Jpn. J. Cancer Res. 85, 869–874, August 1994

Semi-quantitative Analysis of DNA Topoisomerase-I mRNA Level Using Reverse Transcription-Polymerase Chain Reaction in Cancer Cell Lines: Its Relation to Cytotoxicity against Camptothecin Derivative

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Expression of DNA topoisomerase (Topo)-I mRNA in various cancer cell lines was detected using the reverse transcription-polymerase chain reaction (RT-PCR) method. The cytoplasmic polyadenylated RNA isolated from cancer cell lines was reverse-transcribed and the complementary DNA was amplified by PCR primed with Topo-I specific primers. Fidelity of the amplified sequence was confirmed by restriction endonuclease digestion and Southern blot hybridization. The level of Topo-I mRNA was correlated positively with the cytotoxicity of a Topo-I inhibitor, a camptothecin derivative. This RT-PCR method may be applicable to the assessment of sensitivity of cells to Topo-I targeted drugs, especially when only small quantities of cell samples are available.

Key words: DNA topoisomerase I — mRNA — RT-PCR — Cancer cell line

Mammalian DNA topoisomerase I (Topo-I) activity is important for semi-conservative replication of double-helical DNA and for other DNA functions such as transcription, recombination and chromosomal decondensation.[1] Camptothecin, an anti-tumor alkaloid isolated from *Camptotheca acuminata*,[2] interferes with the DNA breakage-reunion reaction of Topo-I by trapping an abortive reaction intermediate, the cleavable complex,[3] and has significant anti-tumor activity against experimental animal tumor models.[4] However, this compound showed a low response rate in clinical trials and caused significant myelotoxicity.[5,6] Thereafter, new camptothecin derivatives with higher anti-tumor activity and less toxicity have been synthesized and subjected to preclinical and clinical trials.[7–9] Giovanella *et al.*[9] reported that a camptothecin analog exerted a potent anti-tumor effect on human colon cancer cells carried by immunodeficient mice, and suggested that the increased levels of Topo-I in colon cancer xenografts might be a factor contributing to the effectiveness. Moreover, several groups[10–12] have suggested that the reduction of Topo-I activity and its mRNA is involved in the development of resistance to camptothecin. The present study was designed to develop a rapid and specific test for the detection of Topo-I mRNA expression based on the reverse transcription-polymerase chain reaction (RT-PCR) method.[13]

MATERIALS AND METHODS

Cell lines The cell lines used in this study were as follows; HeLa S3 (uterine cervical carcinoma), HHUA, Ishikawa (endometrial carcinoma) and Caov-3 (ovarian adenocarcinoma), which were maintained in culture medium as described previously.[14]

Oligonucleotides The 21-base oligonucleotide primers and internal probes for Topo-I complementary DNA (cDNA) were designed according to the report of D'Arpa *et al.*[15] The sequences of the primers and internal probes are illustrated in Fig. 1. The predicted size of the Topo-I PCR product is 680 bp. The internal probe was end-labeled using biotinylated dATP (biotin-14-dATP; Bethesda Research Laboratories, Gaithersburg, MD) by the terminal deoxynucleotidyl transferase method[16] and purified by chromatography through Sephadex G-25.

Isolation of RNA Tumor cells in their exponential growth phase were recovered and washed three times with cold phosphate-buffered saline. The cells (1×10^6) were pelleted and resuspended in 100 μl of lysis buffer [10 m*M* Tris-HCl, pH 8.6, 0.14 *M* NaCl, 1.5 m*M* $MgCl_2$, 0.5% Nonidet P-40, 1 m*M* dithiothreitol and 1,000 units/ml ribonuclease (RNase) inhibitor (RNasin; Promega, Madison, WI)]. The cell suspension was placed on ice for 5 min and centrifuged at 12,000*g* for 2 min at 4°C. The nucleus-free supernatant (100 μl) was transferred to another tube containing 0.5 mg of oligo(dT)-bound superparamagnetic polystyrene beads [Dynabeads Oligo-$(dT)_{25}$; Dynal A.S., Oslo, Norway] in 100 μl of 2× binding buffer (20 m*M* Tris-HCl, pH 7.5, 1 *M* LiCl and 2 m*M* EDTA). The tube was then held for 5 min at room temperature for binding of polyadenylated ($polyA^+$) RNA to $oligo(dT)_{25}$ on the bead surface (0.5 mg of beads can bind about 1 μg of $polyA^+$ RNA) and subsequently placed in a magnetic particle concentrator (Dynal MPC-E; Dynal A.S.) for 1 min. The bead-free superna-

