Jpn. J. Cancer Res. **92**, 778–784, July 2001

Expression of Drug Resistance Genes in VP-16 and mAMSA-selected Human Carcinoma Cells

Yoshihito Matsumoto,[1, 2, 3] Hiroshi Takano,[2] Katsuzo Kunishio,[1] Seigo Nagao[1] and Tito Fojo[2]

[1]Department of Neurological Surgery, Kagawa Medical University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793 and [2]Medicine Branch, Bldg 10, Rm 12N226, DCS, NCI, NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA

The cell lines described in the present study were isolated as part of an effort to understand resistance to topoisomerase (topo) II inhibitors. To that end, 50 sublines were isolated from four human breast cancer cell lines, i.e., MCF-7, T47D, MDA-MB-231, and ZR-75B. As an initial step, a concentration that would be lethal to the majority of cells (IC99) was selected for both VP-16 and mAMSA, for each cell line. The identification of an increasing number of putative drug resistance-related proteins provided the opportunity to examine expression of the corresponding genes in the selected cell lines. Northern blot analysis revealed different responses to the selecting agents in the different cell lines. Previous studies examining expression of multidrug resistance (MDR)-1 in resistant cell lines had found undetectable levels in all cells. In the ZR-75B sublines, increased expression of MDR-associated protein (MRP) and canalicular multispecific organic anion transporter (cMOAT) was observed, and when the relative levels of overexpression were compared, a high correlation was found. In contrast, increased expression of MRP was observed in some of the MDA-MB-231 sublines, without a concomitant increase in cMOAT expression. Finally, in both T47D and MCF-7 sublines, increased expression of cMOAT or MRP was observed infrequently, and where it occurred, was of a much smaller magnitude. In the analysis of expression of MRP, the highest levels were found in the ZR-75B and MDA-MB-231 sublines, with lower levels in the MCF-7 and T47D clones. Similarly, differences in the expression of topo IIα were observed among the sublines. Although the differences in expression appear to depend on the parental cell line from which the resistant sublines were derived, a strong correlation was observed between the expression of MRP and the levels of topo IIα. Cell lines with low levels of MRP had lower levels of topo IIα, while those with high levels of MRP maintained higher levels of topo IIα. While a reduced topo IIα level was common, there did not appear to be a compensating increase in the expression of topo IIβ or topo I or casein kinase (CK) IIα in any of the cell lines. While the possibility that such compensation could occur has been discussed and even reported in some cell lines, such an adaptation was not observed in the present study, suggesting that it is not common.

Key words: Topoisomerase II — MRP — cMOAT — CK II — MDR-1

The epipodophyllotoxins (VP-16 and VM-26) and the aminoacridine (mAMSA) are useful antineoplastic agents with activity against both hematologic malignancies and solid tumors.[1, 2] The anticancer activity of these agents is thought to result at least in part from stabilization of the cleavable complex, an intermediate in which topoisomerase (topo) II is covalently bound to DNA in a step that precedes DNA religation.[3–6] This mechanism of action is shared by other antitumor agents including the anthracyclines and the ellipticines.[7–10] Previous studies have demonstrated the presence of two isozyme forms of topo IIα, *Mr* 170 000 enzyme (topo IIα) and *Mr* 180 000 enzyme (topo IIβ), which are the products of two different genes.[11–13] In tumor cell lines, these two isozymes appear have different sensitivity to several antineoplasmic drugs. Topo IIα was found to be 3-fold more sensitive to VM-26 than topo IIβ. Therefore, the levels and ratio of topo IIα and topo IIβ may be important factors determining the sensitivity of tumor cells to topo II-directed drugs.[14] But, information regarding the expression and drug sensitivity of topo I in these models is lacking.

The efficacy of the epipodophyllotoxins and aminoacridine are limited by the occurrence of drug resistance in the tumor cell population. Cellular insensitivity to drugs that stabilize the cleavable complex is frequently expressed as multidrug resistance (MDR).[15] In some cell lines, overexpression of MDR-1/P-glycoprotein or the MDR-associated protein (MRP) has been demonstrated and implicated as the mechanism of resistance.[16–20] Typically, these cells have reduced drug accumulation, secondary to increased drug efflux. Recently, the cDNA of a new member of the

[3] To whom correspondence should be addressed at the Department of Neurological Surgery, Kagawa Medical University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793.
E-mail: mizaya@kms.ac.jp

ATP binding cassette superfamily named canalicular multispecific organic anion transporter (cMOAT) was isolated.[21, 22] In other cell lines, an atypical MDR phenotype has been identified, with the predominant mechanism of resistance shown to be qualitative and/or quantitative changes in the levels and activity of topo II.[9, 23–25]

Phosphorylation by casein kinase (CK) IIα of topo II occurs primarily on serine residues in the carboxy-terminal domain of the protein.[1, 26–28] Studies using both synchronized cells and purified topo II have demonstrated a correlation between topo II activity and phosphorylation by CKII, suggesting that this post-translational modification may regulate catalytic activity.[29–31] Both increased and decreased activity have been reported following phosphorylation, consistent with differential effects of phosphorylation at divergent sites.

