[CANCER RESEARCH 54, 539–546, January 15, 1994]

Elevation of Topoisomerase I Messenger RNA, Protein, and Catalytic Activity in Human Tumors: Demonstration of Tumor-type Specificity and Implications for Cancer Chemotherapy

Intisar Husain, James L. Mohler, Hillard F. Seigler, and Jeffrey M. Besterman[1]

Department of Cell Biology, Glaxo Inc. Research Institute, Research Triangle Park, North Carolina 27709 [I. H., J. M. B.]; Division of Urology, Department of Surgery, and University of North Carolina-Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599 [J. L. M.]; and the Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710 [H. F. S.]

ABSTRACT

Topoisomerase I has been identified as an intracellular target of camptothecin, a plant alkaloid with anticancer activity. Various lines of evidence suggest that the sensitivity of cells to this drug is directly related to the topoisomerase I content. In humans, the levels of topoisomerase I have been shown to be elevated in colorectal tumors, compared to normal colon mucosa. The aim of our study was to determine whether (*a*) topoisomerase I levels are elevated in other solid tumors, (*b*) the elevated enzyme is catalytically active in these tumors, and (*c*) the increase in topoisomerase I levels in colorectal tumors is a result of increased transcription or translation. Topoisomerase I levels were quantitated in crude extracts from colorectal, prostate, and kidney tumors and their matched normal counterparts by Western blotting and by direct determination of catalytic activity, and mRNA levels were determined by Northern blotting. By Western blotting, colorectal tumors showed 5–35-fold increases in topoisomerase I levels, compared to their normal colon mucosa. In the case of prostate tumors, the increase was 2–10-fold, compared with benign hyperplastic prostate tissue from the same patients. However, no difference was observed in topoisomerase I levels in kidney tumors, compared to their normal counterparts. The catalytic activity of topoisomerase I was determined by a quantitative ^{32}P-transfer assay in crude homogenates, without isolating nuclei. Colorectal and prostate tumors exhibited 11–40- and 4–26-fold increases, respectively, in catalytic activity. However, kidney tumors did not show any alteration in catalytic activity, compared to their normal matched samples. Thus, for all three tumor types there was a good correlation between enzyme levels and catalytic activity. Finally, colorectal tumors were analyzed for steady state mRNA levels. A 2–33-fold increase in mRNA levels was found in colorectal tumors, compared to normal colon mucosa. These results suggest that alterations in topoisomerase I expression in humans are tumor type specific and that the increase in topoisomerase I levels results from either increased transcription of the topoisomerase I gene or increased mRNA stability.

INTRODUCTION

Topoisomerases are nuclear enzymes that control the topological states of DNA by catalyzing the concerted breaking and rejoining of DNA strands. Two major types of topoisomerases, topoisomerase I and topoisomerase II, have been identified in all eukaryotic cells. Topoisomerase I creates a transient single-strand nick and passes the intact strand through the break before resealing. Topoisomerase II makes a double-strand break and passes an intact double strand through the nick before the nick is sealed. These enzymes have been implicated in many important cellular processes such as replication, transcription, recombination, DNA repair, and chromosome segregation at mitosis (1, 2).

Received 8/5/93; accepted 11/15/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Department of Cell Biology, Glaxo Inc. Research Institute, Five Moore Drive, Research Triangle Park, NC 27709.

Both types of topoisomerases have generated extensive clinical interest after their identification as molecular targets of several antitumor drugs (2, 3). Topoisomerase II-directed drugs are diversified in structure and include both DNA intercalators and nonintercalators. Many of these drugs, *i.e.*, Adriamycin, mitoxantrone, and etoposide, are in clinical use.

Recently, topoisomerase I has been identified as a molecular target of camptothecin, a plant alkaloid that shows antitumor activity in various animal tumor models (4, 5). Camptothecin and its derivatives interfere with the DNA breakage-reunion reaction by stabilizing a key covalent intermediate between DNA and the enzyme, resulting in the cleavage of DNA (4, 6).

The sensitivity of cells to the topoisomerase-targeted drugs appears to be related to the level of topoisomerases in the nucleus (7–11). Selective toxicity of topoisomerase II inhibitors toward rapidly proliferating tumor cells might be due to the high levels of topoisomerase II in these cells, compared to low levels of this enzyme in quiescent cells (12, 13). In contrast to topoisomerase II, the levels of topoisomerase I do not change with the cell cycle. However, the recent finding by Giovenella *et al.* (14) that topoisomerase I levels appear elevated in advanced stages of human colorectal adenocarcinoma, compared to normal colonic mucosa, suggests that topoisomerase I may also be an important target of antitumor drugs for tumors showing elevated levels of this enzyme.

Increased expression of topoisomerase I in tumors, compared to normal tissue, could provide selective therapeutic cytotoxicity of drugs directed against topoisomerase I. This prediction is supported by the following observations: (*a*) baby hamster kidney cells overexpressing topoisomerase I are hypersensitive to camptothecin (9); and (*b*) camptothecin-resistant tumor cell lines express decreased levels of topoisomerase I (10).

In spite of the clinical importance of topoisomerase I, data concerning the quantitation of this enzyme in human tumor biopsies are very limited (14–16). Furthermore, in these studies topoisomerase I levels have not been correlated with the catalytic activity of the enzyme and its expression at the mRNA level.

In an attempt to understand the regulation of DNA topoisomerase I, we studied the expression of topoisomerase I in human tumor specimens and their normal counterparts. Specifically, we wanted to answer the following questions. (*a*) Are the levels of topoisomerase I, which are elevated in colorectal adenocarcinoma, also elevated in other solid tumors or is this phenomenon specific to colorectal tumors? (*b*) Is the elevated topoisomerase I catalytically active in these tumors? (*c*) Are increased levels of topoisomerase I due to increased transcription or translation? Our results indicate that in tumors of the colon and prostate, but not kidney, topoisomerase I catalytic activity, protein levels, and mRNA content were significantly elevated, compared to matched normal controls. The increase in the topoisomerase I levels results from either increased transcription of the topoisomerase I gene or increased mRNA stability.

MATERIALS AND METHODS

PMSF,[2] leupeptin, pepstatin A, and Sephadex G-50 quick-spin columns were purchased from Boehringer Mannheim. Plasmid pBR322, DNase I, and the random primer labeling kit were supplied by Bethesda Research Laboratories. [^{32}P]dCTP (3000 and 6000 Ci/mmol) and nick translation kits were from DuPont New England Nuclear Corporation. ^{125}I-labeled Protein A was supplied by Amersham. Klenow fragment, polynucleotide kinase, T_4 ligase, and restriction enzymes were purchased from either BRL or Promega Corporation. The Sequenase kit and cDNA synthesis kit were supplied by United States Biochemical Corporation. Immobilon-P and Nytran membranes were purchased from Millipore (Bedford, MA). Human scleroderma sera containing antitopoisomerase I antibodies (17) were a gift from Dr. Paul Agris, North Carolina State University. Polyclonal antibodies against purified topoisomerase were a gift from Dr. Leroy F. Liu, The Johns Hopkins University School of Medicine (Baltimore, MD). Peptides for raising antibodies were synthesized at the Department of Microbiology and Immunology, University of North Carolina (Chapel Hill, NC). The IgG purification kit and Sulfolink coupling gel for preparation of the affinity column were obtained from Pierce Chemical Company.

Tissues. Samples of colorectal tumors and normal colon were obtained from patients who underwent surgery at Duke University Medical Center (Tables 1, 2, and 3). When the tumor was dissected, care was taken to ensure that normal components of bowel wall were not included. For normal colon, just the mucosa was dissected away and elements of bowel wall including the muscle layers and serosa were not included. Samples of prostatic adenocarcinoma and grossly normal peripheral zone from the centralateral lobe were obtained at radical retropubic prostatectomy, for clinically localized carcinoma, from nine patients treated at the University of North Carolina (Table 1). Eight patients with clinically organ-confined renal masses had tumor and normal renal cortical tissue sampled at radical nephrectomy at the University of North Carolina (Table 1). All specimens were frozen immediately in liquid nitrogen and stored at −100°C. The identity of all tissues studied was confirmed microscopically and all specimens were free of necrosis and preservation or preparation damage. The clinical and pathological characteristics of patients with colorectal, prostate, and renal malignancies are summarized in Tables 1 and 3.