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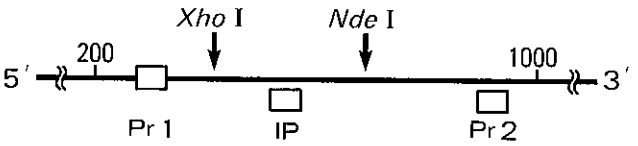

Fig. 1. Positions of the primers and the internal probe for Topo-I cDNA. Pr1: a sense primer, 5′-GAGGAAAAGGTT-CGAGCCTCT-3′ from nucleotide position 271 to 291; Pr2: an antisense primer, 5′-TTCCGAGCTTCCGTCTGGGCT-3′ from nucleotide position 931 to 950; and IP: an internal probe, 5′-CTTGTTATGTGGCTCAGGAAC-3′ from nucleotide position 553 to 573.[13] The predicted size of Topo-I PCR product is 680 bp. The target sequence contains a cleavage site for *Xho* I at nucleotide position 416 (C ↓ TCCGAG) and one for *Nde* I at nucleotide position 690 (CA ↓ TATG).

tant was removed by aspiration, while the tube remained in the concentrator. The polyA$^+$ RNA-bound beads were washed three times with 200 μl of washing buffer (10 m*M* Tris-HCl, pH 7.5, 0.15 *M* LiCl and 1 m*M* EDTA) using the concentrator. After washing, the beads were incubated with 200 μl of 2 m*M* EDTA, pH 7.5, for 2 min at 65°C and the supernatant containing polyA$^+$ RNA was transferred to another tube. The RNA was precipitated by adding 20 μg of glycogen as a carrier, 30 μl of 2 *M* sodium acetate and 600 μl of cold absolute ethanol, and stored at −70°C for 15 min. The RNA solution was then centrifuged at 12,000*g* for 15 min at 4°C and the precipitate was washed with 80% ethanol, dried by evaporation and resuspended in 10 μl of buffer [50 m*M* Tris-HCl, pH 7.4, 10 m*M* $MgCl_2$, 5 units of RNase-free deoxyribonuclease (DNase) I (Boehringer Mannheim Yamanouchi, Tokyo) and 40 units of RNase inhibitor]. The reaction was allowed to proceed for 1 h at 37°C and terminated by heating for 5 min at 90°C. The concentration of RNA was determined using a colorimetric assay (DNA DipStick kit; Invitrogen, San Diego, CA) and the RNA sample was diluted with diethyl pyrocarbonate-treated water at a final volume of 50 μl. Approximately 0.5 μg of cytoplasmic polyA$^+$ RNA per 1×10^6 cells was obtained.

RT A portion of the polyA$^+$ RNA solution (9.5 μl) was heated at 90°C for 5 min to disrupt the region of secondary structure that might involve the polyA$^+$ tail. To generate the first strand of cDNA from the polyA$^+$ RNA, 600 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories), 6 μl of 5×buffer (250 m*M* Tris-HCl, pH 8.3, 375 m*M* KCl and 15 m*M* $MgCl_2$, 10 m*M* dithiothreitol), 1.5 μl of dNTP mix (10 m*M* each of dATP, dCTP, dTTP and dGTP; Pharmacia LKB Biotechnology, Uppsala, Sweden), 1.5 μl of oligo(dT)$_{12-18}$ (Pharmacia LKB Biotechnology), 40 units of RNase inhibitor, 3 μg of nuclease-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and sterile distilled water were added to give a final volume of 30 μl. The mixture was incubated at 42°C for 1 h, heated to 95°C for 5 min to denature the reverse transcriptase and flash-cooled to 4°C.