Using a single clone selection process, we have isolated and characterized 50 human carcinoma cell lines isolated for resistance to VP-16 or mAMSA. These cell lines have been characterized by expression levels of topo I, topo IIα, topo IIβ , CKIIα, MRP, MDR-1, and cMOAT with northern analysis.

MATERIALS AND METHODS

Cell lines and cell cultures The cell lines described in the present report were initially isolated as single clones. Four parental human carcinoma cell lines were used: ZR-75B, T47D, MCF-7, and MDA-MB-231. The selections were performed at different drug concentrations for each cell line (ZR-75B: 300 n*M* VP-16 or 30 n*M* mAMSA; T47D: 300 n*M* VP-16 or 50 n*M* mAMSA; MDA-MB-231: 500 n*M* VP-16 or 50 n*M* mAMSA; and MCF-7: 500 n*M* VP-16 or 50 n*M* mAMSA). These concentrations were those that were toxic to most cells (IC99), and resulted in the survival of only a few cells that grew as isolated colonies. Clones were picked as single colonies and subsequently, a population of cells from each of these initial isolates was maintained in the concentration of drug which had been used in the initial selection.

Northern blotting Total RNA (20 mg/lane) was separated on a 1% formaldehyde gel and transferred to Hybond N+ with 10× SSC. The membranes were hybridized at 42°C overnight with the radiolabeled probe in Hybrisol I (Oncor, Gaithersburg, MD).

RESULTS

To characterize the frequency and nature of acquired mutations in topo IIα, sublines were isolated from four human carcinoma cell lines, MCF-7, T47D, MDA-MB-231, and ZR-75B, by exposing them to VP-16 and mAMSA at concentrations that would be lethal to the majority of cells (IC99). For VP-16, these concentrations

Table I. Fold Resistance Relative to Parental Cells

Cell lines	VP	AM	MX	AD	VC
ZR-75B	1	1	1	1	1
ZR-VP2 (300)	317	70	24	21	2
ZR-VP3 (300)	647	25	7	37	2
ZR-VP13 (300)	172	47	20	9	2
ZR-VP16 (300)	810	11	10	29	3
ZR-VP20 (30)	84	6	10	6	2
ZR-m1 (30)	320	48	8	9	2
ZR-m3 (30)	79	14	10	7	1
ZR-m4 (30)	56	16	16	9	1
ZR-m6 (30)	255	33	10	17	3
ZR-m7 (30)	59	26	12	11	2
ZR-m8 (30)	95	15	14	6	1
ZR-m9 (30)	68	16	17	8	1
MDA-MB-231	1	1	1	1	1
MDA-VP4 (500)	20	10	8	13	4
MDA-VP7 (500)	227	22	96	54	4
MDA-VP11 (500)	18	7	11	6	4
MDA-VP12 (500)	35	11	33	13	4
MDA-VP13 (500)	89	45	16	15	2
MDA-VP7 (1000)	67	26	33	22	1
MDA-m4 (100)	44	22	9	9	1
MDA-m5 (100)	68	35	14	8	1
MDA-m6 (100)	51	32	14	7	1
MDA-m14 (100)	87	38	11	10	1
MDA-m15 (100)	98	48	26	8	2
MCF-7	1	1	1	1	1
MCF-7-VP3 (500)	22	11	12	9	2
MCF-7-VP6 (500)	61	11	14	14	2
MCF-7-VP7 (500)	17	10	15	8	3
MCF-7-VP8 (500)	49	6	12	25	3
MCF-7-VP10 (500)	47	5	9	8	1
MCF-7-VP13 (500)	37	10	11	9	2
MCF-7-VP15 (500)	25	18	19	10	1
MCF-7-VP17 (500)	14	9	9	8	1
MCF-7-m1 (50)	12	27	17	6	2
MCF-7-m2 (50)	20	28	23	11	2
MCF-7-m2 (50)	15	29	18	7	1
MCF-7-m4 (50)	23	37	31	13	1
MCF-7-m6 (50)	58	24	25	14	2
MCF-7-m10 (50)	47	22	14	16	2
MCF-7-m11 (50)	33	20	18	10	2
T47D	1	1	1	1	1
T47D-VP1 (300)	44	15	5	15	1
T47D-VP4 (300)	120	40	19	28	1
T47D-VP5 (300)	93	36	25	36	2
T47D-VP7 (300)	312	39	23	62	3
T47D-VP10 (300)	85	26	21	19	2
T47D-VP15 (300)	89	27	18	13	1
T47D-m2 (50)	47	48	6	17	1
T47D-m6 (50)	85	92	8	31	2
T47D-m7 (50)	56	71	8	19	1
T47D-m9 (50)	89	69	24	26	2
T47D-m15 (50)	48	26	39	28	1
T47D-m19 (50)	85	135	10	29	1