Plasmid containing GAPDH cDNA was a gift from Dr. Cheryl Walker, Chemical Industry Institute of Toxicology (Research Triangle Park, NC). HeLa cells were obtained from the Lineberger Comprehensive Cancer Center, University of North Carolina (Chapel Hill, NC). The HT-29 human colon adenocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). The cells were grown in McCoy's 5A medium containing 10% fetal bovine serum and 2 mM glutamine, in an atmosphere of 5% CO_2 at 37°C.

Preparation of Nick-translated ^{32}P-Labeled DNA. Nick-translated pBR322 DNA was prepared using [^{32}P]dCTP (6000 Ci/mmol) and a nick translation kit, according to the manufacturer's instructions. The reaction was stopped by adding EDTA to a final concentration of 20 mM. Unincorporated dCTP was removed with G-50 Sephadex quick-spin columns.

Preparation of Crude Extracts from Human Tissues and HT-29 Cells for Topoisomerase I Activity. All steps during extract preparation were performed at 4°C. Frozen tissues (0.15–0.30 g) were first ground with mortar and pestle in the presence of liquid nitrogen, suspended in buffer A (10 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol, 10 mM EDTA, 0.25% Triton X-100, 1 mM PMSF, 1 μM leupeptin, 1 μM pepstatin) at 0.19 g tissue/ml, and homogenized with a Potter-Elvehjem Teflon-glass homogenizer. The extract was kept on ice for 30 min, an equal volume of nuclear lysis buffer B (buffer A containing 2 M NaCl) was added slowly, and the mixture was stirred gently on ice for 2 h. The suspension was centrifuged at 10,000 rpm in an SS-34 rotor for 2 h. Polyethylene glycol 8000 was added slowly to the supernatant (final concentration, 5%), the mixture was stirred on ice for 30 min, and the supernatant was cleared by centrifugation in a Beckman microfuge. The supernatant was dialyzed against buffer containing 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM 2-mercaptoethanol, 1 mM EDTA, 10% glycerol, 1 mM PMSF, 1 μM pepstatin, and 1 μM leupeptin, for 12 h at 4°C. The dialyzed supernatant was centrifuged in a microfuge to remove any suspended material and assayed for topoisomerase I activity. Crude extracts from HT-29 cells were prepared after the cells were washed 3 times with phosphate-buffered saline containing 1 mM PMSF, 1 μM pepstatin, and 1 μM leupeptin, as described for tissue homogenates. Protein was estimated by the bicinchoninic acid protein assay reagent (Pierce), using bovine serum albumin as a standard.

Table 1 *Topoisomerase I levels in colorectal, prostate, and kidney tumors expressed as the ratio of tumor to normal and the relationship between the topoisomerase I levels and staging of tumors*

Topoisomerase I levels were estimated by Western blot analysis using equal protein from tissue homogenates of tumor and matched normal samples from the same patients as described in "Materials and Methods" and Fig. 1. The catalytic activity of topoisomerase I was determined by the ^{32}P transfer assay using three protein concentrations from tumors and corresponding normal tissue extracts as described in "Materials and Methods" and Fig. 6. Values in the linear range were used to calculate the ratio. ND, not determined. The results of Western blot analysis on the colorectal tumor of patient 7 are shown in Fig. 1, *Lanes 1* and *2*, on the prostate tumors of patient 12 in Fig. 1, *Lanes 3* and *4*, on the kidney tumor of patient 18, in Fig. 1, *Lanes 5* and *6*. The catalytic activity measurements for the colorectal tumor of patient 2, the prostate tumor of patient 12, and kidney tumor of patient 18 are shown in Fig. 6, *Lanes 1* and *2*, *Lanes 3* and *4*, and *Lanes 5* and *6*, respectively.

Tumor	Patient	Catalytic activity	Western blot	Dukes staging	Grade	Stage
Colorectal	1	17.0	8.0	C2		
	2	13.0	5.5	B2		
	3	11.0	5.0	B2		
	4	40.0	20.6	Adenoma		
	5	15.0	6.5	C2		
	6	ND	5.0	B1		
	7	ND	18.5	C2		
	8	ND	35.0	B1		
Prostate[a]	9	4.0	2.4		5 + 4 = 9	$P_{3c}N_0M_0$
	10	8.5	5.2		4 + 3 = 7	$P_{3a}N_0M_0$
	11	1.0	2.3		4 + 3 = 7	$P_{3c}N_0M_0$
	12	26.0	10.6		3 + 3 = 6	$P_{3a}N_0M_0$
	13	9.5	6.6		3 + 4 = 7	$P_{3c}N_0M_0$
	14	1.0	0.9		5 + 4 = 9	$P_{3c}N_1M_0$
	15	ND	6.0		3 + 3 = 6	$P_{3a}N_0M_0$
	16	ND	3.3		3 + 4 = 7	$P_{2b}N_0M_0$
	17	ND	2.3		4 + 4 = 8	$P_{3a}N_0M_0$
Kidney[b]	18	1.9	1.5		1	$P_1N_0M_0$
	19	1.8	1.0		1	$P_{3a}N_0M_0$
	20	2.5	1.0		2	$P_2N_0M_0$
	21	ND	1.0		2	$P_{3a}N_0M_0$
	22	ND	0.9		2	$P_1N_0M_0$
	23	ND	0.7		2	$P_1N_0M_0$
	24	ND	1.6		2	$P_{3a}N_0M_0$
	25	ND	1.0		3	$P_{3a}N_2M_0$

[a] Grade as reported in Ref. 37; stage as reported in Ref. 38.
[b] Stage as reported in Ref. 39.

Preparation of Nuclear Extracts from HT-29 Cells. Cells were lysed in lysis buffer (30 mM Tris-HCl, pH 7.5, 0.3 M sucrose, 2 mM $MgCl_2$, 2 mM dithiothreitol, 15 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.25% Triton X-100, 1 mM PMSF, 1 μM leupeptin, 1 μM pepstatin) by homogenization in a Potter-Elvehjem Teflon-glass homogenizer. The suspension was kept on ice for 15 min and centrifuged at 1500 rpm in an SS-34 rotor for 10 min. The nuclear pellet was washed three times with buffer A and resuspended in the same buffer. The nuclei were lysed by adding an equal volume of buffer B. After this step, the protocol for preparation of crude homogenates was followed.

Antibodies and Immunoblot Analysis. The following peptides from the predicted amino acid sequence for topoisomerase I (18) were used for raising polyclonal antibodies: (*a*) CNHQRAPPKTFEKSMMNLQTK (amino acids 630–650), (*b*) CERYPEGIKWKFLEHKGPVF (amino acids 209–227), and (*c*) CFSSPPQIKDEPEDDGYFVP (amino acids 110–128). For raising polyclonal antibodies against topoisomerase II, peptide CLNSGVSKPDPAKTKN (amino acids 1446–1461) from the predicted amino acid sequence for topoisomerase II (19) was used. Peptides were coupled to the carrier protein keyhole limpet hemocyanin, via *m*-maleimidobenzoic acid *N*-hydroxylsuccinimide ester, through a cysteine residue, injected intradermally into rabbits after mixing with Freund's complete adjuvant, and subsequently injected i.m. at multiple sites in incomplete Freund's adjuvant. IgG was purified from high-titer antisera using an ImmunoPurePlus (A) IgG purification kit. Topoisomerase I-specific antibodies were purified by passing the IgG fraction over an affinity column

[2] The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SSC, saline-sodium citrate buffer; SSPE, saline-sodium phosphate-EDTA buffer; cDNA, complementary DNA; poly(A)$^+$, polyadenylated; TBST, Tris-buffered saline/Tween.

Table 2 *Topoisomerase I content (ng/mg protein)*

Topoisomerase I contents from human surgical specimens were determined by Western blot analysis. Quantitation was done by separating equal amounts of protein from normal and tumor tissue homogenates along with different concentrations of HeLa cell lysates. A value of 0.6 ng topoisomerase I/10^5 HeLa cells (20) was used to calculate the topoisomerase I contents. Values are means ± SD.

