Establishment and Characterization of 6-[[2-(Dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride (TAS-103)-resistant Cell Lines

Yoshimi Aoyagi,1 Takashi Kobunai, Teruhiro Utsugi, Konstanty Wierzba and Yuji Yamada
Taiho Pharmaceutical Co., Ltd., Hanno Research Center, 1-27 Misugidai, Hanno, Saitama 357-8527

6-[[2-(Dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride (TAS-103) is a novel anticancer agent that was developed to target both topoisomerase (Topo) I and Topo II. To elucidate its mechanism of action, we have established and characterized TAS-103-resistant cells, derived from mouse leukemia (P388), human colon cancer (DLD-1), and human lung adenocarcinoma (A549) cell lines, by exposure to stepwisely increasing concentrations of TAS-103 in the culture medium. P388/TAS cells showed only cross-resistance to VP-16 and adriamycin (ADR). The Topo II activity in these cells was decreased to below one-fourth of that in the parental cells, while the Topo I activity remained unchanged. DLD/TAS cells appeared to be cross-resistant to VP-16, ADR, camptothecin (CPT), SN-38 and vincristine (VCR). The enzymatic activities of both Topo I and Topo II in these cells were decreased to one-fourth of that observed in the parental cells. Furthermore, the decreased activities were accompanied by lower expression at the mRNA and protein levels. A549/TAS cells acquired cross-resistance to VP-16, ADR and VCR, though the Topo activities were virtually unchanged. In this cell line, the intracellular accumulation of TAS-103 was significantly decreased and the expression of multidrug resistance associated protein (MRP) was elevated when compared with the parental cells. The results indicate that the affected activities of Topo I and/or Topo II, and in some instances decreased accumulation of TAS-103, are associated with the development of resistance to TAS-103, although the main mechanism of resistance to TAS-103 varied among cell lines.

Key words:    TAS-103 — Topoisomerases — Resistance

The DNA topoisomerases (Topo), nuclear enzymes controlling DNA topology, have been identified as important targets for cancer chemotherapy. CPT-11 and topotecan were developed as inhibitors of Topo I, while VP-16, VM-26 and some intercalating agents widely used in cancer chemotherapy (adriamycin (ADR), amsacrin, mitoxantrone), were identified as Topo II inhibitors.5, 6) The novel anticancer agents targeting both Topo I and Topo II are now considered as a promising approach in finding clinically more effective antitumor agents than agents targeting only one Topo. We have reported that 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride (TAS-103) inhibited Topo I and Topo II in in vitro enzymatic assays, and showed potent antitumor activity against various human tumor xenografts and metastatic tumors.3, 4) TAS-103 is under clinical evaluation in the USA.

In this study, to elucidate the mechanism of action of TAS-103, we have established TAS-103-resistant cell lines and characterized their cross-resistance to various anticancer agents, the changes in Topo levels and the intracellular accumulation of TAS-103. Cell lines resistant to Topo inhibitors have been reported to acquire the resistance because of reduced expression levels or mutation of the enzymes.5-11) The resistance phenotype was associated with alteration in both Topo I and Topo II following sequential exposure to either Topo I or Topo II inhibitor.12) Another possible mechanism of resistance is a decrease of drug accumulation caused by the presence of drug transporter proteins (P-glycoprotein (P-gp) and multidrug resistance related protein)13-15).

The multifunctional changes, decreased levels of Topo I and/or Topo II and intracellular accumulation of TAS-103 should be taken into account as factors contributing to differences in the mechanisms of resistance among various cell lines.

MATERIALS AND METHODS

Chemicals TAS-103 (Fig. 1) and SN-38 were synthesized by Taiho Pharmaceutical Co., Ltd. (Tokyo). Other drugs were purchased as follows: VP-16 and cis-diammine-dichloroplatinum (CDDP) from Nippon Kayaku Co., Ltd. (Tokyo), ADR from Kyowa Hakko Co., Ltd. (Tokyo),
aclacinomycin (ACR) from Yamanouchi Co., Ltd. (Tokyo), vincristine (VCR) from Eli Lilly, Ltd. (Tokyo), 5-fluorouracil (5-FU) from Wako Pure Chemical Industries, Ltd. (Tokyo), and camptothecin (CPT) from Sigma (St. Louis, MO). [3H]TAS-103 was obtained from Moravek Biochemicals, Inc. (Tokyo).