VP, etoposide; AM, m-amsacrine; MX, mitoxantrone; AD, adriamycin; VC, vincristine.

were 300 n*M* for ZR-75B and T47D and 500 n*M* for MDA-MB-231 and MCF-7. For mAMSA, 30 n*M* was used with ZR-75B, 50 n*M* with T47D and MCF-7 and 100 n*M* with MDA-MB-231. A population of cells was then exposed to either VP-16 or mAMSA, and after a period of several weeks, individual colonies appeared. These were isolated using a sterile ring and trypsin, and transferred to a 25 cm^2 flask, where after an initial period of about one week in drug-free medium, the cells were again placed in the concentration of drug in which they had survived, and allowed to expand. Using this approach, 50 sublines were isolated, i.e., 13 from ZR-75B, 11 from MDA-MB-231,12 from T47D, and 14 from MCF-7. Of these, 26 were isolated with VP-16 and 24 with mAMSA.

Cytotoxicity assays were performed as previously described.[32] Briefly, 300 to 1000 cells/well plated in 96-well dishes were incubated overnight, after which time a drug was added and the cells were incubated for an additional 5 days. At the end of the incubation period, cells were fixed with trichloroacetic acid, and stained with 0.4% sulforhodamine B dissolved in acetic acid. Unbound dye was removed by washing four times with 1% acetic acid, and protein-bound dye was extracted with 10 m*M* unbuffered Tris base. Optical density was determined on a 96-well microtiter plate reader. Cross resistance to the screening agents, VP-16 and mAMSA, is shown along with data for mitoxantrone, adriamycin, and vincristine. Cross resistance to the four topo II poisons was observed with little or no cross resistance to the microtubule active agent, vincristine. Although there was variability, on the whole, the ZR-75B subline showed higher levels of cross resistance, with preferential resistance to VP-16 independent of the