PCR The primers used to amplify glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were 5′-TGAAGGT-CGGAGTCAACGGATTTGGT-3′ (G3PDH-5′; 71–96, exon I) and 3′-CACCACCTGGAGTACCGGGTG-TAC-5′ (G3PDH-3′; 1030–1053, exon VIII) (Clontech Laboratories, Palo Alto, CA).[17] The size of PCR products for G3PDH was 983 bp. Seventeen μl of PCR mixture was added to 1 μl of the cDNA pool from the RT reaction. The PCR mix consisted of 0.1 μl (0.5 units) of recombinant *Taq* DNA polymerase (AmpliTaq DNA polymerase; Perkin-Elmer Cetus, Norwalk, CT), 1.6 μl of dNTP mix (10 m*M* each of dATP, dCTP, dTTP and dDTP; Pharmacia LKB Biotechnology), 2 μl of 10× reaction buffer (100 m*M* Tris-HCl, pH 8.3, 500 m*M* KCl, 10 m*M* $MgCl_2$ and 0.01% gelatin) and 13.3 μl of sterile distilled water. One μl each of 5′- and 3′-primers was added at the final concentration of 1 μM and the mixture was subjected to PCR amplification using the DNA Thermal Cycler 480 (Perkin-Elmer Cetus). This solution was subjected to 30 cycles of amplification. The amplification profile involved denaturation at 94°C for 1 min, primer annealing at 60°C for 2 min and primer extension at 72°C for 3 min. To detect the presence of contaminants, all the PCRs were accompanied by negative controls having the necessary components for PCR, but without the addition of template cDNA. Following amplification, 8 μl of the reaction product was mixed with 2 μl of gel loading solution (0.025% bromophenol blue and 30% glycerol in distilled water) and 8 μl of the mixture was applied to 3% NuSieve/1% Agarose (FMC BioProducts, Rockland, ME) gel in Tris-borate/EDTA buffer (45 m*M* Tris-borate, pH 8.0, 1 m*M* EDTA). A 50 to 1,000 bp DNA ladder (BioMarker Low; Bioventures Inc., Murfreesboro, TN) was used as size markers. The gels were electrophoresed at 4 V/cm for 90 min, stained with ethidium bromide and photographed. When desired, approximately 1 μg of the cDNA amplified with Topo-I specific primers was cleaved with 100 units of *Xho* I (Takara Shuzo Co., Kyoto) and/or *Nde* I (Takara Shuzo Co.) for 12 h at 37°C.

Southern blot analysis The gel was denatured and neutralized and the DNA was transferred to a nylon membrane (PhotoGene Nylon Membrane; Bethesda Research Laboratories) by capillary blotting for 12 h using 20×standard saline citrate (SSC, 3 *M* NaCl, 0.3 *M* sodium citrate, pH 7.0). After blotting, the membrane was baked at 80°C for 2 h and prehybridized at 42°C for 2 h in 1 *M* NaCl, 50 m*M* Tris-HCl, pH 7.5, 1% sodium

dodecyl sulfate (SDS), 10% dextran sulfate and 100 μg/ml denatured salmon sperm DNA. In the hybridization, the same solution was used, except that it also included the biotinylated probe at a final concentration of 100 ng/ml. After hybridization at 42°C for 18 h, the membrane was washed twice at 55°C for 30 min in 6×SSC, and 0.1% SDS. The blots were blocked by incubation in 3% bovine serum albumin, 1 *M* NaCl, and 0.1 *M* Tris-HCl, pH 7.5, for 4 h at 55°C. The hybridized nucleic acids were detected with a nonradioactive detection kit (PhotoGene Nucleic Acid Detection System; Bethesda Research Laboratories).