Tumor type	Normal	Tumor
Colorectal	2.7 ± 1.8 (n = 7)	24.6 ± 10.2 (n = 6)
Prostate	3.7 ± 1.0 (n = 7)	15.5 ± 10.1 (n = 7)
Kidney	4.9 ± 0.6 (n = 4)	4.7 ± 1.0 (n = 4)

Table 3 *Topoisomerase I mRNA levels in colorectal tumors expressed as the ratio of tumor to normal and clinical and pathological characteristics of these patients*

mRNA from normal colon and colon tumor from each patient (2 μg) was analyzed as described in Fig. 8. Values represent ratio of tumor to normal. Patients 26–30 in whom mRNA levels were measured were different from patients 1–8 who were used for quantitating topoisomerase I levels and its catalytic activity

Patient	Age (yr)	Sex	Dukes staging	Topoisomerase I mRNA levels
26	70	Male	B2	17.0
27	53	Female	B1	33.0
28	75	Female	C2	10.0
29	72	Male	B2	2.0
30	63	Male	B1	3.0

prepared by coupling peptide to Sulfolink coupling gel through the cysteine residue of the peptide. Affinity-purified antibodies were dialyzed and stored at −100°C in phosphate-buffered saline containing 0.5 mg/ml bovine serum albumin and 0.02% sodium azide.

For immunoblot analysis, tissues were homogenized in buffer A at 4°C and then extracted with an equal volume of SDS sample buffer (4% SDS, 10% 2-mercaptoethanol, and 0.004% bromphenol blue in 160 mM Tris-HCl, pH 6.8). Proteins were separated electrophoretically by 7.5% SDS-PAGE. SDS-polyacryamide gels and Immobilon-P membranes were equilibrated in transfer buffer (20 mM Tris, 192 mM glycine, pH 8.2, containing 0.08% SDS and 20% methanol) for 30 min. The proteins were transferred to Immobilon-P membrane at 45 V for 25 min in transfer buffer. The blots were incubated overnight in blocking buffer, 3% powdered nonfat milk, in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20, 0.02% sodium azide). The blots were then incubated for 6 h with topoisomerase I-specific affinity-purified antibodies diluted 1:200 in blocking buffer; they were washed three times with TBST and incubated with ^{125}I-labeled Protein A in blocking buffer (1 μCi/ml) for 45–60 min. After the blots were washed three times with TBST, they were subjected to autoradiography and the topoisomerase I-related bands were quantitated by densitometry, or the bands were quantitated directly from the filters by phosphoimaging (Molecular Dynamics).

Preabsorption of Autoimmune Serum or Topoisomerase I Peptide Antibodies with HeLa Cell Extract Topoisomerase I. HeLa cell lysate was fractionated by preparative SDS-PAGE and the proteins were transferred to Immobilon-P. A strip of the membrane was cut away and incubated with the anti-topoisomerase I peptide antibodies or autoimmune serum, and the topoisomerase I-related band was detected with ^{125}I-labeled Protein A. This membrane piece was aligned with the remaining untreated membrane. The membrane band corresponding to the topoisomerase I band was cut out. Autoimmune serum or anti-topoisomerase I peptide antibodies were preabsorbed on this membrane containing topoisomerase I.

Assay of Topoisomerase I Activity in Crude Extracts. Topoisomerase I catalytic activity was determined by a specific, sensitive, and quantitative assay based on the formation of a covalent enzyme-DNA intermediate (20, 21). The cycle of strand breakage and religation during topoisomerase I action proceeds through an intermediate in which a covalent linkage is formed between a tyrosine residue on the enzyme and a 3′-phosphoryl residue at the cleavage site in DNA. This assay measures the transfer of ^{32}P from ^{32}P-labeled DNA to topoisomerase I. ^{32}P-labeled topoisomerase I molecules are identified by SDS-PAGE and quantitated by phosphoimaging or by densitometry.

The reaction mixture contained 50 mM Tris-HCl, pH 7.5, 60 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 μg pBR322 nick-translated DNA (specific activity, 4–5 × 10^8 cpm/μg DNA), 30 μg/ml nuclease-free bovine serum albumin, and 1–5 μl of tissue homogenate, in a total volume of 30 μl. After incubation at 37°C for 30 min, DNase I was added at a concentration of 125 μg/ml and the incubation was continued for an additional 15 min. The reaction was stopped by adding an equal volume of buffer containing 0.16 M Tris-HCl, pH 6.8 4% SDS, 30% glycerol, and 10% 2-mercaptoethanol. The samples were boiled for 5 min and proteins were separated by 7.5% SDS-PAGE at 200 V for 45 min. The gels were dried onto Whatman No. 3MM paper and autoradiographed. The topoisomerase I-related bands were quantitated by use of either a phosphoimager or a densitometer. The addition of protease inhibitors during enzyme incubation did not have any significant effect on either purified topoisomerase I or topoisomerase I catalytic activity from tissue homogenates. The enzyme activity was stable at least for 1 week when tissue extracts were stored at −100°C. At 4°C, about 80% of the topoisomerase I activity was lost in about 2 weeks.

Preparation of a Topoisomerase I-specific cDNA Probe. The cDNA was synthesized from 2 μg total RNA from HeLa cells using random hexamers and Moloney murine leukemia virus reverse transcriptase. The topoisomerase I-specific cDNA was amplified by polymerase chain reaction using CAGAATTCGACCTCGAGATGAGGATGATGTTG and GCAAGCTTGTATTTCTGCCAGTCCTTCTCACC forward and backward primers, respectively. These primers were selected from the topoisomerase I cDNA sequence (18) and synthesized with *Eco*RI and *Hind*III sites. The 930-base pair amplified cDNA was purified and cloned into *Eco*RI/*Hind*III sites of pGEM3Zf$^+$ (Promega Corporation). Identity of the topoisomerase I cDNA was confirmed by digesting the DNA with *Eco*RI and *Hind*III to confirm its expected size, by sequencing 5′ and 3′ ends, and by determining the presence of sites for restriction enzymes.

Preparation and Analysis of Poly(A)$^+$ RNA. Total RNA from human tissues was prepared by the guanidinium isothiocyanate method as described by Chirgwin *et al.* (22). Polyadenylated RNA was purified by three cycles of oligothymidylate-cellulose chromatography. Poly(A)$^+$ RNA was also isolated directly from tissues using the Poly(A) tract 1000 kit from Promega Corporation. RNA was quantitated by its absorbance at 260 nm. Poly(A)$^+$ RNA (2 μg/lane) was denatured at 65°C for 10 min in 50% formamide, 6.5% formaldehyde, 40 mM 3-(*N*-morpholino)propanesulfonic acid and 1 mM EDTA pH adjusted to 7.0 with acetic acid buffer and separated on a 1% agarose gel containing 0.74% formaldehyde. The RNA was transferred onto a Nytran membrane in 20× SSC overnight and immobilized by exposure of the filter to UV in Stratalinker UV cross-linker (Stratagene Inc.). Filters were prehybridized in 5× Denhardt's reagent, 5× SSPE, 0.5% SDS, 50% formamide, 0.25 mg/ml heat-denatured salmon sperm DNA, at 42°C for 18 h. Hybridization was performed at 42°C for 48 h in 1× Denhardt's reagent, 5× SSPE, 0.25% SDS, 50% formamide, with 0.25 mg/ml salmon sperm DNA and ^{32}P-labeled topoisomerase I-specific probe (both heat denatured by boiling for 10 min). The filters were washed in the following sequence: once in 2× SSC/0.1% SDS at room temperature for 10 min, twice in 1× SSC/0.1% SDS at 42°C for 15 min each time, twice in 0.1× SSC/0.1% SDS at 50°C for 15 min, and finally twice in 0.1× SSC/0.1% SDS at 60°C for 15 min each time. The filters were partially dried and exposed to X-ray film at −100°C with an intensifying screen. The topoisomerase I-specific probe was removed by washing the filter in 0.01× SSPE/0.5% SDS at 85°C for 20 min. To normalize for loading differences, the same filter was hybridized with a GAPDH probe. The topoisomerase I-specific 4.1-kilobase bands were quantitated by scanning autoradiographs with a densitometer.