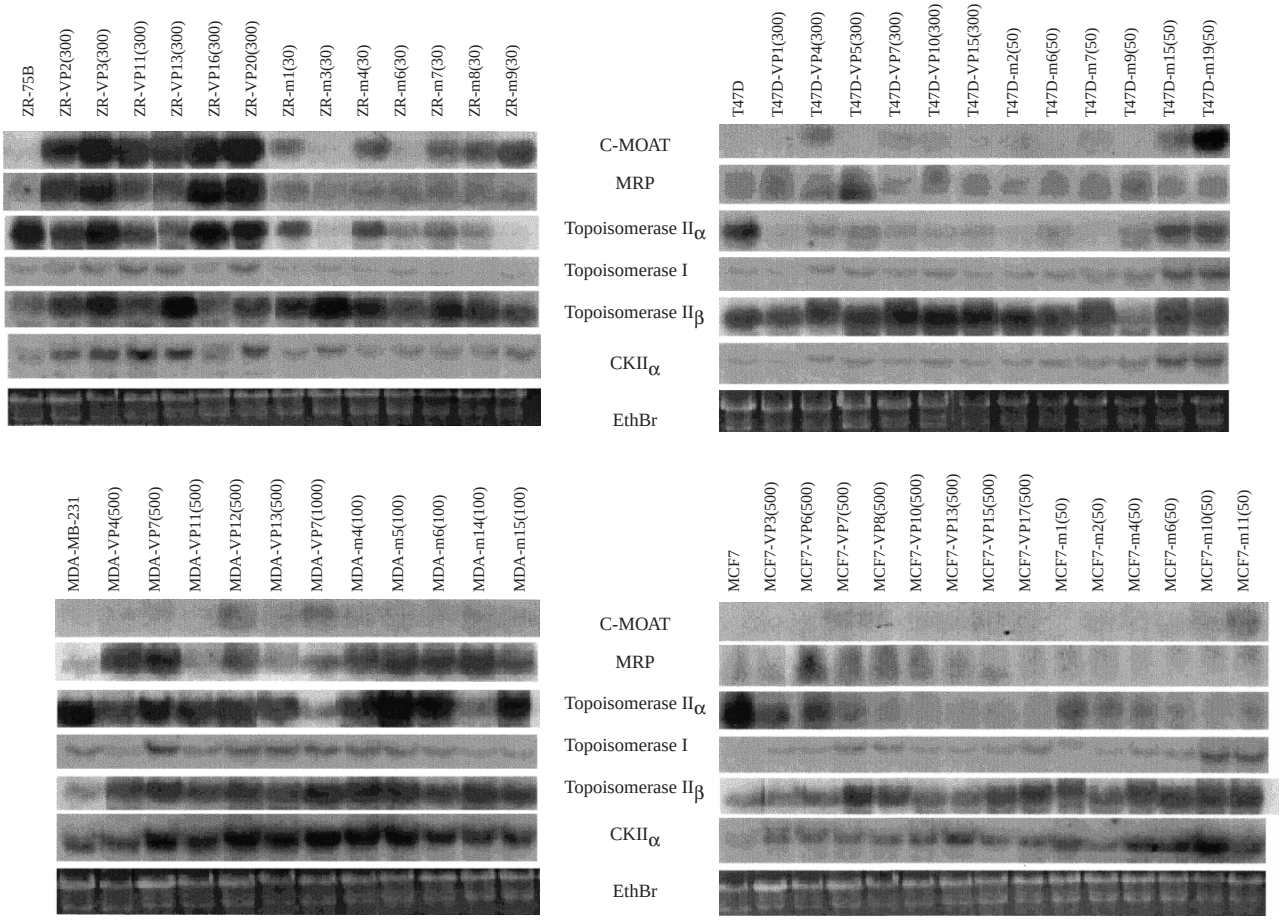

Fig. 1. Expression of drug-resistance genes. First panel: northern analysis using cMOAT cDNA (residues 2986 to 3867). Second panel: northern analysis using MRP cDNA (residues 251 to 965). Third panel: northern analysis using topo IIα cDNA (residues −91 to +449). Fourth panel: northern analysis using topo I cDNA (residues 211 to 573). Fifth panel: northern analysis using topo IIβ cDNA (residues 3119 to 3806). Sixth panel: northern analysis using CKIIα cDNA (residues 275 to 1079). Seventh panel: ethidium bromide stained RNA prior to transfer.

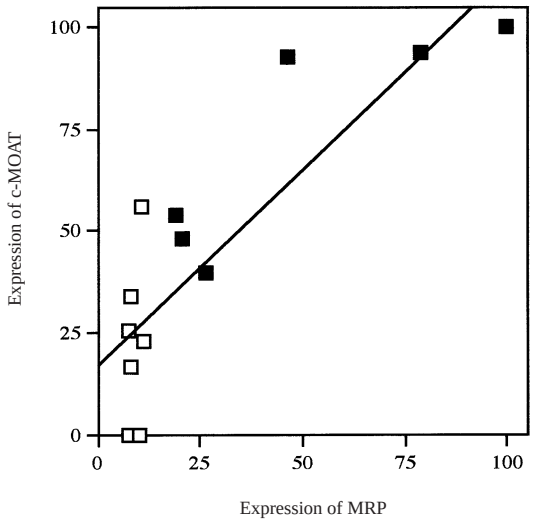

Fig. 2. Correlation in the thirteen drug-resistant ZR-75B cell lines between MRP and cMOAT expression as determined by northern analysis. ■, ZR-VPs; □, ZR-mAMSAs. $y=0.956x+18.805$, $r=0.847$.

selecting agent. Cross resistance to mitoxantrone and adriamycin was also observed, with minimal to absent cross resistance to vincristine (Table I).