Semi-quantitative analysis The ethidium bromide-stained PCR products were analyzed densitometrically from a photo by calculating the area in arbitrary units (AU) using a Bio Image instrument (Millipore Corporation, Benfold, MA). The relationship between the starting amount of cDNA and the PCR products was analyzed with G3PDH mRNA expression as an internal standard in triplicate. Topo-I and G3PDH mRNA levels were analyzed by scanning densitometry of PCR products at 1:1 dilution in triplicate and Topo-I mRNA/G3PDH mRNA levels (corrected AU) were plotted on a log-log scale to obtain IC_{50} for camptothecin derivative, irinotecan hydrochloride (CPT-11). CPT-11 was kindly provided by Yakult Co., Tokyo.

RESULTS

We first determined the optimal concentration of Mg^{2+} in PCR buffer, because the presence of divalent cations is critical for amplification.[18] The yields of Topo-I PCR products generated from a set of reactions containing various concentrations of Mg^{2+} are shown in Fig. 2. Since a single strong band of the predicted size (689 bp) was observed at a concentration of 1.0 m*M* $MgCl_2$, this level of $MgCl_2$ was used in the PCR reaction buffer thereafter.

Next, the fidelity of the amplified sequence for Topo-I cDNA, which was selected to contain cleavage sites for two restriction endonucleases, *Xho* I and *Nde* I (Fig. 1) was confirmed. The PCR product was subjected to digestion by *Xho* I and/or *Nde* I, and fractionated by electrophoresis. As shown in Fig. 3A, these treatments cleaved the uncut PCR product (lane 1) into the expected fragments; 534 and 146 bp (*Xho* I digestion, lane 2), 420 and 260 bp (*Nde* I digestion, lane 3), and 274, 260 and 146 bp (*Xho* I and *Nde* I digestion, lane 4). The uncut and cut PCR products were then transferred to a nylon membrane and probed with an internal probe that was complementary to a region within the sequence between the cleavage site for *Xho* I and that for *Nde* I (Fig. 1). As shown in Fig. 3B, a single fragment that contained sequences complementary to the internal probe was detected by autoradiography; 680 bp (uncut, lane 1), 534 bp (*Xho* I digestion, lane 2), 420 bp (*Nde* I digestion, lane 3), and 274 bp (*Xho* I and *Nde* I digestion, lane 4).

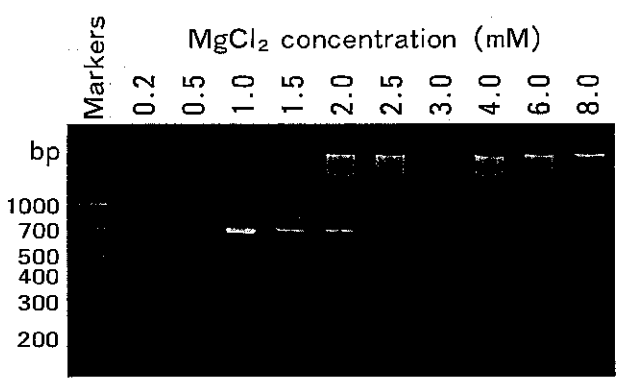

Fig. 2. Effect of Mg^{2+} concentration on the yield of Topo-I PCR product. The single preparation of polyA$^+$ RNA isolated from Caov-3 cells was reverse-transcribed and cDNA was amplified by PCR with Topo-I-specific primers as described in "Materials and Methods." The reactions were performed under the same conditions except for the concentrations of $MgCl_2$ in PCR buffer.