RESULTS

Detection and Quantitation of Topoisomerase I in Human Tumors by Immunoblotting. Human topoisomerase I is a single polypeptide chain of M_r 100,000 and is known to produce smaller degradation products (M_r 60,000–70,000) in cell extracts because of proteolysis (23–25). Topoisomerase I has been purified from a variety of human cells (10, 23), including the human colon tumor cell line HT-29 (10). Therefore, throughout this work, HT-29 cells were used as a standard of comparison. Topoisomerase I levels in matched pairs of human tumors and normal tissue counterparts were analyzed using an affinity-purified polyclonal antibody raised against peptide *a* (see "Materials and Methods"). As seen in Fig. 1, this antibody reacted with M_r 54,000, 68,000, and 100,000 bands from normal colon and

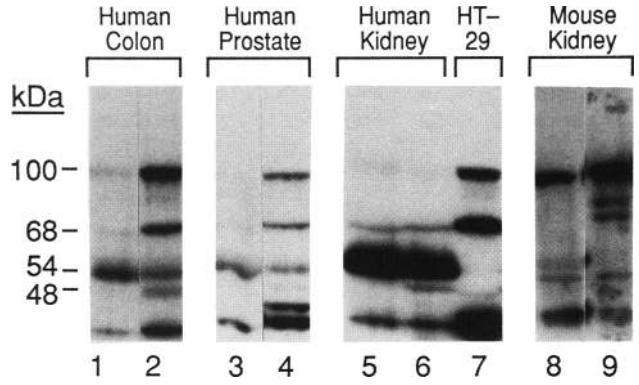

Fig. 1. Analysis of topoisomerase I in human and mouse tissues by Western blot analysis. Protein from tissue homogenates (60–120 μg) was separated by 7.5% SDS-PAGE and transferred to Immobilon-P transfer membranes. The blots were treated with affinity-purified anti-topoisomerase I peptide antibodies. The bands were detected with ^{125}I-labeled Protein A. *Lanes 1* and *2*, normal colon and colon tumor; *lanes 3* and *4*, benign prostatic hyperplasia and prostate tumor; *lanes 5* and *6*, normal kidney and kidney tumor; *lane 7*, HT-29 cell extract; *lanes 8* and *9*, mouse kidney tissue homogenate and kidney nuclear extract, respectively. Results for colorectal, prostate, and kidney tumors are for patients 7, 12, and 18, respectively.

colon tumors, benign prostatic tissue and prostate tumor, and normal kidney and kidney tumors. The M_r 54,000 band appeared specific to human tissues, because the band was almost undetectable in crude or nuclear extracts from mouse liver (data not shown) and mouse kidney (Fig. 1, *Lanes 8* and *9*). A variety of anti-topoisomerase I antibodies cross-reacted with these three bands, *i.e.*, human autoimmune sera containing autoantibodies against topoisomerase I from four different scleroderma patients, polyclonal antibodies raised against peptides *b* and *c* of the topoisomerase I molecule, and polyclonal antibodies raised against purified topoisomerase I from HeLa cells (data not shown). However, preabsorption experiments suggested that the M_r 54,000 band is not derived from topoisomerase I but that the M_r 68,000 band is. As shown in Fig. *a2*, preabsorption of the polyclonal antibodies raised against peptide *a* with a molar excess of peptide I inhibited reactivity with purified calf thymus topoisomerase I (Fig. 2, *Lane 8 versus Lane 12*) and the topoisomerase I-specific M_r 68,000 (Fig. 2, *Lanes 1* and *2 versus Lanes 3* and *4* and *Lane 6 versus Lane 10*) and M_r 100,000 (Fig. 2, *Lane 5 versus Lane 9*) bands from human tissues and the HT-29 human colon tumor cell line (Fig. 2, *Lane 7 versus Lane 11*) but did not inhibit reactivity with the M_r 54,000 band in human tissues (Fig. 2, *Lanes 1* and *2 versus Lanes 3* and *4* and *Lanes 5* and *6 versus Lanes 9* and *10*). Similar results were observed when the polyclonal antibodies were preabsorbed with HeLa cell extract topoisomerase I before blotting (data not shown). Preabsorption of the autoimmune sera with HeLa cell extract topoisomerase I also did not inhibit reactivity with the M_r 54,000 protein band (Fig. 2, *Lane 13 versus Lane 16*), while inhibiting reactivity with purified calf thymus topoisomerase I and topoisomerase I from HeLa cell lysate (compare Fig. 2, *Lanes 14* and *15* with *Lanes 17* and *18*). Preabsorption of antibodies raised with a peptide derived from topoisomerase II had no effect (data not shown). Therefore, these results strongly suggest that the M_r 100,000 and 68,000 bands, but not the M_r 54,000 band, from the human tumors are, indeed, topoisomerase I. These observations are consistent with the M_r 100,000 band being intact topoisomerase I (23–25) and the M_r 68,000 band being a degradation product. Indeed, a M_r 68,000 carboxyl-terminal fragment of topoisomerase I is produced during purification from HeLa cells (23). Therefore, in general, peptide antibodies detected only a M_r 68,000 form of topoisomerase I in human kidney tissues (Fig. 1, *Lane 5* and *6*; Fig. 2, *Lanes 1* and *2*) and M_r 68,000 and 100,000 forms in colorectal and prostatic tissues (Fig. 1, *Lanes 1–4*). However, occasionally only the M_r 68,000 band was observed in both colorectal and prostatic tissues (Fig. 2, *Lane 6*, and Fig. 3, *Lane 1* for colon; data for prostate not shown).

In all tumor types studied, occasionally an additional M_r 48,000 band was detected which was not present in the normal tissue counterparts (Fig. 1, *Lanes 2, 4*, and *6*; Fig. 2, *Lanes 2, 5*, and *6*). This band was also inhibited by preabsorption of antibodies with peptide I (Fig. 2, *Lane 2 versus Lane 4* and *Lanes 5* and *6 versus Lanes 9* and *10*), suggesting that the M_r 48,000 protein band is also related to topoisomerase I. The M_r 48,000 band was also recognized by autoimmune serum (Fig. 3, compare *Lanes 1* and *2* with *Lanes 4* and *5*). The identity of other lower molecular weight bands observed in human tissues and HT-29 cell extracts has not been reported, although partial inhibition of these bands was observed upon preabsorption of antibodies with peptide (Fig. 2, *Lanes 3*, *4*, and *9–11*). However, these other bands were not detected with autoimmune serum (Fig. 3, *Lanes 4–6*); therefore, we believe it is unlikely that these other lower molecular weight bands represent topoisomerase I.

Having identified the M_r 100,000, 68,000, and 48,000 bands as being topoisomerase I, by Western blotting, we quantitated topoisomerase I levels in human colon, prostate, and kidney tumors using affinity-purified polyclonal antibodies raised against peptide I. As summarized in Tables 1 and 2 and Fig. 4, elevated levels of topoisomerase I were observed in eight colorectal tumors and eight of nine prostate tumors. Colon tumors exhibited 5–35-fold increases in topoisomerase I levels, compared to the normal colon mucosa from the same patient. In prostate tumors, topoisomerase I levels were 2–10-fold higher, compared to benign hyperplastic prostate tissue from the same patients. In one prostate tumor, topoisomerase I levels were the