The identification of an increasing number of putative drug-resistance proteins provided the opportunity to examine expression of their genes in the selected cell lines. To that end, total RNA was isolated from parental cells and the resistant sublines. Based on the expected strength of the signal, hybridization was performed first with a cMOAT probe, then with a probe for MRP, and this was followed by probes for topo IIα and topo IIβ, as well as topo I (not shown) and CKIIα (also not shown). Previous studies examining expression of MDR-1 in the resistant cell lines had found undetectable levels in all cells.[33)]

Examination of the northern blot analyses revealed different responses to the selecting agents in the different cell lines (Fig. 1). In the ZR-75B sublines, increased expression of MRP and cMOAT was observed, and when the relative levels of overexpression were compared, a high correlation was found, as shown in Fig. 2. In contrast, increased expression of MRP was observed in some of the MDA-MB-231 sublines without a concomitant increase in cMOAT expression. Finally, in both T47D and MCF-7 sublines, increased expression of cMOAT or MRP was observed infrequently, and where it occurred, was of a much smaller magnitude. For this analysis, the levels of MRP expression are expressed relative to that in the line which had the highest levels (ZR-VP20). As can be seen, higher levels are found in the ZR-75B and MDA-MB-231 sublines, with lower levels in the MCF-7 and T47D

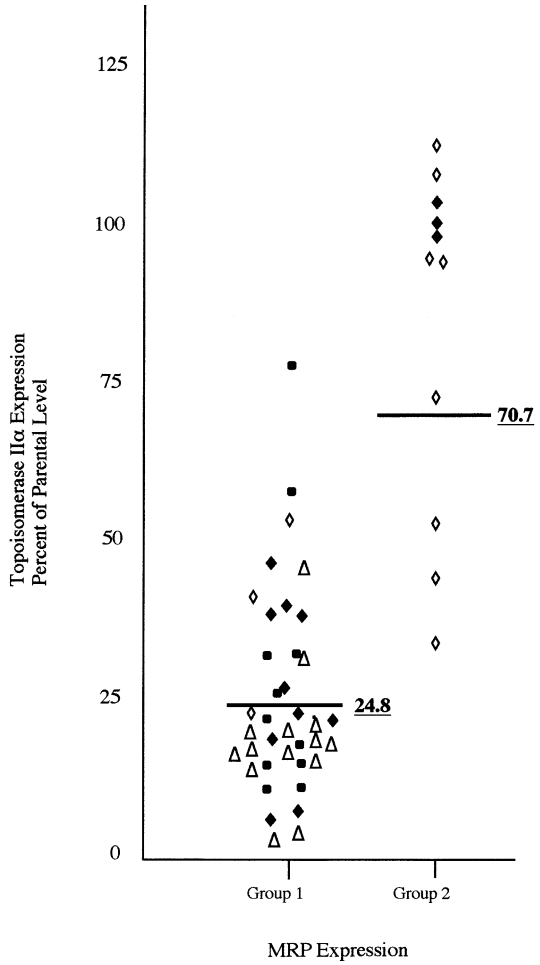

Fig. 3. Quantitative analysis of topo IIα and MRP expression. Group 1: low expression of MRP [less than 30% of MRP expression of ZR-VP20]. Group 2: high expression of MRP [more than 30% of MRP expression of ZR-VP20]. ◆, ZR-75B; ◇, MDA-MB-231; ■, T47D; △, MCF-7.

clones. Similarly, differences in the expression of topo IIα were observed among the sublines. Although the differences in expression appear to depend on the parental cell line from which the resistant sublines were derived, a strong correlation was observed between the expression of MRP and the levels of topo IIα. Thus, as shown in Fig. 3, cell lines with low levels of MRP had lower levels of topo IIα, while those with high levels of MRP maintained higher levels of topo IIα.

DISCUSSION

The present study describes the characterization of multidrug-resistant sublines derived from four parental carcinoma cell lines by exposure to VP-16 and mAMSA.

Characterization of 50 clones is described. The results demonstrate the divergent profiles that can arise with drug selection, and the greatest differences appear to be cell line-specific, as do the adaptations which appear to occur during the course of drug selections. Several observations can be made from these selections which we think provide some insight into the diversity of drug-resistance profiles.

First, it appears that properties inherent to the parental cell line from which the resistant isolates were derived are important in the acquired phenotype, and may be as important as the drug used in the selection process. This preliminary observation is based on the profiles seen in the northern blot analyses, which showed that expression of MRP (and cMOAT) occurred preferentially in the ZR-75B sublines, and to be a lesser extent in the MDA-MB-231 clones, but infrequently in the T47D and MCF-7 isolates. These observations were not isolated instances, but appeared to represent a recurring pattern.