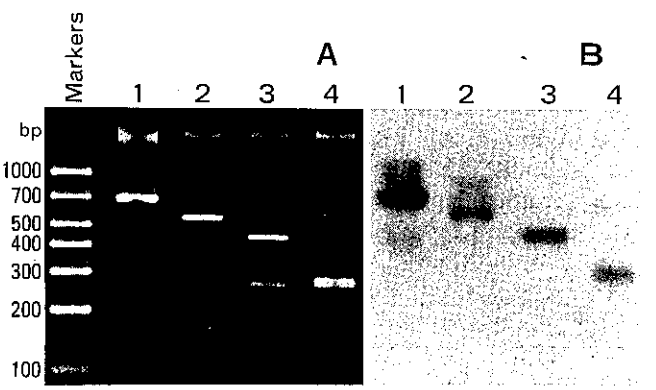

Fig. 3. Analysis of the digested Topo-I PCR products by gel electrophoresis (A) and Southern blot hybridization (B). The Topo-I PCR products were obtained by amplifying the cDNA from Caov-3 cells with Topo-I-specific primers, and cut with *Xho* I and/or *Nde* I. The uncut and cut PCR products were fractionated by gel electrophoresis, blotted onto a nylon membrane and hybridized with an internal probe. The sequences of the primers and the internal probe are shown in Fig. 1. Lane 1: uncut; lane 2: *Xho* I digestion; lane 3: *Nde* I digestion; lane 4: *Xho* I and *Nde* I digestion. Digestions by *Xho* I, *Nde* I and both of them should cleave the PCR product of 680 bp into fragments of 534 and 146 bp, of 420 and 260 bp, and of 274, 260 and 146 bp, respectively. Hybridization of the digested PCR products with the internal probe should yield only one band of the fragment containing sequences complementary to the probe.

Amplified products derived from genomic DNA remaining in the RNA preparations are the same in size as those from the cDNA synthesized in the RT reaction, because Topo-I is encoded by a single gene.[15] Fig. 4 shows the importance of pretreating the polyA$^+$ RNA sample with DNase I. When polyA$^+$ RNA was subjected to the RT-PCR procedure in the absence of DNase I pretreatment, the PCR product of the predicted size (680 bp) was detected. However, even when RT was omitted from the reaction, a faint band of the same size was observed, implying that a portion of the product resulted from contaminating DNA in the polyA$^+$ RNA preparation. Indeed, no specific DNA amplicon was observed when the same polyA$^+$ RNA was treated with DNase I and subjected to the PCR procedure. Therefore, pretreatment of the polyA$^+$ RNA with DNase I followed by the RT-PCR procedure resulted in an amplicon that could only be generated by amplification of the cDNA synthesized in the RT reaction.

In order to quantitate the relative levels of Topo-I mRNA expressions in the four gynecologic tumor cell lines, cDNA was obtained from those cells and seven serial 1:2^n dilutions were subjected to PCR (Fig. 5). There was a linear relationship between the amount of the PCR product formed and that of cDNA, which depends on the amount of initial mRNA present in the sample of Topo-I (Fig. 6). As described previously,[14] the four cell lines tested are responsive to CPT-11, a new derivative of camptothecin. The level of Topo-I mRNA expression is correlated positively with the sensitivity to CPT-11 cytotoxicity. Relative quantitation of Topo-I mRNA level in AU with G3PDH mRNA level and IC_{50} values to CPT-11 is shown in Table I. There was a good linear relationship between IC_{50} values to CPT-11 and relative Topo-I mRNA levels (Fig. 7).

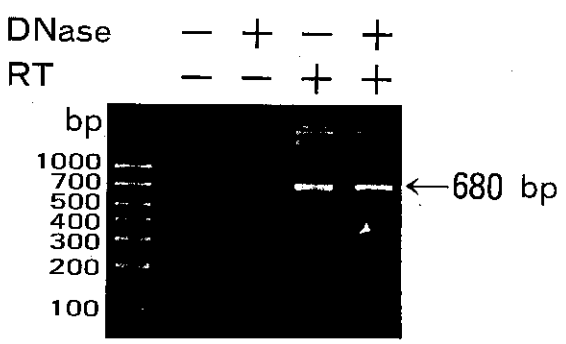

Fig. 4. Effectiveness of DNase I pretreatment of polyA$^+$ RNA preparation in the RT-PCR analysis of Topo-I mRNA. The polyA$^+$ RNA isolated from Caov-3 cells was pretreated with DNase I (+) or not pretreated (−). Each was then subjected to the RT-PCR procedure in the presence (+) or absence (−) of reverse transcriptase.