Cell culture and establishment of TAS-103-resistant cell lines The following cell lines were used to derive TAS-103-resistant cell lines; DLD-1 human colon cancer, A549 human lung adenocarcinoma (obtained from the Japanese Cancer Research Resources Bank, Tokyo) and P388 murine leukemia cell line (provided by Dr. T. Tsuruo, Institute of Molecular and Cellular Biology, University of Tokyo). The cells were propagated in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) in an incubator under a humidified atmosphere of 5% CO₂ at 37°C. Each TAS-103-resistant cell line was selected after long-term exposure to stepwisely increasing concentrations of TAS-103. This procedure has been continued for about 1 year. TAS-103-resistant cell lines were established using the limiting dilution technique. The selected DLD/TAS showed doubling times of 29.0 h compared to 23.3 h of parental DLD-1/p cells. A549/TAS and parental A549/p cells are characterized by doubling times of 22.1 h and 22.9 h, respectively.

Cytotoxicity test Cells were plated into 96-well microplates. After overnight incubation, medium containing a serially diluted test compound was added to each well in triplicate, and incubation was continued for 72 h. Cell growth was assessed using the modified colorimetric tetrazolium-formazan (MTT) assay. The IC₅₀ was defined as the drug concentration needed to produce a 50% reduction of growth relative to control cells. Colony-forming assay was also performed with DLD/TAS and DLD/p cells. The cells, seeded at a density of 300 cells/well in 35 mm dishes, were incubated overnight, then treated for 6 days with various concentrations of drugs. Colonies were counted after crystal-violet staining. Results were expressed as a percentage of the control number of colonies formed in the absence of drug.

**Topo catalytic activities of nuclear extracts** Crude nuclear extracts were prepared using 0.35 M salt extraction as described,17) and the protein concentration was determined using the Bradford method (Bio-Rad, Tokyo). Topo I enzymatic activity was measured in terms of the relaxation of supercoiled pBR322 DNA as described previously.16) Supercoiled pBR322DNA (0.1 µg) (TaKaRa Shuzo Co., Ltd., Tokyo) and a crude nuclear extract were mixed in 20 µl of 35 mM Tris-HCl buffer, pH 8.0, containing 72 mM KCl, 5 mM MgCl₂, 5 mM dithiothreitol, 5 mM spermidine, and 0.01% bovine serum albumin. Topo II catalytic activity was measured in terms of the decatenation of kinetoplast DNA as described previously.16) Kinetoplast DNA (0.1 µg) (TaKaRa Shuzo Co., Ltd.) and a crude nuclear extract were added to 20 µl of the reaction mixture (50 mM Tris-HCl, pH 7.5, 8.5 mM KCl, 10 mM MgCl₂, 0.5 mM dithiothreitol, 0.5 mM EDTA, 1 mM ATP and 30 µg/ml bovine serum albumin (BSA)). After incubation at 37°C for 30 min, the reaction was terminated by addition of 5 µl of solution consisting of 2.5% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue and 50% glycerol. The reaction products were subjected to electrophoresis in 1% agarose gel, performed using the Mupid 2 gel system (Advance Co., Ltd., Tokyo) in TBE buffer (90 mM Tris–borate, pH 8.3, 2.5 mM EDTA). The gel was removed and stained in 2 µM ethidium bromide (EtBr) solution for 30 min, and then photographed under ultraviolet light using Polaroid film.

**Western blotting** Equivalent amounts of nuclear extracts from all the cell lines were loaded on 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) gels and electrophoresed, then transferred to polyvinylidene difluoride (PVDF) membranes (“Immobilon,” Millipore, Tokyo). The membranes were incubated overnight at 4°C with anti-Topo antibodies. The antibodies used were anti-Topo I, a sclerosis patient’s serum (Topo Gen, Inc., Columbus, Ohio) or Topo II, a polyclonal antibody to human Topo Htz (Topo Gen, Inc.). The membranes were rinsed with phosphate buffer solution (PBS) containing 0.05% Tween 20 and incubated with secondary antibodies, horseradish peroxidase (HRP)-conjugated protein A and a goat anti-rabbit IgG for Topo I and Topo II, respectively. Immunocomplexes were detected with an enhanced chemiluminescence (ECL) system (Amersham Corp., Tokyo), using an ARGUS-50 chemiluminiometer (Hamamatsu Photonics, Tokyo).