Second, the co-expression of cMOAT and MRP in the ZR-75B sublines showed a high correlation coefficient, suggesting that expression of these two genes may be similarly regulated in the ZR-75B cell line. While it is possible that both genes can confer resistance to the drugs used in the isolation procedure, and that overexpression occurred concordantly, the high correlation suggests that in ZR-75B, similar regulatory mechanisms may have been responsible for the coordinate expression. This possibility implies that overexpression of one gene, possibly MRP for VP-16 may have occurred as an adaptation to drug exposure, and that this was mediated through an increase in transcription, which similarly affected expression of cMOAT. Expression of several ABC transporters which mediate drug resistance can be coordinately regulated by transcription factors identified as “gain of function” genes in selections for drug resistance.

Third, while more than one mechanism can contribute to the drug-resistance phenotype, it can be argued that one mechanism predominates, although divergent machanisms can interact constructively to confer sufficient drug tolerance.[33] Thus, for example, while the most common observation in these selections was the nearly universal decrease in the levels of topo IIα, the magnitude of this decrease depended to a certain extent on the level of MRP expression. Thus, cell lines with increased levels of MRP, which would be expected to have reduced intracellular drug concentrations, did not need to have as large a fall in topo IIα to allow for faster growth. Indeed, it might be argued that the ideal phenotype would consist of MRP overexpression with reduced drug exposure and a stable topo II level. The fact that this was not observed in the majority of cases, and indeed was principally observed only in the ZR-75B and MDA-MB-231 sublines, suggests that the resultant phenotype might be dictated in large part by the parental (or sensitive) cell.

Fourth, while a reduced topo IIα level was common, there did not appear to be a compensating increase in the expression of topo IIβ in any of the cell lines.[34] While the possibility that such compensation could occur has been discussed and even reported in some cell lines, such an adaptation was not observed in the present study, suggesting it is not common. It is well known that the two topo enzymes have differential sensitivity to drugs, and for the drugs used in the selections described herein, the sensitivity of topo IIβ is considerably less than that of topo IIα.[35] Consequently, one might predict that substitution of the β-isoform might be advantageous. However, this was not observed. The reason why this did not occur is not clear at present. However, it is possible that these two topoisomerases share some transcription factors, which decrease as a part of the adaptation to drug exposure and the decrease in the topo IIα, and this prevents a substantial increase in expression of the β isoform.

The results described in the present study support and extend previous observations. The most frequent change observed was a decrease in the expression of topo IIα. Previous studies have documented reduced expression of topo IIα with drug selection and this has been proposed as a mechanism of drug resistance. A reduction of topo levels effectively diminishes the main intracellular target of VP-16 and mAMSA, and can confer broad cross resistance to these agents.

Previous reports describing characterization of MRP-overexpressing cell lines have reported modest relative resistance ratios of 3.5 to 28.4 for VP-16 and 6 to 22.5 for adriamycin in the majority of cell lines, with higher and more variable ratios in a cell line which previous studies had identified as having altered topo II levels.[36] The high levels of cross resistance achieved in the resistant sublines, as shown for the ZR-75B and MDA-MB-231 sublines, exceeds the majority of reported relative resistance values. High levels of resistance may have been achieved because more than one mechanism contributed to the resistant phenotype. This demonstrates how two mechanisms can synergize and confer very high levels of drug tolerance.

In the cell lines without significant changes in the expression of MRP (or cMOAT), it was somewhat surprising to find that reductions in topo II levels could confer such a high degree of cross resistance. The results suggest that reduced topo IIα levels can confer a high degree of resistance and cross resistance.

In summary, the present study demonstrates how cellular adaptation to drug exposure occurs, and how more than one mechanism can contribute to the resistant phenotype. Reduced expression of topo IIα was the most frequent change observed in the selection process. Additional changes included increased expression of MRP and in some cell lines, also of cMOAT. The divergent phenotypes observed were principally dependent on the parental cell

lines, suggesting that the cell of origin may contribute significantly to the drug-resistant phenotype, even when the cell lines being compared arise from the same tissue. Preliminary results suggest that expression of MRP and cMOAT may be coordinary regulated in some cells, and that compensatory changes in topo IIβ levels occur infrequently in cells with reduced levels of topo IIα.