DISCUSSION

Although conventional methods such as Northern blot and *in situ* hybridization can be used to analyze mRNA expression, those methods are technically difficult and have practical problems of time needed and sensitivity of detection as well as numbers of cells required. The RT-PCR method has recently been introduced as a highly sensitive tool for the study of gene expression at the RNA level. For RT-PCR analysis, the successful isolation of undamaged RNA and the specific priming of

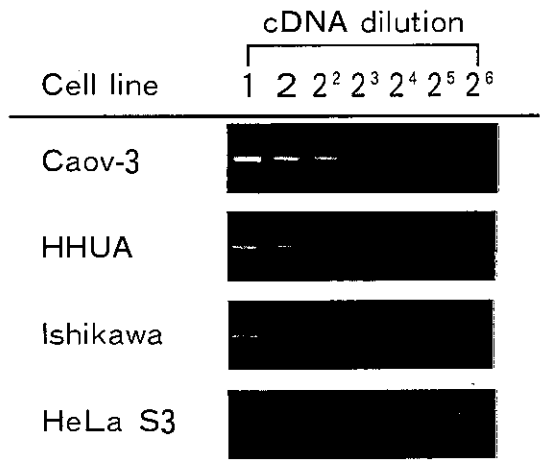

Fig. 5. Seven serial 1:2^n cDNA dilutions in four cell lines are shown.

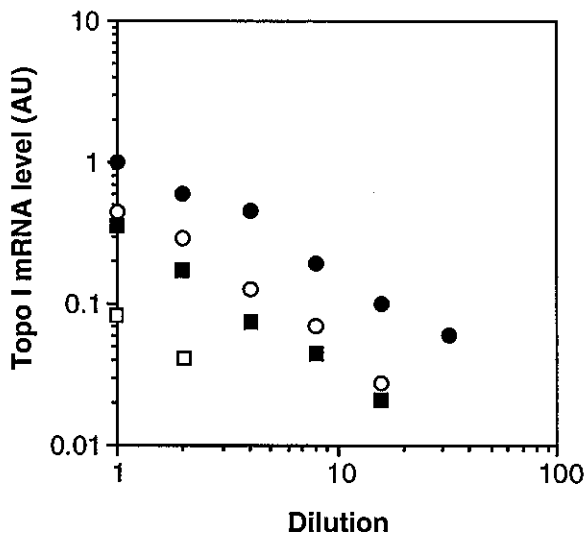

Fig. 6. Relationship between amount of cDNA and the PCR products formed in four cell lines (●: Caov-3, ○: HHUA, ■: Ishikawa, □: HeLa S3). There are linear relationships between the above two values in the four cell lines.

Table I. Topo-I mRNA/G3PDH mRNA Levels and IC_{50} for CPT-11 in Four Cell Lines

	Topo-I mRNA level (AU)	G3PDH mRNA level (AU)	Topo-I mRNA/G3PDH mRNA (corrected AU)	IC_{50} for CPT-11[a]
Caov-3	1.005	0.288	3.49	4.77
HHUA	0.436	0.234	1.86	6.76
Ishikawa	0.375	0.256	1.46	7.59
HeLa S3	0.082	0.284	0.29	21.93

a) IC_{50} values were taken from our previous report.