**Quantification of PCR products** The mRNA levels of Topo were quantified by the real time-PCR method using an ABI7700 Sequence Detector System.20,21) The primers for Topo quantification to be used with the ABI Prism 7700 Sequence Detector (PE Applied Biosystems, Foster, CA) were selected to optimize the PCR for a two-step pro-

---

**Fig. 1. Structure of TAS-103.**

![Structure of TAS-103](image-url)
Establishment of TAS-103-resistant Cell Lines

file, which is preferred by TaqMan chemistry (see Table II).\(^20, 21\) The internal oligonucleotide probes were labeled with the fluorescent dyes 5-carboxyfluorescein (FAM) on the 5' end and N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA) on the 3' end. Typically, PCR was carried out in a 50 \(\mu\)l incubation mixture containing 50 ng of total RNA from each cell line isolated using the SV total RNA isolation system (Promega, Tokyo), and 1 \(\times\) TaqMan amplification. Detection was performed with the ABI 7700 system with the following cycling profile: 1 cycle of 50 \(^\circ\)C for 2 min, 1 cycle of 95 \(^\circ\)C for 5 min, and 45 cycles of 94 \(^\circ\)C for 30 s and 68 \(^\circ\)C for 1 min. The fluorescence emission data of each sample were available for analysis immediately after the completion of PCR. The amount of each PCR product of Topo was normalized with respect to the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Accumulation of TAS-103 in various cells

Exponentially growing cells (1x10^6 cells/ml) were incubated in RPMI1640 medium containing 0.1 \(\mu\)M \(^3\)H[TAS-103 for 2 h. An aliquot of 0.2 ml of cell suspension was layered on warmed silicon oil (0.2 ml) and centrifuged (12000 \(g\), 5 min). The cell pellet was dissolved by sonication in Soluene (1.5 ml) and then HIONIC-FLUOR (10 ml) was added to each tube. The radioactivity was measured in a liquid scintillation counter.

Immunocytochemical staining

Cytocentrifuge preparations of the cells were air-dried, fixed in cold acetone for 5 min, and incubated with a blocking solution containing 10% rabbit serum (Histofine, Nichirei, Tokyo) at room temperature for 10 min. The slides were incubated with antibodies to multidrug resistance associated protein (MRP), LAR or P-gp (MRPm6, LRP-56 or JSB-1, Nichirei) overnight at 4 \(^\circ\)C. Subsequently, the staining was visualized by an avidin-biotin complex immunoperoxidase method as described earlier.\(^22, 23\)

RESULTS

Sensitivities of TAS-103-resistant cell lines to various anticancer drugs

Table I shows the cytotoxicity (IC_{50} values) of various anticancer drugs to the TAS-103-resistant and parental cell lines. P388/TAS, DLD/TAS and A549/TAS cells appeared to be 62-, 26- and 27-fold resistant to TAS-103, respectively. P388/TAS cells, having a high degree of resistance, were found to be cross-resistant only to Topo II inhibitors, VP-16 (44-fold) and ADR (20-fold). DLD/TAS acquired a partial cross-resistance to Topo I inhibitors, CPT (6.0-fold) and SN-38 (5.4-fold), and Topo II inhibitors, ADR (8.3-fold) and VCR (11-fold), and cross-resistance to VP-16 (33-fold). This cell line was not cross-resistant to ACR, a catalytic inhibitor of Topo II, or to CDDP, mitomycin C (MMC) or 5-FU. A549/TAS cells showed partial cross-resistance to VP-16 (6.9-fold), ADR (6.9-fold), 5-FU (5.1-fold) and VCR (13-fold). The dose-response curves of DLD/TAS cells, as determined by MTT assay (Fig. 2a) and by colony-forming assay (Fig. 2b) were shifted to a higher concentration of TAS-103 when compared with those for the parental DLD-1/p cells.