(Received January 17, 2001/Revised April 5, 2001/Accepted April 16, 2001)

REFERENCES

1) Caron, P. R., Watt, P. and Wang, J. C. The C-terminal domain of *Saccharomyces cerevisiae* DNA topoisomerase II. *Mol. Cell. Biol.*, **14**, 3197–3207 (1993).
2) Nelson, W. G., Liu, L. F. and Coffey, D. S. Newly replicated DNA is associated with DNA topoisomerase II in cultured rat prostatic adenocarcinoma cells. *Nature*, **322**, 187–189 (1984).
3) Berger, J. C., Gamblin, S. J., Harrison, S. C. and Wang, J. C. Structure and mechnism of DNA topoisomerase II. *Nature*, **379**, 225–232 (1996).
4) Ross, W., Rowe, T., Glisson, B., Yalowich, J. and Liu, L. F. Role of topoisomerase II in modifying epipodophyllotoxin-induced DNA damage. *Cancer Res.*, **44**, 5857–5860 (1984).
5) Vosberg, V. P. DNA topoisomerase: enzymes that control DNA conformation. *Curr. Top. Microbiol. Immunol.*, **114**, 19–102 (1985).
6) Wang, J. C. DNA topoisomerase. *Annu. Rev. Biochem.*, **54**, 655–697 (1985).
7) Maxwell, A. and Gellert, M. Mechanistic aspects of DNA topoisomerases. *Adv. Protein Chem.*, **38**, 69–109 (1986).
8) Nelson, E. M., Tewey, K. M. and Liu, L. F. Mechanisms of antitumor drug action: poisoning of mammalian DNA topoisomerase II on DNA by 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide. *Proc. Natl. Acad. Sci. USA*, **81**, 1361–1365 (1984).
9) Tewey, K. M., Chen, G. L., Nelson, E. M. and Liu, L. F. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *J. Biol. Chem.*, **259**, 9182–9187 (1984).
10) Zwelling, L. A. DNA topoisomerase II as a target of antineoplastic drug therapy. *Cancer Metastasis Rev.*, **4**, 263–276 (1985).
11) Austin, C. A., Patel, S. and Fisher, L. M. Novel HeLa topoisomerase II is the II-b isoform: complete coding sequence and homology with other type II topoisomerases. *Biochim. Biophys. Acta*, **172**, 283–291 (1993).
12) Chung, T. D. Y., Drake, F. H., Tan, K. B., Per, S. R., Crooke, S. T. and Mirabelli, C. K. Characterization and immunological identification of cDNA clones encoding two human DNA topoisomerase II isozymes. *Proc. Natl. Acad. Sci. USA*, **86**, 9431–9435 (1989).
13) Drake, F. H., Hofmann, G. A., Bartus, H. F., Mattern, M. R., Crooke, S. T. and Mirabelli, C. K. Biochemical and pharmocological properties of p170 and p180 forms of topoisomerase II. *Biochemistry*, **28**, 8154–8160 (1989).
14) Woessner, R. D., Chung, T. D. Y., Hofmann, G. A., Mattern, M. R., Mirabelli, C. K., Drake, F. H. and Johnson, R. K. Differences between normal and ras-transformed NIH-3T3 cells on expression of the 170 kD and 180 kD forms of topoisomerase II. *Cancer Res.*, **50**, 2901–2908 (1990).
15) Danks, M. K., Warmouth, M. R., Friche, E., Granzen, B., Bugg, B. Y., Harker, W. G., Zwelling, L. A., Futscher, B. W., Suttle, D. P. and Beck, W. T. Single-strand conformational polymorphism analysis of the Mr 170,000 isozyme of DNA topoisomerase II in human tumor cells. *Cancer Res.*, **53**, 1373–1379 (1993).
16) Cole, S. P. C., Chanda, E. R., Dicke, F. P., Gerlach, J. H. and Mirski, S. E. L. Non P-glycoprotein-mediated multidrug resistance in a small cell lung cancer cell line: evidence for decreased susceptibility to drug induced DNA damage and reduced levels of topoisomerase II. *Cancer Res.*, **51**, 3345–3352 (1991).
17) Cole, S. P. C., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M. V. and Deeley, R. G. Overexpression of a transporter gene in a multidrug resistant human lung cancer cell line. *Science*, **258**, 1650–1654 (1992).
18) Grant, C. E., Vadimarsson, G., Hipfner, D. R., Almquist, K. C., Cole, S. P. C. and Deeley, R. G. Overexpression of multidrug resistance associated protein increases resistance to natural product drugs. *Cancer Res.*, **54**, 357–361 (1994).
19) Lee, J. S., Scala, S., Matsumoto, Y., Dickstein, B., Robey, R., Zhan, Z., Altenberg, G. and Bates, S. E. Reduced drug accumulation and multidrug resistance in human breast cancer cells without associated P glycoprotein or MRP overexpression. *J. Cell. Biochem.*, **65**, 513–526 (1997).
20) Lorico, A., Rappa, G., Srimatkandada, S., Catapano, C. V., Fernandes, D. J., Germino, J. F. and Sartorelli, A. C. Increased rate of VP-16 efflux in a murine leukemia cell line overexpressing the multidrug resistance associated protein gene. *Cancer Res.*, **55**, 4352–4360 (1995).
21) Paulusma, C. C., Bosma, P. J., Zaman, G. J., Bakker, C. T., Otter, M., Scheffer, G. L., Scheper, R. J. and Borst, P. Congenital jaundice in rats with a mutation in a multidrug resistance associated protein gene. *Science*, **271**, 1126–1127 (1996).
22) Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kagotani, K., Okumura, K., Akiyama, S. and Kuwano, M. A human canalicular multispecific organic anion transporter gene is overexpression in cisplatin resistant human cancer cell lines with decreased drug accumulation. *Cancer Res.*, **56**, 4124–4129 (1996).
23) Beck, W. T., Kim, R. and Chen, R. R. Novel actions of inhibitors of DNA topoisomerase II in drug-resistant tumor