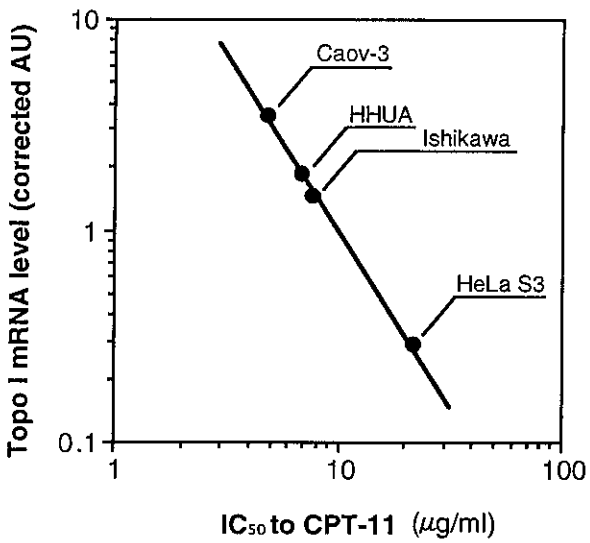

Fig. 7. The PCR-evaluated Topo-I mRNA level and IC_{50} for CPT-11 were plotted on a log-log scale. There is a good linear relationship between them.

RT might be the limiting factors determining the specificity and sensitivity of cDNA amplification. Although the RT-PCR procedure used is essentially based on the method described by Rappolee *et al.*,[13] a few modifications have been made. First, we isolated polyA$^+$ RNA from cytoplasm using oligo(dT)$_{25}$-bound magnetic beads without an intermediate total RNA purificaion step. This method is simple and rapid, and applicable to extremely small samples of clinical materials. Secound, we pretreated polyA$^+$ RNA with DNase I to digest genomic DNA that remained as a trace contaminant in the RNA preparation. The RT-PCR technique, due to its high sensitivity, often generates false-positive reactions that could result from genomic DNA contamination of the isolated RNA. In order to avoid this problem, target sequences should contain an intron/exon border so that genomic DNA and mRNA can be distinguished. This strategy, however, cannot be applied to the Topo-I gene, which lacks introns.[15] The DNase I pretreatment is a simple and well-tested procedure for RT-PCR to analyze expression of intronless genes.[19,20] Fidelity of the amplified sequence was confirmed by PCR product restriction enzyme analysis and by Southern blot hybridization.

Topo-I has been identified as a principal target of Topo-I inhibitors such as camptothecin.[1,3] CPT-11 is a new derivative of camptothecin, and a good candidate for clinical trials because of its high anti-tumor activity, low toxicity and high aqueous solubility.[7,8] The results shown in Figs. 5–7 and Table I suggest that the responsiveness of tumor cells to CPT-11 depends on the steady-state level of Topo-I mRNA in the cells. Since the RT-PCR technique provides a readout of mRNA being produced by cells at a given point of time, it is not necessarily clear whether the expression of Topo-I mRNA in the cells is correlated with the level of Topo-I activity. In this regard, Eng *et al.*[10] have reported that resistance to camptothecin is due to reduced activity of Topo-I in the cells, and that Topo-I activity is directly related to the mRNA level when analyzed by Western and Northern blotting, respectively. If the sensitivity to Topo-I inhibitors is proportional to the Topo-I mRNA level, the development of an assay to detect Topo-I gene expression in tumor cells could help in the clinical monitoring of drug sensitivity. Several possibilities could explain the difference in the levels of Topo-I mRNA among the cell lines, e.g., differences in gene amplification, gene transcription rate or mRNA stability, and these are presently under investigation.

Although the numbers of samples examined in the present study were small, we have been able to detect specifically the expression of Topo-I mRNA using RT-PCR in cell lines. The procedure for isolating polyA$^+$ RNA might be applicable to processing of tissue samples, from which small amounts of RNA are often obtained, to assay sensitivity to Topo-I-targeted drugs.

ACKNOWLEDGMENTS

This work was supported in part by Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (#04670995). We would like to thank Dr. Jiro Fujimoto for helpful discussions and technical advices.