Analysis of Topo catalytic activities

The Topo I activity (Fig. 3A) was measured in terms of the relaxation of supercoiled pBR322 plasmid DNA, and Topo II activity (Fig. 3B) was measured in terms of the decatenation assay of kinetoplast DNA in the presence of nuclear extracts from each cell line. As presented in Fig. 3A, 7.81 ng of nuclear protein from DLD-1/p showed slightly higher Topo I activity than 31.25 ng of nuclear protein from DLD/TAS, indicating that the Topo I activity present in DLD/TAS cells was reduced to about one-fourth of that seen in DLD-1/p cells. However, no apparent differences in Topo I activity were observed between P388/TAS or A549/TAS and their parental cell lines. As presented in Fig. 3B, minicircle forms of DNA were observed in the

| Drug    | IC_{50} value: \(\mu\)M (relative resistance)^\(a\) | P388/p | P388/TAS | DLD-1/p | DLD/TAS | A549/p | A549/TAS |
|---------|-----------------------------------------------|--------|----------|---------|---------|--------|---------|
| TAS-103 | 0.00052                                        | 0.032 (62) | 0.034 | 0.89 (26) | 0.019 | 0.52 (27) |
| CPT     | 0.0086                                         | 0.011 (1.3) | 0.020 | 0.12 (6.0) | 0.049 | 0.069 (1.4) |
| SN-38   | 0.0017                                         | 0.0059 (3.5) | 0.024 | 0.13 (5.4) | 0.14 | 0.11 (0.79) |
| CPT-11  | 0.61                                           | 0.95 (1.6) | 8.4 | 24 (2.9) | 34 | 22 (0.65) |
| VP-16   | 0.020                                          | 0.88 (44) | 0.95 | 31 (33) | 3.2 | 22 (6.9) |
| ADR     | 0.0034                                         | 0.067 (20) | 0.12 | 1.0 (8.3) | 0.16 | 1.1 (6.9) |
| ACR     | 0.0021                                         | 0.0038 (1.8) | 0.010 | 0.010 (1.0) | 0.0061 | 0.0086 (1.4) |
| VCR     | 0.0011                                         | 0.0022 (2.0) | 0.012 | 0.13 (11) | 0.031 | 0.41 (13) |
| CDDP    | 0.12                                           | 0.29 (2.4) | 5.0 | 2.9 (0.58) | 9.7 | 4.7 (0.48) |
| MMC     | 0.027                                          | 0.051 (1.9) | 0.30 | 0.75 (2.5) | 0.12 | 0.21 (1.8) |
| 5-FU    | 0.45                                           | 0.48 (1.1) | 7.7 | 6.4 (0.83) | 6.8 | 35 (5.1) |

^a^ Relative resistance represents the ratio of IC_{50} for the resistant line to IC_{50} for the parental line.
presence of 500 ng nuclear protein of P388/p, whereas the nuclear extract from P388/TAS cells did not decatenate kinetoplast DNA, even the presence of 2000 ng protein, indicating that Topo II activity in P388/TAS cells was reduced to below one-fourth of that of P388/p cells. Topo II activity in DLD/TAS cells was also reduced to about one-fourth of that present in DLD-1/p cells. However, the differences in Topo II activity of A549/TAS and A549/p cells were minimal.

**mRNA and protein expression levels of topoisomerases** We examined the mRNA levels of Topo I, Topo Iα and Topo Iβ by real-time PCR (Fig. 4A) using primers specific for each transcript (Table II), and the cellular contents of Topo I and Topo II by western blotting (Fig. 4B). The expression of mRNAs of Topo I, Topo Iα and Topo Iβ was greatly reduced in DLD/TAS cells to 33, 16 and 8.4%, respectively, when compared with the parental DLD-1/p cells. A similar result was obtained
from western blotting: a marked reduction in the amounts of protein of Topo I and Topo II was detected in DLD/TAS, when compared with the parental cells (Fig. 4B). Based on these results, it appeared that the observed reduction of activity of Topo I and II in DLD/TAS cells was caused by decreased levels of the respective proteins and mRNAs.

Intracellular accumulation of TAS-103 The accumulation of TAS-103 in the cells after 2 h incubation with 0.1 \( \mu M \) \[^{3}H\]TAS-103 is shown in Fig. 5. The accumulations of TAS-103 by DLD/TAS and DLD-1/p cells were very similar. However, a significant reduction of TAS-103 accumulation by A549/TAS cells was observed when compared with the parental cell line, A549/p. Such a dramatic decrease of the intracellular concentration of TAS-103 in A549/TAS cells indicates the presence of altered mechanisms of drug intracellular accumulation.