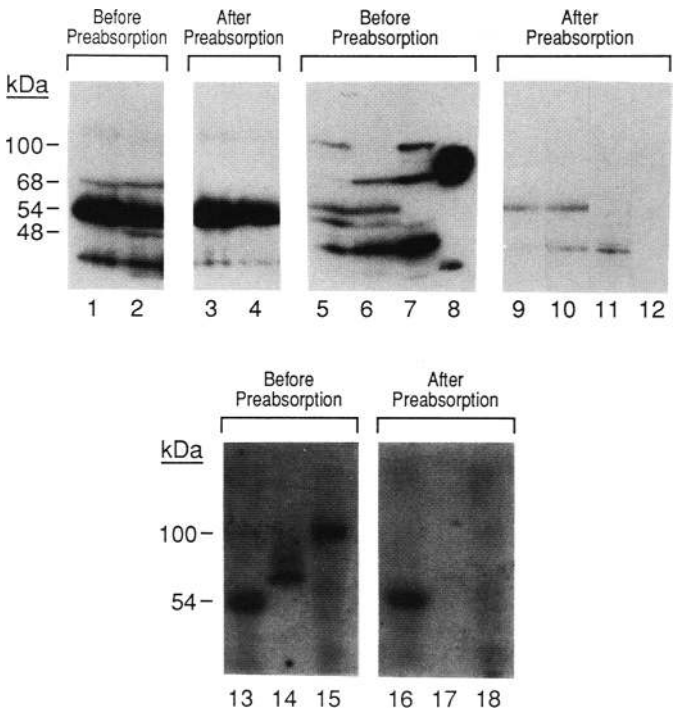

Fig. 2. Identification of topoisomerase I-related bands in human tumors by immunoblotting. Immunoblotting was performed as described in Fig. 1. For preabsorption, peptide antibodies (1:200 dilution) were first incubated with a 50-fold molar excess of peptide I at 4°C for 1 h before incubation with the blots. Autoimmune serum (1:6000 dilution) was preabsorbed with HeLa cell extract as described in "Materials and Methods." *Lanes 1* and *2*, normal kidney and kidney tumor; *lanes 3* and *4*, normal kidney and kidney tumor after preabsorption; *lane 5*, prostatic tumor; *lane 6*, colon tumor; *lane 7*, HT-29 cell extract; *lane 8*, purified calf thymus topoisomerase I; *lanes 9–12*, same as *lanes 5–8* except antibodies were preabsorbed with peptide; *lane 13*, human kidney tumor; *lane 14*, purified calf thymus topoisomerase I; *lane 15*, HeLa cell lysates; *lanes 16–18*, same as *lanes 13–15* except autoimmune serum was preabsorbed with the HeLa cell extract. Occasionally a M_r 100,000 topoisomerase I band was detected in kidney tissues. The kidney homogenates used in *lane 2* and *lane 13* are from different kidney tumor samples.

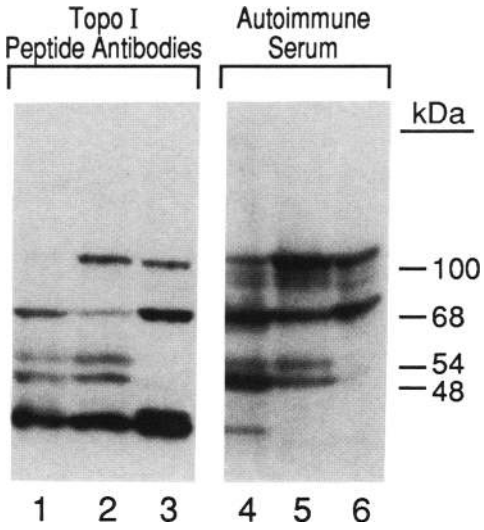

Fig. 3. Detection of topoisomerase I in tissue homogenates by immunoblotting using affinity-purified anti-topoisomerase I peptide antibodies and autoimmune serum from scleroderma patients. Immunoblotting was performed as described in Fig. 1. *Lane 1*, colon tumor; *lane 2*, prostate tumor; *lane 3*, HT-29 extract; *lanes 4–6*, same as *lanes 1–3* except autoimmune serum (1:2000 dilution) was used instead of anti-topoisomerase I peptide antibodies. A weak M_r 100,000 band (*lane 1*) detected by antipeptide antibodies, compared to a strong M_r 100,000 band in *lane 4* detected by autoimmune serum, is probably due to the high affinity of these autoimmune antibodies.

same as in normal tissue. In contrast, eight kidney tumors did not exhibit any elevation of this enzyme, compared to their corresponding normal matched samples. Normal colorectal, prostate, and kidney tissues showed similar levels of topoisomerase I (Table 2).

Quantitation of Topoisomerase I Catalytic Activity in Crude Extracts from Human Tumors. To determine whether the topoisomerase I which is elevated in human tumor tissues is enzymatically active, we measured the catalytic activity of the enzyme in tumor tissues by a specific, sensitive, and quantitative assay based on the formation of a covalent enzyme-DNA intermediate through a tyrosine residue on the enzyme and a 3′-phosphoryl residue at the cleavage site on DNA (20). This assay has been used previously for quantitating topoisomerase I in nuclear extracts from cultured cells (20, 21) but not in cell or tissue extracts without first preparing nuclei. Here, we have adapted this assay and demonstrate that it is possible to quantitate topoisomerase I catalytic activity in crude extracts from human tumor specimens without preparing nuclei.

We first measured topoisomerase I activity in crude extracts from the HT-29 human colon tumor cell line without isolating nuclei. As shown in Fig. 5, an ATP-independent topoisomerase I-specific band of M_r 84,000 was detected (Fig. 5, *Lanes 1* and *2*). This single M_r 84,000 band probably results from the degradation of the M_r 100,000 band to the M_r 68,000 form during the extraction procedure, and the M_r 68,000 band then migrates as an M_r 84,000 species due to the attachment of a short oligonucleotide after DNase I digestion (see "Discussion"). Preabsorption of the crude extracts with polyclonal antibodies raised against peptide I of topoisomerase I or with scleroderma serum reduced or prevented the appearance of the topoisomerase I-specific band (Fig. 5, *Lanes 3–6*), whereas preabsorption with antibodies against topoisomerase II had no effect (Fig. 5, *Lanes 7* and *8*), suggesting that the assay can measure topoisomerase I activity directly in crude extracts. The total activity of topoisomerase I measured in the crude extract from HT-29 cells was similar to that measured in nuclear extracts from the same number of HT-29 cells (data not shown). Using this approach, we detected a topoisomerase I-specific band in crude extracts from human colon, prostate, and kidney tumors (Fig. 6). Results of topoisomerase I estimation in colon, prostate, and kidney tumors by the ^{32}P transfer assay are summarized in Table 1 and Fig. 4 for comparison with the Western blotting measurements. Compared to paired normal tissue, colon and prostate tumors showed 11–40- and 4–26-fold increases, respectively, in catalytic activity of topoisomerase I. Kidney tumors did not show a significant change in catalytic activity, compared to their normal counterparts. In general, there was a good correlation between catalytic activity of the enzyme and its level in tumor tissue (Fig. 7).

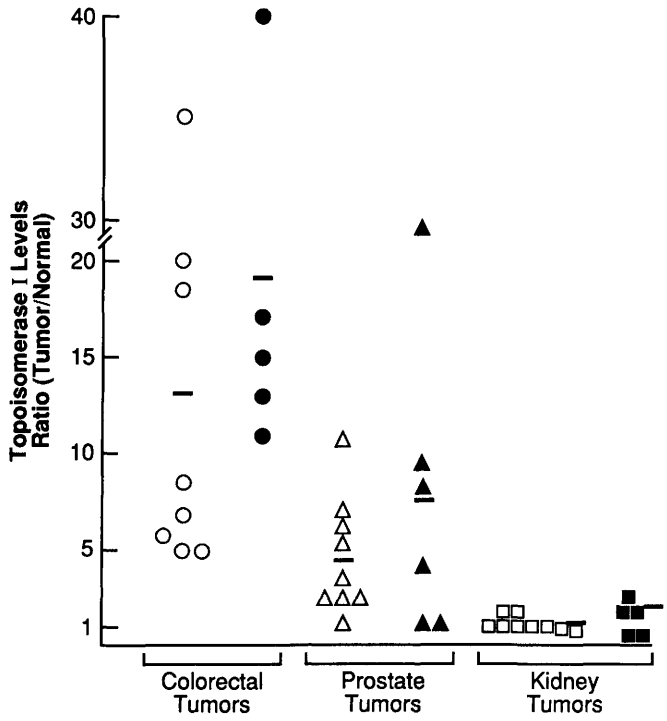

Fig. 4. Topoisomerase I levels in colorectal, prostate, and kidney tumors. Topoisomerase I levels were estimated in human tumor and matched normal samples by Western blotting (○, △, □). The catalytic activity of topoisomerase I in the same samples was determined by the ^{32}P-transfer assay (●, ▲, ■). Topoisomerase I levels are expressed as the ratio of tumor to normal values. — denotes mean values for each tumor type. In two kidney samples, the ratio of tumor to normal values for catalytic activity was <1. In these samples the intensity of topoisomerase I-related bands in the tumors was much lower than in their matched normal tissues, making it difficult to quantitate accurately. Therefore, the values for these two kidney tumor samples were not included in determination of a mean value.

