genes between small cell and non-small cell lung cancer. *Jpn. J. Cancer Res.*, 89, 855–861 (1998).

19) Ohashi, N., Fujiwara, Y., Yamaoka, N., Katoh, O., Satow, Y. and Yamakido, M. No alteration in DNA topoisomerase I gene related to CPT-11 resistance in human lung cancer. *Jpn. J. Cancer Res.*, 87, 1280–1287 (1996).

20) Giacone, G., Gazdar, A. F., Beck, H., Zunino, F. and Capranico, G. Multidrug sensitivity phenotype of human lung cancer cells associated with topoisomerase II expression. *Cancer Res.*, 52, 1666–1674 (1992).

21) Giovannella, B. C., Stehlin, J. S., Wall, M. E., Wani, M. C., Nicholas, A. W., Liu, L. F., Silber, R. and Potmesil, M. DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science*, 246, 1046–1048 (1989).

22) Bader, P., Fuchs, J., Wenderoth, M., von Schweinitz, D., Niethammer, D. and Beck, J. F. Altered expression of resistance associated genes in hepatoblastoma xenografts incorporated into mice following treatment with Adriamycin or cisplatin. *Anticancer Res.*, 18, 3127–3132 (1998).

23) Kooke, K., Abe, T., Hisano, T., Kubo, T., Wada, M., Kohn, K. and Kuwano, M. Overexpression of multidrug resistance protein gene in human cancer cell lines selected for drug resistance to epipodophyllotoxins. *Jpn. J. Cancer Res.*, 87, 765–772 (1996).

24) Oguri, T., Fujiwara, Y., Isobe, T., Katoh, O., Watanabe, H. and Yamakido, M. Expression of γ-glutamylcysteine synthetase (γ-GCS) and multidrug resistance-associated protein (MRP), but not human canicular multispecific organic anion transporter (cMOAT), genes correlates with exposure of human lung cancer to platinum drugs. *Br. J. Cancer*, 77, 1089–1096 (1998).

25) Abe, Y., Nakamura, M., Ota, E., Ozeki, Y., Tamai, S., Inoue, H., Ueyama, Y., Ogata, T. and Tamaoki, N. Expression of the multidrug resistance gene (MDR1) in non-small cell lung cancer. *Jpn. J. Cancer Res.*, 85, 536–541 (1994).

26) Kreisholt, J., Sorensen, M., Jensen, P. B., Nielsen, B. S., Anderson, C. B. and Sehested, M. Immunohistochemical detection of DNA topoisomerase IIα, P-glycoprotein and multidrug resistance protein (MRP) in small-cell and non-small-cell lung cancer. *Br. J. Cancer*, 77, 1469–1473 (1998).

27) Oguri, T., Fujiwara, Y., Ochiai, M., Fujitaka, K., Miyazaki, M., Takahashi, T., Yokozaki, M., Isobe, T., Ohune, T., Tsuya, T., Katoh, O. and Yamakido, M. Expression of lung-resistance protein gene is not associated with platinum drug exposure in lung cancer. *Anticancer Res.*, 18, 4159–4162 (1998).

28) Kim, W. J., Kakahi, Y., Kinoshita, H., Arao, S., Fukumoto, M. and Yoshida, O. Expression patterns of multidrug-resistance (MDRI), multidrug resistance-associated protein (MRP), glutathione S-transferase-π (GST-π) and topoisomerase II (TopoII) genes in renal cell carcinomas and normal kidney. *J. Urol.*, 156, 506–511 (1996).

29) Kakahi, Y., Wu, W.-J., Kim, W.-J., Arao, S., Fukumoto, M. and Yoshida, O. Comparison of multidrug resistance gene expression levels with malignant potentials and influence of chemotheraphy in urothelial cancers. *Int. J. Urol.*, 2, 309–315 (1995).

30) Sandri, M. I., Hochhauser, D., Ayton, P., Campliejohn, R. C., Whitehouse, R., Turley, H., Gatter, K., Hickson, I. D. and Harris, A. L. Differential expression of the topoisomerase Iα and β genes in human breast cancers. *Br. J. Cancer*, 73, 1518–1524 (1996).

31) Schneider, J., Rubio, M. P., Barbazan, M. J., Rodriguez, E. F. J., Seizinger, B. R. and Castresana, J. S. P-Glycoprotein, HER-2/neu, and mutant p53 expression in human gynecologic tumors. *J. Natl. Cancer Inst.*, 86, 850–858 (1994).

32) Izquierdo, M. A., van der Zee, A. G. J., Vermorken, J. B., van der Valk, P., Beliné, J. A. M., Giacone, G., Scheffer, G. L., Flens, M. J., Pinedo, H. M., Kenemans, P., Meijer, C. J. L. M., de Vries, E. E. G. and Schepere, R. J. Drug resistance-associated marker LRP for prediction of response to chemotherapy and prognoses in advanced ovarian carcinoma. *J. Natl. Cancer Inst.*, 87, 1230–1237 (1995).

33) van der Zee, A. G. J., Hollema, H., de Jong, S., Boonstra, H., Gouw, A., Willemse, P. H. B., Zijlstra, J. G. and de Vries, E. E. G. P-Glycoprotein expression and DNA topoisomerase I and II activity in benign tumors of the ovary and in malignant tumors of the ovary, before and after platinum/cyclophosphamide chemotherapy. *Cancer Res.*, 51, 5915–5920 (1991).