Expression of drug transporter proteins, P-gp, MRP and LRP We compared the immunocytochemical reactivity of TAS-103-resistant and their parental cell lines to antibodies to MRP, LRP or P-gp (Fig. 6). No apparent differences in the expression levels of P-gp and LRP were observed between A549/TAS and A549/p cells. However, the staining with MRP antibody of A549/TAS cells was more intense than that observed in the parental cells. There was no marked difference in the staining with P-gp and MRP between DLD/TAS and DLD-1/p cells (data not shown).

DISCUSSION

Simultaneous inhibition of Topo I and Topo II was our main goal in the development of a new anticancer agent. As a result, TAS-103, a compound showing a broad and
potent antitumor activity against various human tumor xenografts and metastatic tumors, was developed. The cytotoxic activity of TAS-103 appeared to be unaffected by overexpression of P-gp, a protein involved in multidrug resistance (MDR). The above properties justified further clinical evaluation, which is in progress in the USA.

In this study, to demonstrate the involvement of the inhibition of Topo I and Topo II in the antitumor activity of TAS-103, we attempted to establish and characterize TAS-103-resistant cell lines. Our intention was to select cell lines with affected Topo I or Topo II enzymes, allowing us to clarify the contribution of both or either enzyme to the cytotoxic effects mediated by TAS-103. Among 10 cancer cell lines exposed to TAS-103 for more than one year, only 3 acquired more than 10-fold resistance to TAS-103. The remaining cell lines failed to gain a sufficient level of resistance, indicating rather low ability of TAS-103 to induce drug resistance. Moreover, it is of particular interest that the cell lines acquiring resistance to TAS-103 differed from each other in the type of mechanism of resistance. A commonly observed mechanism of resistance is present in the case of P388/TAS cells. This cell line appeared to be cross-resistant only to Topo II inhibitors, VP-16 and ADR. The determination of Topo activities in the nuclear extracts showed a significant reduction of Topo II activity only, to below one-fourth of that present in parental cells. The Topo I activity remained essentially at the level of the parental cells, exhibiting similar sensitivity to Topo I inhibitors. DLD/TAS cells, showed cross-resistance to Topo II inhibitors, VP-16 and ADR. They were also partially resistant to Topo I inhibitors, CPT and SN-38. The intracellular uptake of TAS-103 in DLD/TAS cells is not affected significantly. The activities of intranuclear Topo I and Topo II in DLD/TAS cells diminished to one-fourth of that found in the parental cell line, and decreased levels of protein and mRNA of Topo I and Topo II were confirmed by western blotting and real-time PCR. These results provide an evidence of a direct link between the cytotoxic effect of TAS-103 and the amount of target molecules in cancer cells. Moreover, our real-time PCR studies showed decreased expression of two subtypes of Topo II, namely Topo IIα and IIβ, in DLD/TAS cells. Several reports have been published on the correlation between decreased levels of Topo IIα and resistance to topo II inhibitors (VP-16, ADR or mitoxantrone). On the other hand, the relation between decreased levels of Topo IIβ and resistance to Topo II inhibitors seems to vary depending on the drug. The expression of Topo IIα occurs in proliferating cells.
Establishment of TAS-103-resistant Cell Lines

and then decreases when the cells become non-cycling, whereas Topo IIβ is expressed in both cycling and non-cycling cells. Methyl N-{4-(9-acridinylamino)-2-methoxyphenyl}carbamate hydrochloride (AMCA) is active against non-cycling cells, therefore contributing to the inhibition of both Topo IIβ and Topo IIX.\(^\text{21,32}\) The cytotoxicity of VP-16 against unstimulated lymphocytes is mediated by Topo IIβ.\(^\text{23}\) The resistance to TAS-103 is associated with decreased levels of Topo IIα and IIβ, indicating that both isoforms are probably inhibited by TAS-103.