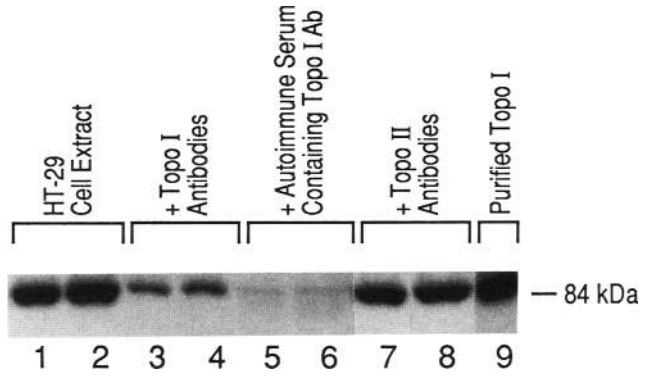

Fig. 5. Determination of topoisomerase I catalytic activity in crude extracts from the HT-29 colon tumor cell line by the ^{32}P-transfer assay. HT-29 cell extracts were incubated with ^{32}P-labeled nick-translated pBR322 DNA. ^{32}P-labeled proteins were analyzed by SDS-PAGE as described in "Materials and Methods." Gels were dried and autoradiographed. *Lanes 1* and *2*, 2 and 4 μl of HT-29 extracts; *lanes 3* and *4*, HT-29 extracts (2 and 4 μl) preabsorbed with anti-topoisomerase I peptide antibodies before incubation with nick-translated DNA; *lanes 5* and *6*, HT-29 extracts (2 and 4 μl) preabsorbed with autoimmune serum containing anti-topoisomerase I antibodies; *lanes 7* and *8*, 2 and 4 μl of HT-29 extracts preabsorbed with affinity-purified anti-topoisomerase II peptide polyclonal antibodies; *lane 9*, purified topoisomerase I from calf thymus.

Detection of Topoisomerase I mRNA by Northern Blot Analysis. To measure the steady state levels of mRNA, we isolated poly(A)$^+$ RNA from five human colon tumors and corresponding normal colon mucosa (these specimens were not from the patients listed in Tables 1 and 2). A 930-base pair DNA topoisomerase I-specific probe hybridized to a single mRNA species of 4.1 kilobases. This corresponds to the size of the topoisomerase I mRNA which has been described previously (18, 26). Fig. 8 is an autoradiogram of a Northern blot. Quantitative analysis by densitometry showed that levels of topoisomerase I mRNA were elevated in all five colon tumors, compared to the normal colon mucosa (Fig. 8; Table 3). Loading differences were corrected for by hybridizing the filters with a GADPH cDNA probe (27). In addition, the gel was stained after the transfer step to ensure equal transfer of RNA from all lanes. These results suggest that increased levels of topoisomerase I in colon tumors might result from either increased transcription of the topoisomerase I gene or increased stability of mRNA in these tumors.

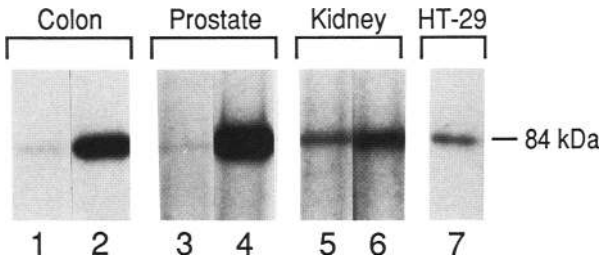

Fig. 6. Estimation of topoisomerase I catalytic activity in human colon, prostate, and kidney tumors and their matched normal counterparts by the ^{32}P-transfer assay. One to 5 μl of crude homogenates from tumors and their normal counterparts, containing equal amounts of protein, were used in assays. Activity was determined as described in "Materials and Methods" and Fig. 5. A single M_r 84,000 topoisomerase I band was detected and quantitated by densitometry. *Lanes 1* and *2*, normal colon and colon tumor homogenate; *lanes 3* and *4*, benign prostatic hyperplasia and prostate tumor homogenates; *lanes 5* and *6*, normal kidney and kidney tumor homogenate; *lane 7*, HT-29 cell extract. The catalytic activity determined in colorectal, prostate, and kidney tumors was for patients 2, 12, and 18, respectively.

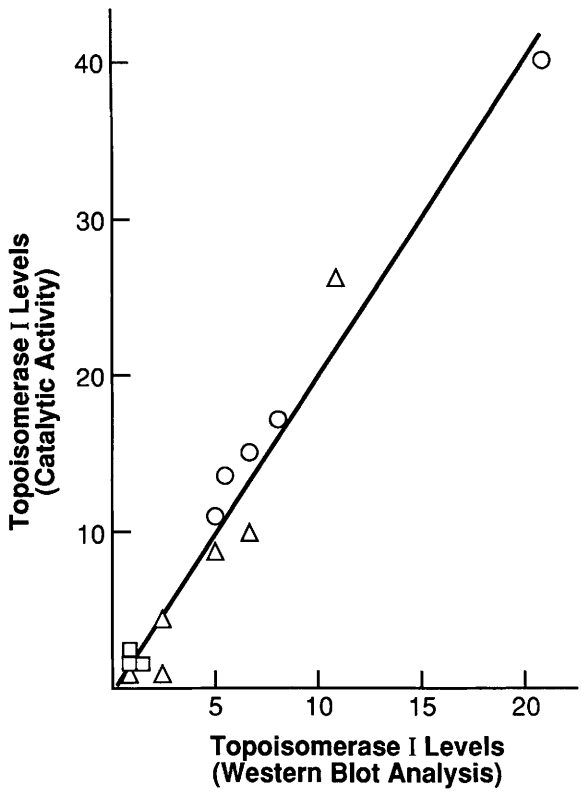

Fig. 7. Relationship between topoisomerase I protein levels and catalytic activity. Topoisomerase I levels determined by Western blotting were plotted against catalytic activity determined by the ^{32}P-transfer assay. The data are from Table 1. ○, colon tumor; △, prostate tumor; □, kidney tumor.

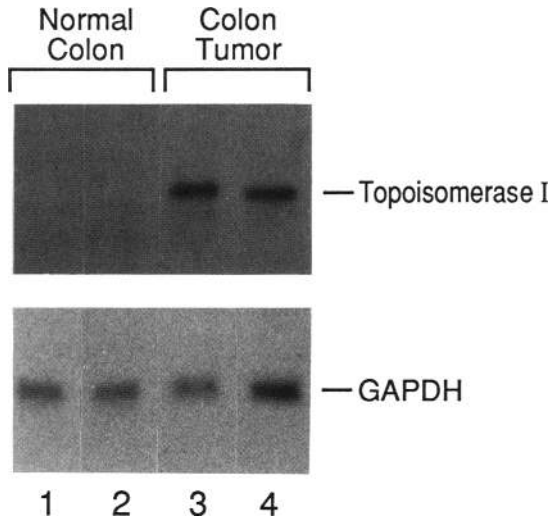

Fig. 8. Northern blot analysis of topoisomerase I mRNA from normal colon and colon tumors. Two μg poly(A)$^+$ RNA from normal colon and colon tumors were fractionated on 1% agarose-formaldehyde gels, transferred to Nytran membranes, and hybridized with the 970-base pair topoisomerase I cDNA fragment as described in "Materials and Methods." The topoisomerase I-specific bands were quantitated by densitometry. The same filter was hybridized with GADPH probe, to normalize the RNA loading differences, after removing the topoisomerase probe. *Lanes 1* and *3*, normal colon and colon tumor from one patient; *lanes 2* and *4*, normal colon and colon tumor from a second patient.