34) Ito, K., Fujimori, M., Kohn, K., Nakagawa, A., Kuwano, M. and Amano, J. Expression of multidrug resistance associated genes in paired cases of breast cancers and adjacent
normal breast tissues. *Proc. Am. Assoc. Cancer Res.*, **38**, 388 (1997).

35) Yamazaki, K., Isobe, H., Hanada, T., Betsuyaku, T., Hasegawa, A., Hizawa, N., Ogura, S. and Kawakami, Y. Topoisomerase IIα content and topoisomerase II catalytic activity cannot explain drug sensitivities to topoisomerase II inhibitors in lung cancer cell lines. *Cancer Chemother. Pharmacol.*, **39**, 192–198 (1997).

36) Nooter, K., Westerman, A. M., Flens, M. J., Zaman, G. J. R., Scheper, R. J., van der Valk, P., Sonneveld, P., Gratama, J. W., Kok, T., Eggermont, A. M. M., Bosman, F. T. and Stoter, G. Expression of the multidrug resistance-associated protein (MRP) gene in human cancers. *Clin. Cancer Res.*, **1**, 1301–1310 (1995).

37) Schadendorf, D., Makki, A., Stahr, C., van Dyck, A., Wanner, R., Scheffer, G. L., Flens, M. J., Scheper, R. and Henz, B. M. Membrane transport proteins associated with drug resistance expressed in human melanoma. *Am. J. Pathol.*, **147**, 1545–1552 (1995).

38) Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Giaccone, G., Broxterman, H. J., Meijer, C. J. L. M., van der Valk, P. and Scheper, R. J. Broad distribution of the multidrug resistance-related vault lung resistance protein in normal human tissues and tumors. *Am. J. Pathol.*, **148**, 877–887 (1996).

39) Kelley, D. J., Pavelic, Z. P., Gapany, M., Stambrook, P., Pavelic, L., Gapany, S. and Gluckman, J. L. Detection of P-glycoprotein in squamous cell carcinomas of the head and neck. *Arch. Otolaryngol. Head Neck Surg.*, **119**, 411–414 (1993).

40) Tsuzuki, H., Saito, H., Ohtsubo, T., Tanaka, N., Noda, I., Sugimoto, C., Tsuda, G. and Imamura, Y. Immunohistochemical detection of P-glycoprotein and chemosensitivity from AT8 assay in human head and neck malignant tumor. *Head Neck Cancer*, **22**, 1–6 (1996).

41) Weiters, M. J. P., Fichtinger-Scheper, A. M. J., Baan, R. A., Flens, M. J., Scheper, R. J. and Braakhuis, B. J. M. Role of glutathione, glutathione S-transferases and multidrug resistance-related proteins in cisplatin sensitivity of head and neck cancer cell lines. *Br. J. Cancer*, **77**, 556–561 (1998).

42) Saito, H., Tanaka, N., Sugimoto, C. and Tsuzuki, H. Anti-cancer drug resistance and approaches to overcoming it in head and neck cancer. *Pract. Otol. (Kyoto)*, **90**, 607–614 (1997).

43) Duensing, S., Dallmann, L., Grosse, J., Buer, J., Hänninen, E. L., Deckert, M., Stördel, S., Kirchner, H., Poliwoda, H. and Atzpodien, J. Immunohistochemical detection of P-glycoprotein: initial expression correlates with survival in renal cell carcinoma patients. *Oncology*, **51**, 309 (1994).

44) Izquierdo, M. A., Shoemaker, R. H., Flens, M. J., Scheffer, G. L., Wu, L., Prather, T. R. and Scheper, R. J. Overlapping phenotypes of multidrug resistance among panels of human cancer-cell lines. *Int. J. Cancer*, **65**, 230–237 (1996).

45) Ishikawa, T., Bao, J. J., Yamane, Y., Akimaru, K., Fridge, R., Wright, C. D. and Kuo, M. T. Coordinated induction of MRP/GS-X pump and gamma-glutamylcysteine synthetase by heavy metals in human leukemia cells. *J. Biol. Chem.*, **271**, 14981–14988 (1996).

46) Horikoshi, T., Danenberg, K. D., Stadlbauer, T. H. W., Volkenandt, M., Shea, L. C. C., Aigner, K., Gustavsson, B., Leichman, L., Frosing, R., Ray, M., Gibson, N. W., Spears, C. P. and Danenberg, P. V. Quantitation of thymidylate synthase, dihydrofolate reductase, and DT-diaphorase gene expression in human tumors using the polymerase chain reaction. *Cancer Res.*, **52**, 108–116 (1992).

47) Ishikawa, Y., Kubota, T., Otani, Y., Watanabe, M., Teramoto, K., Kumai, K., Kitajima, M., Takechi, T., Okabe, H. and Fukushima, M. Dihydropyrimidin dehydrogenase activity and messenger RNA level may be related to the anticancer effect of 5-fluorouracil on human tumor xenografts in nude mice. *Clin. Cancer Res.*, **5**, 883–889 (1999).