Another type of resistance was observed in A549/TAS cells. The resistance of A549/TAS cells was associated with a markedly decreased intracellular accumulation of TAS-103. A549/TAS was distinguishable from MDR cells, because of the lack of overexpression of P-gp, a finding which is supported by the earlier report that MDR cells (P388/ADR, MCF-7/Ad10 and KB/VCR cells) did not show cross-resistance to TAS-103.\(^\text{4}\) Decreased intracellular accumulation of TAS-103 may be related to an increased expression of MRP in A549/TAS cells. It has been reported that the increased expression of MRP in the cells was associated with a decreased accumulation of VP-16 or glutathione.\(^\text{34,35}\) MRP can actively transport structurally diverse glutathione (GSH)- and glucuronide-conjugated molecules, to a greater extent than their unmodified forms, e.g., VCR and VP-16.\(^\text{36}\) The glucuronide conjugate of TAS-103 has been observed in vivo and if such a conjugate is formed in vitro, the presence of MRP may contribute to its decreased accumulation in the cells. For this to occur, the cell must have the ability to form a conjugate and further to excrete it from the intracellular space. However, partial cross-resistance of A549/TAS cells to VP-16 indicates that increased elimination of a drug mediated by MRP may be only a contributory factor. An ongoing study indicates possible involvement of biotransformation of TAS-103 by the tumor cell line concerned.

Our results suggest that decreased levels of Topo I and/or Topo II, and in some cases, a decrease of drug accumulation, are associated with resistance to TAS-103. The involvement of multiple mechanisms presumably accounts for the differences in the mechanisms of resistance among various cell lines.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor M. Kuwano for valuable discussions and a critical review of the manuscript.

(Received December 25, 1999/Revised February 22, 2000/ Accepted February 29, 2000)

REFERENCES

1) Chen, A. Y. and Liu, L. F. DNA topoisomerases: essential enzymes and lethal targets. Annu. Rev. Pharmacol. Toxicol., \textbf{34}, 191–218 (1994).
2) Takano, H., Kohno, K., Matsuo, K., Matsuda, T. and Kuwano, M. DNA topoisomerase-targeting antitumor agent and drug resistance. Anticancer Drugs, \textbf{3}, 323–330 (1992).
3) Utsugi, T., Aoyagi, K., Asao, T., Okazaki, S., Aoyagi, Y., Sano, M., Wierzba, K. and Yamada, Y. Antitumor activity of a novel quinoline derivative, TAS-103, with inhibitory effects on topoisomerases I and II. Jpn. J. Cancer Res., \textbf{88}, 992–1002 (1997).
4) Aoyagi, Y., Kobunai, T., Utsugi, T., Yamada, R. and Yamada, Y. \textit{In vitro} antitumor activity of TAS-103, a novel quinoline derivative that targets topoisomerase I and II. Jpn. J. Cancer Res., \textbf{90}, 578–588 (1999).
5) Sugimoto, Y., Tsukahara, S., Oh-hara, T., Isoe, T. and Tsurow, T. Decreased expression of DNA topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. Cancer Res., \textbf{50}, 6925–6930 (1990).
6) Matsuo, K., Kohno, K., Takano, H., Sato, S., Kiue, A. and Kuwano, M. Reduction of drug accumulation and DNA topoisomerase II activity in acquired teniposide-resistant human cancer KB cell lines. Cancer Res., \textbf{50}, 5919–5924 (1990).
7) Long, B. H., Wang, L., Lorico, A., Wang, R. C. C., Brattain, M. G. and Casazza, A. M. Mechanisms of resistance to etoposide and teniposide in acquired resistant human colon and lung carcinoma cell lines. Cancer Res., \textbf{51}, 5725–5824 (1991).
8) Kubota, N., Nishio, T., Kakeda, Y., Ohmori, T., Fundayama, Y., Ogasaarwa, H., Ohira, T., Kunikake, H., Terashima, Y. and Saijo, N. Characterization of an etoposide-resistant human ovarian cancer cell line. Cancer Chemother. Pharmacol., \textbf{34}, 183–190 (1994).
9) Tamura, H., Kohchi, C., Yamada, R., Ikeda, T., Koizumi, O., Patterson, E., Keeve, D. J., Okada, K., Kjeidsen, E., Nishikawa, K. and Andoh, T. Molecular cloning of a cDNA of a camptothecin-resistant human DNA topoisomerase I and identification of mutation sites. Nucleic Acids Res., \textbf{19}, 69–75 (1991).
10) Kubota, N., Kanzawa, F., Nishio, T., Kakeda, Y., Ohmori, T., Fujii, Y., Terashima, Y. and Saijo, N. Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. Biochem. Biophys. Res. Commun., \textbf{188}, 571–577 (1992).
11) Bugg, B. Y., Danks, M. K., Beck, W. T. and Stutle, D. P. Expression of a mutant DNA topoisomerase II in CCRF-CEM human leukemia cells selected for resistance to teniposide. Proc. Natl. Acad. Sci. USA, \textbf{88}, 7654–7658 (1991).
12) Saleem, A., Ibrahin, N., Patel, M., Li, X., Gupta, E., Mendoza, J., Pantazis, P. and Rubin, H. E. Mechanisms of resistance in a human cell line exposed to sequential topoi-
550