Relationship between Topoisomerase I Levels in Colorectal, Prostate, and Kidney Tumors and Their Staging. When the patients' tumor samples were stratified according to their staging, there was no correlation between levels of topoisomerase I in tumors and their staging (Table 1). However, a larger number of samples are probably required to establish such a relationship.

DISCUSSION

Topoisomerase I has been identified as an intracellular target of camptothecin, a plant alkaloid with anticancer activity. Various lines of evidence suggest that the sensitivity of cells to camptothecin is directly related to the topoisomerase I content (9–11). Giovanella *et al.* (14) suggested that the topoisomerase I levels were elevated in human colon adenocarcinoma, based on immunoblotting. We have confirmed and significantly extended the findings of those investigators. In this report we have quantitated the expression of topoisomerase I at the level of catalytic activity, protein content, and mRNA content in surgical specimens of human colorectal, prostate, and kidney tumors and in matched normal tissue from the same patients. Results indicate that in tumors of the colon and prostate, but not kidney, topoisomerase I catalytic activity, protein expression, and mRNA content (colon only) were significantly elevated, compared to matched controls. There was a good correlation between mRNA levels, enzyme levels, and catalytic activity. However, no correlation was seen between levels of topoisomerase I in tumor samples and their staging.

To our knowledge this is the first report to utilize crude extracts and the quantitative ^{32}P-transfer assay for determination of topoisomerase I catalytic activity in human tissues. This is also the first report wherein levels of topoisomerase I protein have been correlated with its catalytic activity in human tumor tissues. In addition to the work reported here, Van der Zee *et al.* (15) measured topoisomerase I catalytic activity in human malignant ovarian tumors by a semiquan-

titative relaxation assay and found the activity to be 8-fold elevated, compared to benign tumors of the ovary.

Human topoisomerase I has a native molecular weight of 100,000 but is known to readily undergo proteolysis during extraction of cells and tissues (23–25). Topoisomerase I levels were quantitated by Western blotting using affinity-purified peptide antibodies. In colon and prostate tissues M_r 68,000 and 100,000 protein bands were detected, while in kidney only the M_r 68,000 band was detected by the antibodies. In all tumor types studied an additional M_r 48,000 band was detected occasionally which was not present in the normal tissue counterparts. Preabsorption of antibodies with the topoisomerase I peptide antigen resulted in inhibition of the M_r 48,000, 68,000, and 100,000 bands, suggesting that all three protein bands are related to topoisomerase I. Thus, even though samples were prepared in buffer containing proteolytic inhibitors and tissues were solubilized by adding boiling SDS sample buffer, proteolysis of topoisomerase I was only partially preventable. Preabsorption experiments suggest that the M_r 54,000 band detected in all tumor types is not related to topoisomerase I.

Topoisomerase I catalytic activity was measured in crude extracts from tumor and normal tissues by a ^{32}P-transfer assay. We used this method because it allowed us to determine topoisomerase I catalytic activity in small surgical specimens without the need to prepare nuclei. This method is sensitive, specific, and quantitative, in contrast to the semiquantitative relaxation assay used by others to estimate topoisomerase I activity. A single topoisomerase I-specific band (M_r 84,000) was detected in crude extracts from HT-29 cells and from colon, prostate, and kidney tissues. It is possible that during extraction of the enzyme all of the M_r 100,000 band is cleaved to the M_r 68,000 form even in the presence of proteolytic inhibitors. Indeed, a M_r 68,000 carboxyl-terminal fragment which retains relaxation activity has been detected during topoisomerase I purification from HeLa cells (23). Furthermore, in a few samples of colon and prostate we detected only the M_r 68,000 band by Western blotting. Champoux (28) has shown that digestion of DNA by DNase I after covalent intermediate formation leaves an oligonucleotide residue approximately 17 ± 8 nucleotides long attached to topoisomerase I, resulting in reduced mobility of the enzyme. This might explain the mobility shift from the M_r 68,000 active form of the enzyme in Western blotting to the M_r 84,000 form in the ^{32}P-transfer assay (Fig. 6). Indeed, calf thymus topoisomerase I (native molecular weight, 84,000) is degraded mostly to the M_r 68,000 form (Fig. 2, *Lane 14*), which then migrates as an M_r 84,000 band in the activity assay because the oligonucleotide residue is attached to it (Fig. 5, *Lane 9*). The M_r 48,000 fragment occasionally observed in tumor tissues by Western blotting might not have catalytic activity, because we never observed a corresponding band in the catalytic activity assay.

Quantitative analysis showed that the catalytic activity of topoisomerase I was elevated in colon and prostate tumors. These results demonstrate that the increase in catalytic activity is due to an increase in the amount of the enzyme. In general, the increase in catalytic activity is slightly more than the increase in enzyme content. We do not have a full explanation for this observation. However, it has been shown that the M_r 68,000 form of topoisomerase I from chicken RBC shows about 2-fold higher activity than the intact M_r 100,000 form of the enzyme (25). In addition, it is possible that intracellular factors present in crude homogenates may modulate the topoisomerase I activity.

Increased levels of mRNA detected in colorectal tumor samples, compared to normal specimens from the same patients, suggest that the increased topoisomerase I levels results from either increased transcription of the topoisomerase I gene or increased mRNA stability. The average increase of 13-fold in topoisomerase I mRNA levels in colorectal tumors is very close to the average 13-fold increase in topoisomerase I protein levels determined by Western blotting. However, increased levels of topoisomerase I in some tumors might also be due to translational regulation or alteration of protein stability, because mRNA and protein levels were determined in different tumor samples and there was a wide range of RNA and protein levels in different samples. The increased transcription of the topoisomerase I gene observed in colorectal tumors might result from transactivation of the topoisomerase I gene by as yet unknown transcription factors through various potential regulatory transcriptional elements present in the promoter region of the topoisomerase I gene (29, 30). One such candidate might be c-*fos*. The c-*fos* gene is one of the earliest known genes activated in response to mitogenic stimuli (31). The 5′ regulatory sequences for topoisomerase I contain an AP-1 binding site (29, 30). Therefore, c-*fos* might activate topoisomerase expression through binding to the AP-1 site. This speculation is supported by the recent observation of Scanlon *et al.* (32) that increased expression of c-*fos* in cisplatin-resistant cell lines is accompanied by enhanced topoisomerase I expression.

Increases in topoisomerase I mRNA levels have been observed in response to partial hepatectomy in rats (33), after serum stimulation of resting HeLa 299 cells (34), and after treatment of human fibroblast cells with phorbol 12-myristate 13-acetate (35). Infection of HeLa cells by adenovirus also resulted in increased topoisomerase I-specific mRNA (36). This increase in mRNA was not accompanied by a corresponding increase in topoisomerase I protein, suggesting that topoisomerase I protein synthesis could be regulated at both the transcriptional and translational levels. The latter results are contradictory to the observation of Chow and Pearson (20), who showed that increased topoisomerase activity after adenovirus infection of HeLa cells was due to a corresponding increase in the amount of enzyme.

In conclusion, we have shown that topoisomerase I levels and catalytic activity are elevated in human colorectal and prostate tumors, but not kidney tumors, probably as a result of either increased transcription or increased mRNA stability. These results and the observation that cells overexpressing active topoisomerase I are hypersensitive to camptothecin (9) suggest that estimation of topoisomerase I in tumor biopsies from patients may be useful as a diagnostic tool for predicting the responsiveness and selective cytotoxicity of topoisomerase I-directed drugs. Specifically, these results suggest that colorectal and prostate carcinomas may be more responsive to topoisomerase I-directed chemotherapeutic agents than carcinoma of the kidney.

ACKNOWLEDGMENTS

We thank Renae Crosby for making the cDNA probe for topoisomerase I, John Bisi for Northern blot analysis of normal colon and colon tumor samples, and Mary Ellen Buchheit for her help in preparing the manuscript.