cells. *Cancer Chemother. Pharmacol.*, **34** (Suppl.), S14–S18 (1994).
24) Lee, M. S., Wang, J. C. and Beran, M. Two independent amsacrine resistant human myeloid leukemia cell lines share an identical point mutation in the 170 kDa form of human topoisomerase II. *J. Mol. Biol.*, **223**, 837–843 (1992).
25) Matsumoto, Y., Morisaki, K., Kunishio, K., Nagao, S., Takano, H. and Fojo, T. Incidence of mutation and deletion in topoisomerase II mRNA of etoposide and m-AMSA resistant cell lines. *Jpn. J. Cancer Chemother.*, **25**, 1145–1149 (1998).
26) Alghisi, G. C., Roberts, E., Cardenas, M. E. and Gasser, S. M. The regulation of DNA topoisomerase II by casein kinase II. *Cell. Mol. Biol. Res.*, **40**, 563–571 (1994).
27) Cardenas, M. E., Dang, Q., Glover, C. V. C. and Gasser, S. M. Casein kinase II phosphorylates the eukaryote-specific c-terminal domain of topoisomerase II *in vitro*. *EMBO J.*, **11**, 1785–1796 (1992).
28) Crenshaw, D. W. and Hsieh, T. S. Function of the hydrophilic carboxyl terminus of type II DNA topoisomerase from *Drosophila melanogaster*. *J. Biol. Chem.*, **268**, 21335–21343 (1993).
29) Wasserman, R. A., Austin, C. A., Fisher, L. M. and Wang, J. C. Use of yeast in the study of anticancer drugs targeting DNA topoisomerases: expression of a functional recombinant human DNA topoisomerase II alpha in yeast. *Cancer Res.*, **53**, 3591–3596 (1993).
30) Wells, N. J., Addison, C. M., Fry, A. M., Ganapathi, R. and Hickson, I. D. Serine 1524 is a major site of phosphorylation on human topoisomerase IIa protein *in vivo* and is a substrate for casein kinase II *in vitro*. *J. Biol. Chem.*, **270**, 29746–29751 (1994).
31) Wells, N. J., Fry, A. M., Guano, F., Norbury, C. and Hickson, I. D. Cell cycle phase-specific phosphorylation of human topoisomerase IIα. *J. Biol. Chem.*, **269**, 28357–28363 (1995).
32) Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, **82**, 1107–1112 (1990).
33) Matsumoto, Y., Takano, H. and Fojo, T. Cellular adaptation to drug exposure: evolution of the drug-resistant phenotype. *Cancer Res.*, **57**, 5086–5092 (1997).
34) Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson, R. K. and Drake, F. H. Proliferation- and cell cycle-dependent differnces in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. *Cell Growth Differ.*, **2**, 209–214 (1991).
35) Harker, W. G., Slade, D. L., Parr, R. L., Feldhoff, P. W., Sullivan, D. M. and Holguin, M. H. Alterations in the topoisomerase IIa gene, messenger RNA, and subcellular protein distribution as well reduced expression of the DNA topoisomerase IIβ enzyme in mitoxantrone-resistant HL-60 human leukemia cell line. *Leukemia*, **20**, 1707–1716 (1996).
36) Cole, S. P. C., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. E. and Wilson, G. M. Pharmacological characterization of multidrug resistant MRP transfected human tumor cells. *Cancer Res.*, **54**, 5902–5910 (1994).