somerase poisoning. Cancer Res., 57, 5100–5106 (1997).
13) Minato, K., Kanazawa, F., Nishio, K., Nakagawa, K., Fujiwara, Y. and Saji, N. Characterization of an eto-
side-resistant human small-cell lung cancer cell line. Cancer Chemother. Pharmacol., 26, 313–317 (1990).
14) Schneider, E., Horton, J. K., Yang, C. H., Nakagawa, M., and Cowan, K. H. Multidrug resistance-associated protein
gene overexpression and reduced drug sensitivity of topoi-
somerase II in a human breast carcinoma MCF7 cell line selected for etoposide. Cancer Res., 54, 152–158 (1994).
15) Lautier, D., Canitrot, Y., Deleley, R. G. and Cole, S. P. C. Multidrug resistance mediated by the multidrug resistance
protein (MRP) gene. Biochem. Pharmacol., 52, 967–977 (1996).
16) Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czvrrwinsky, M. J., Fine, D. L., Mayo, J. G., Shoemaker, R.
H. and Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazo-
lum assay. Cancer Res., 48, 589–601 (1988).
17) Deffie, A. M., Batra, J. K. and Goldenberg, G. J. Direct
correlation between DNA topoisomerase II activity and
cytotoxicity in adriamycin-sensitive and -resistant P388 leuko-
emia cells. Cancer Res., 49, 58–62 (1989).
18) Utsugi, T., Matern, M. R., Mirabelli, C. K. and Hanna, N. Potentiation of topoisomerase inhibitor-induced DNA strand
breakage and cytotoxicity by tumor necrosis factor: enhancement of topoisomerase activity as a mechanism of potentiation.
Cancer Res., 50, 2636–2640 (1990).
19) Utsugi, T., Shibata, J., Sugimoto, Y., Aoyagi, K., Wierzba,
K., Kobunai, T., Terada, T., Oh-hara, T., Tsuruo, T. and Yamada, Y. Antitumor activity of a novel podophyllotoxin
derivative (TOP-53) against lung cancer and lung metastatic
cancer. Cancer Res., 56, 2809–2814 (1996).
20) Desjardins, E. L., Chen, Y., Perkins, D. M., Teixeira, L.,
Cave, D. M. and Eisenach, D. K. Comparison of the ABI
7700 system (TaqMan) and competitive PCR for quantifica-
tion of IS6110 DNA in sputum during treatment of tubercu-
llosis. J. Clin. Microbiol., 36, 1964–1968 (1998).
21) Luthra, R., McBride, J. A., Cabanillas, F. and Sarris, A. Novel 5’-exonuclease-based real-time PCR assay for detection
of t(14;18)(q32;q21) in patients with follicular lymphoma.
Am. J. Pathol., 153, 63–68 (1998).
22) van der Valk, P., van der Kalken, C. K., Ketelaars, H.,
Boxterman, H. J., Scheffer, G. L., Kuiper, C. M., Tsuruo,
T., Lankema, C. J. L. M., Pinedo, H. M. and Scherer, R. J.
Distribution of multidrug resistance-associated P-glucopro-
tein in normal and neoplastic human tissue. Ann. Oncol., 1,
56–64 (1990).
23) Scherer, R. J., Broxterman, H. J., Scheffer, G. L., Kaaijik,
P., Dalton, W. S., van Heijningen, T. H. M., van Kalken, C.
K., Slovak, M. L., de Vries, E. G. E., van der Valk, P.,
Meijer, C. J. L. M. and Pinedo, H. M. Overexpression of a
Mr 110,000 vesicular protein in non-P-glycoprotein-mediated
multidrug resistance. Cancer Res., 53, 1475–1479 (1993).
24) Yabuki, N., Sasano, H., Kato, T., Ohara, S., Toyota, M.,
Nagura, H., Miyake, A., Nozaki, N. and Kikuchi, A. Immunohistochemical study of DNA topoisomerase II in