REFERENCES

1. Wang, J. C. DNA topoisomerases. Annu. Rev. Biochem., *54:* 665–697, 1985.
2. Wang, J. C. Recent studies on DNA topoisomerases. Biochim. Biophys. Acta, *909:* 1–9, 1987.
3. Liu, L. F. DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem., *58:* 351–375, 1989.
4. 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:* 14873–14878, 1985.
5. Kunimoto, T., Nitta, K., Tanaka, T., Uchara, N., Baba, H., Takeuchi, M., Yokokura, T., Sawoda, S., Miyasaka, T., and Mutai, M. Antitumor activity of 7-ethyl-10-[4–(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, a novel water soluble derivative of camptothecin, against murine tumors. Cancer Res., *47:* 5944–5947, 1987.
6. Schneider, E., Hsiang, Y-H., and Liu, L. F. DNA topoisomerases as anticancer drug targets. Adv. Pharmacol., *21:* 149–183, 1990.
7. Davies, S. M., Robson, C. N., Davies, S. L., and Hickson, I. D. Nuclear topoisomerase II levels correlate with the sensitivity of mammalian cells to intercalating agents and epipodophyllotoxins. J. Biol. Chem., *263:* 17724–17729, 1988.
8. Fry, A. M., Chresta, C. M., Davis, S. M., Walker, M. C., Harris, A. L., Hartley, J. A.,

Masters, J. R. W., and Hickson, I. D. Relationship between topoisomerase II levels and chemosensitivity in human tumor cell lines. Cancer Res., *51:* 6592–6595, 1991.
9. Madden, K. R., and Champoux, J. J. Over-expression of human topoisomerase I in baby hamster kidney cells: hypersensitivity to clonal isolates to camptothecin. Cancer Res., *52:* 525–532, 1992.
10. Sugimoto, Y., Tsukahara, S., Ohhara, 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.
11. Bjornsti, M-A., Benedetti, P., Vigilianti, G. A., and Wang, J. C. Expression of human DNA topoisomerase I in yeast cells lacking yeast DNA topoisomerase I: restoration of sensitivity of the cells to the antitumor drug camptothecin. Cancer Res., *49:* 6318–6323, 1989.
12. Heck, M. S., and Earnshaw, W. C. Topoisomerase II: a specific marker for cell proliferation. J. Cell. Biol., *103:* 2569–2581, 1986.
13. D'Arpa, P., and Liu, L. F. Topoisomerase-targeting antitumor drugs. Biochim. Biophys. Acta, *989:* 163–177, 1989.
14. Giovanella, B. C., Steblin, J. S., Wall, M. E., Wani, M. C., Nicholas, A. W., Liu, L. F., Silber, R., and Pomesil, M. DNA topoisomerase I targeted chemotherapy of human colon cancer in xenografts. Science (Washington DC), *246:* 1046–1048, 1989.
15. Van der Zee, A. G. J., Hollema, H., Jong, S., Boonstra, H., Gouw, A., Willemse, P. H. B., Zijlstra, J. G., and Varies, E. G. E. 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.
16. Holden, J. A., Rolfson, D. H., and Wittwer, C. T. Human DNA topoisomerase II: evaluation of enzyme activity in normal and neoplastic tissues. Biochemistry, *29:* 2127–2134, 1990.
17. Shero, J. H., Bordwell, B., Rothfield, N. F., and Earnshaw, W. C. High titers of autoantibodies to topoisomerase I (scl 70) in sera from scleroderma patients. Science (Washington DC), *231:* 737–740, 1986.
18. D'Arpa, P., Machlin, P. S., Ratrie, H., III, Rothfield, N. F., Cleveland, D. W., and Earnshaw, W. C. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67.7 kd carboxyl-terminal fragment. Proc. Natl. Acad. Sci. USA, *85:* 2543–2547, 1988.
19. Tsai-Pflufelder, M., Liu, L. F., Liu, A. A., Tewey, K. M., Whang-Peng, J., Knutsen, T., Huebner, K., Croce, C. M., and Wang, J. C. Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q,21–22. Proc. Natl. Acad. Sci. USA, *85:* 7177–7181, 1988.
20. Chow, K., and Pearson, G. D. Adenovirus infection elevates levels of cellular topoisomerase I. Proc. Natl. Acad. Sci. USA, *82:* 2247–2251, 1985.
21. Crespi, M. D., Meadovan, A. G., and Baldi, A. Increment of DNA topoisomerase in chemically and virally transformed cells. Exp. Cell Res., *175:* 206–215, 1988.
22. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J., and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry, *18:* 5294–5299, 1979.
23. Liu, L. F., and Miller, K. G. Eukaryotic DNA topoisomerases: two forms of type I DNA topoisomerases from HeLa cell nuclei. Proc. Natl. Acad. Sci. USA, *78:* 3487–3491, 1981.
24. Champoux, J. J. Proteins that affect DNA conformation. Annu. Rev. Biochem., *47:* 449–479, 1978.
25. Tricole, T., and Kowalski, D. Topoisomerase I from chicken erythrocytes: purification, characterization and detection by a deoxyribonucleic acid binding assay. Biochemistry, *22:* 2025–2031, 1983.
26. Juan, C-C., Hwang, J., Liu, A. A., Whang-Peng, J., Knutsen, T., Huebner, K., Croce, C. M., Zhang, H., Wang, J. C., and Liu, L. F. Human DNA topoisomerase I is encoded by a single-copy gene that maps to chromosome region 20q12–13.2. Proc. Natl. Acad. USA, *85:* 8910–8913, 1988.
27. Fort, P., Marty, L., Pisechaezyk, M., Elsabrouty, S., Dani, C., Jeanteur, P., and Blanchard, J. M. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res., *13:* 1431–1442, 1985.
28. Champoux, J. J. DNA is linked to the rat liver DNA nicking-closing enzyme by a phosphodiester bond to tyrosine. J. Biol. Chem., *256:* 4805–4809, 1981.
29. Kunze, N., Klein, M., Richter, A., and Knippers, R. Structural characterization of the human DNA topoisomerase I gene promoter. Eur. J. Biochem., *194:* 323–330, 1990.
30. Kunze, N., Yang, G., Dolberg, M., Sundarp, R., Knippers, R., and Richter, A. Structure of the human type I DNA topoisomerase gene. J. Biol. Chem., *266:* 9610–9616, 1991.
31. Greenberg, M. E., and Ziff, E. B. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (Lond.), *311:* 433–438, 1984.
32. Scanlon, K. J., Jiao, L., Funato, T., Wang, W., Tone, T., Rossi, J. J., and Kashani-Sabet, M. Ribozyme mediated cleavage of c-*fos* mRNA reduces gene expression of DNA synthesis enzyme and metallothionein. Proc. Natl. Acad. Sci. USA, *88:* 10591–10595, 1991.
33. Sobczak, J., Tournier, M. F., Lotti, A. M., and Douquet, M. Gene expression in regenerating liver in relation to cell proliferation and stress. Eur. J. Biochem., *180:* 49–53, 1989.
34. Romig, H., and Richter, A. Expression of the topoisomerase I gene in serum stimulated human fibroblasts. Biochim. Biophys. Acta, *1048:* 274–280, 1990.
35. Hwong, C. L., Chen, M. S., and Hwang, J. Phorbol ester transiently increases topoisomerase I mRNA in human skin fibroblasts. J. Biol. Chem., *264:* 14923–14926, 1989.
36. Romig, H., and Richter, A. Expression of the type I DNA topoisomerase gene in adenovirus-5 infected human cells. Nucleic Acids Res., *18:* 801–808, 1990.
37. Gleason, D. F., Mellinger, G. T., and the VA Cooperative Urological Research Group. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J. Urol., *111:* 58, 1974.
38. Schroder, F. H., Hermanck, P., Denis, L., Gospodarowicz, M. K., and Pavone-Macaluso, M. The TNM classification of prostate cancer. Prostate, *4* (Suppl.)*:* 129–138, 1992.
39. Hermanck, P., Sobin, L. H. (eds.) TNM Classification of Malignant Tumors, Ed. 4, Berlin: Springer Verlag, 1987.