(Received January 31, 1994/Accepted May 17, 1994)

REFERENCES

1) Kornberg, A. and Baker, T. A. Topoisomerases. *In* "DNA Replication," ed. A. Kornberg and T. A. Baker, pp. 379–401 (1991). WH Freeman and Company, New York.
2) Wall, M. E., Wani, M. C., Cook, C. E., Palmar, K. H., MacPhail, A. T. and Sim, G. A. Plant antitumor agents. 1. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.*, **88**, 3888–3890 (1966).
3) Hsiang, Y. H., Hertzberg, R., Hecht, S. and Liu, L. F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.*, **260**, 1483–1488 (1985).
4) Gallo, R. C., Whang-Peng, J. and Adamson, R. H. Studies on the antitumor activity, mechanism of action, and the cell cycle effects of camptothecin. *J. Natl. Cacner Inst.*, **46**, 789–795 (1971).
5) Gottlieb, J. A. and Luce, J. K. Treatment of malignant melanoma with camptothecin (NSC-100880). *Cancer Chemother. Rep.*, **56**, 103–105 (1972).
6) Muggia, F. M., Creaven, P. J., Hansen, H. H., Cohen, M. H. and Selawry, O. S. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother. Rep.*, **56**, 515–521 (1972).
7) Burris, H. A., Rothenberg, M. L., Kuhn, J. G. and Von-Hoff, D. D. Clinical trials with the topoisomerase I inhibitors. *Semin. Oncol.*, **19**, 663–669 (1992).
8) Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C. and Kaufmann, S. H. The current status of camptothecin analogues as antitumor agents. *J. Natl. Cancer Inst.*, **85**, 271–291 (1993).
9) Giovanella, 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).
10) Eng, W. K., McCabe, F. L., Tan, K. B., Mattern, M. R., Hofmann, G. A., Woessner, R. D., Hertzberg, R. P. and Johnson, R. K. Development of a stable camptothecin-resistant subline of P388 leukemia with reduced topoisomerase I content. *Mol. Pharmacol.*, **38**, 471–480 (1990).
11) Sugimoto, Y., Tsukahara, S., Oh-hara, T., Isoe, T. and Tsuruo, T. Decreased expression of DNA topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. *Cancer Res.*, **50**, 6925–6930 (1990).
12) Sugimoto, Y., Tsukahara, S., Oh-hara, T., Liu, L. F. and Tsuruo, T. Elevated expression of DNA topoisomerase II in camptothecin-resistant human tumor cell lines. *Cancer Res.*, **50**, 7962–7965 (1990).
13) Rappolee, D. A., Wang, A., Mark, D. and Werb, Z. Novel method for studying mRNA phenotypes in single or small numbers of cells. *J. Cell Biochem.*, **39**, 1–11 (1989).
14) Mori, H., Sawairi, M., Itoh, N., Hanabayashi, T., Kondoh, H. and Tamaya, T. Augmentation of antiproliferative activity of CPT-11, a new derivative of camptothecin, by tumor necrosis factor against proliferation of gynecologic tumor cell lines. *Anti-cancer Drugs*, **2**, 469–474 (1991).
15) D'Arpa, P., Machlin, P. S., Ratrie, H., Rothfield, N. F., Cleveland, D. W. and Earnshaw, W. C. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67.7-kDa carboxyl-terminal fragment. *Proc. Natl. Acad. Sci. USA*, **85**, 2543–2547 (1988).
16) Karger, B. D. Preparation and use of biotinylated oligonucleotide probes. *Focus*, **11**, 57–58 (1989).
17) Arcali, P., Martinelli, R. and Salvatore, F. The complete sequence of a full length cDNA for human liver glyceraldehyde-3-phosphate dehydrogenase: evidence for multiple mRNA species. *Nucleic Acids Res.*, **153**, 1012 (1984).
18) Innis, M. A. and Gelfand, D. H. Optimization of PCRs. *In* "PCR Protocols: A Guide to Methods and Applications," ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, pp. 3–12 (1990). Academic Press, San Diego.
19) Kashani-Sabet, M., Rossi, J. J., Lu, Y., Ma, J. X., Chen, J., Miyachi, H. and Scanlon, K. J. Detection of drug resistance in human tumors by *in vitro* enzymatic amplification. *Cancer Res.*, **48**, 5775–5778 (1988).
20) Grillo, M. and Margolis, F. L. Use of reverse transcriptase polymerase chain reaction to monitor expression of intronless genes. *BioTechniques*, **9**, 262–268 (1990).