human gastric disorder. Am. J. Pathol., 149, 997–1007 (1996).
25) Witthoft, S., Vries, E. G. E., Keith, W. N., Nienhuis, E. F.,
Greef, W. T. A., Uges, D. R. A. and Mulder, N. H. Differential
expression of DNA topoisomerase II α and β on P-
gp and MRP-negative VM26, mAMSA and mitoxantrone-
resistant sublines of the human SCLC cell line GCL4. Br.
J. Cancer, 74, 1869–1876 (1996).
26) Hazlehurt, L. A., Foley, N. E., Gleason-Guzman, M. C.,
Hacker, M. P., Cress, A. E., Greenberger, L. W., De Jong,
W. C. and Dalton, W. S. Multiple mechanisms confer drug resistance to mitoxantrone in the human 8226 myeloma cell
line. Cancer Res., 59, 1021–1028 (1999).
27) Deruddere, S., Dalaporte, C. and Jacquemin-Sablon, A. Role of topoisomerase II β in the resistance of 9-OH-ellipti-
cine-resistant Chinese hamster fibroblast to topoisomerase
II inhibitors. Cancer Res., 57, 4301–4308 (1997).
28) Austin, C. A. and Marsh, K. L. Eukaryotic DNA topoi-
somerase IIβ. BioEssays, 20, 215–226 (1998).
29) Woessner, R. D., Mattern, M. R., Mirabelli, C. K., Johnson,
R. K. and Drake, F. H. Proliferation- and cell cycle-depen-
dent differences in expression of the 170 kDa and 180 kDa
forms of topoisomerase II in NIH-3T3 cells. Cell Growth
Differ., 2, 209–214 (1991).
30) Prosperi, E., Marchese, G. and Astaldi-Ricotti, G. C. B. Expression of the 170-kDa and 180-kDa isoforms of DNA
topoisomerase II in resting and proliferating human lympho-
cytes. Cell Prolif., 27, 257–267 (1994).
31) Turnbull, R. M., Mezes, E. L., Perenna Rogers, M., Lock,
R. B., Sullivan, D. M., Finlay, G. J., Bagulay, B. C. and Austin,
C. A. Carbamates analogues of ansamycin active against non-cycling cells: relative activity against topoi-
somerase II α and β. Cancer Chemother. Pharmacol., 44,
275–282 (1999).
32) Baguley, B. C., Leteurtre, F., Riou, J. F., Finlay, G. J. and Pommier, Y. A carbamate analogue of ansamycin with
activity against non-cycling cells stimulates topoisomerase II
cleavage at DNA sites distinct from ansamycin. Eur. J.
Cancer, 33, 272–279 (1997).
33) Tronov, V. A., Konoplyannikov, M. A., Nikolskaya, T. A.
and Konstantinov, E. A. Apoptosis of unstimulated human lymphocyte and DNA strand breaks induced by the topoi-
somerase II inhibitor etoposide (VP-16). Biochemistry, 46,
1041–1045 (1999).
34) Young, L. C., Campling, B. G., Voskoglou-Nomikos, T.,
Cole, S. P. C., Deleley, R. G. and Gerlach, J. H. Expression
of multidrug resistance protein-related genes in lung cancer:
correlation with drug response. Clin. Cancer Res., 5, 673–
680 (1999).
35) Lorico, A., Rappa, G., Finchi, A. R., Yang, D., Flavell, A.
R. and Sartorelli, C. A. Disruption of the murine MRP
(non-malignant resistance protein) gene leads to increased sen-
sitivity to etoposide (VP-16) and increased levels of glu-
thione. Cancer Res., 57, 5238–5242 (1997).
36) Rappa, G., Lorico, A., Flavell, A. R. and Sartorelli, C. A. Evidence that the multidrug resistance protein (MRP)
functions as a co-transporter of glutathione and natural product
toxins. Cancer Res., 57, 5232–5237 (1997).