Vol. 195, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1993 Pages 409-414

Higher Expression of Topoisomerase II in Lung Cancers Than Normal Lung Tissues: Different Expression Pattern From Topoisomerase I

Tadao Hasegawa,* Ken-ichi Isobe,† Izumi Nakashima,†
and Kaoru Shimokata*

*First Department of Internal Medicine and †Department of Immunology, Nagoya University School of Medicine, Nagoya, Japan

Received July 11, 1993

To examine the expression of topoisomerase I and topoisomerase II in primary lung cancer specimens at mRNA level, we carried out Northern blot analysis. As for topoisomerase I expression, there was no remarkable difference between lung cancer specimens and non-cancerous lung tissues. On the other hand, we could detect topoisomerase II mRNA in almost all lung cancer specimens, but not in non-cancerous tissues. By Southern blot analysis, we could not detect large deletion nor rearrangement in DNA level. These results suggest that the expression of topoisomerase II is highly increased in lung cancer at mRNA level and drugs against topoisomerase II might be more tumor-specific than those against topoisomerase I.

Topoisomerase I (topo I) and topoisomerase II (topo II) are nuclear enzymes that catalyze the topological changes of single (topo I)- and double (topo II)-stranded DNA. These enzymes are implicated to be involved in the replication, transcription and recombination of DNA. Recent studies have identified the topoisomerases as targets of a number of anticancer drugs. Topo I was specifically inhibited with camptothecin, an alkaloid isolated from *Camptotheca acuminata* (1), which has a strong antitumor activity against a wide range of experimental tumors (2,3). As for clinical relevance, topo I was found to be increased in human colon cancers suggesting a role of the enzyme in the therapeutic activity of camptothecin (4). A derivative of camptothecin, CPT-11, is now under clinical studies and is one of the new antitumor agents for lung cancer (5). Topo II was considered as a potent target of antineoplastic agents including epipodophyllotoxins, amsacrine and antracyclines(6-8). These drugs stabilize the cleavable complex and inhibit further reaction (9,10).

The abbreviations used are: TopoI, topoisomerase I; Topo II, topoisomerase II.

0006-291X/93 $4.00

Resistance to anticancer drugs could result from several different mechanisms, including alternation in the intracellular target and changes in the cellular uptake/efflux of the drug. Mammalian tumor cell lines resistant to drugs that act on topo I (11-13) or topo II (14-21) have been described and shown to be associated with decreased enzyme activities. The common mechanism of drug resistance, uptake/efflux change, has not yet been demonstrated for camptothecin. Some reports suggest that the modifications of topoisomerase activities is related to gene alterations (22).

In lung cancer patients, topo I and topo II inhibitor anti-tumor drugs are used in clinical studies, but the effect of these drugs are not complete. The drug resistance exists in primary cancers. So far, there have been little reports that analyzed the expression of topo I or topo II in primary *in vivo* lung cancer specimens compared to non-cancerous lung tissues. We carried out the analysis of topo I and topo II mRNA expressions in primary lung cancer specimens and non-cancerous lung tissues.

MATERIALS AND METHODS

Tumor and non-tumor specimens from lung cancer patients. Sixteen lung cancer tissues and non-cancerous lung tissues of the same patients were freshly obtained from surgical specimens at Nagoya University Hospital, Nagoya, Japan. Cancer types are listed in Table 1.

DNA probes. A 2.0 kb EcoRI-EcoRV fragment of phtopI (23) was used as a topo I probe. λhTOP2/Z2, a 1.8 kb EcoRI-XbaI fragment of the human topo II cDNA clone was used as a topo II probe (24). Human ß-actin probe(25) was used as an internal control.

RNA isolation and Northern blot analysis. Total RNA was extracted from lung specimens following the methods described by Chomczynski and Sacchi (26). About 10 µg of each RNA preparation was electrophoresed in 1% agarose gels containing 1.1 M formaldehyde. The RNA was transferred to Hybond-N nylon membranes (Amersham). Detection of mRNA with ^{32}P-labeled (using Multiprime labeling system; Amersham) probe was carried out by hybridization for 18 h at 42^{o}C in 5xSSPE (1xSSPE is composed of 0.18 M NaCl, 10 mM sodium phosphate (pH 7.7) and 1 mM

Table 1. Characteristics of the patients

Lung cancer type	Pt. No.
squamous	Pt. 1, 2, 6, 7, 12, 13
adeno	Pt. 4, 5, 8, 9, 10, 11, 15, 16
large	Pt. 14
mucoepidermoid	Pt. 3
normal lung tissues	Pt. 1-16

EDTA), 5xDenhart's solution (1xDenhart's solution is composed of 0.02% bovine serum albumin, 0.02% Ficoll and 0.02% polyvinyl pyrollidone), 50% formamide, 0.1% SDS, 50 μg/ml heat denatured salmon testis DNA, and radioactive probe. Membranes were washed for 15 min at 65°C in a solution containing 2xSSC (1xSSC is composed of 0.15 M NaCl and 15 mM sodium citrate) with 0.1% SDS twice, followed by washing in 1xSSC with 0.1% SDS for 30 min once at 65°C and a final washing for 15 min in 0.1% SSC with 0.1% SDS twice at room temperature. Autoradiography of the membranes was then performed at -70°C using Fuji RX film.

DNA extraction and Southern blot analysis. Genomic DNA from lung tissues was extracted as follows: Each tissue was homogenized in a solution containing 50 mM Tris (pH 7.5), 100 mM EDTA, 100 mM NaCl and 1% SDS. Proteinase K was added to a final concentration of 0.5%, and the solutions were incubated at 55°C overnight. Digests were extracted with phenol and chloroform three times and precipitated by ethanol. DNA samples were digested by restriction enzymes, electrophoresed through 0.8% agarose gels and transferred to Hybond-N nylon membranes (Amersham). Hybridization protocol was performed same as Northern blot analysis mentioned above.

RESULTS

Topo I and topo II mRNA expressions in in vivo primary lung cancers and non-cancerous lung tissues. Firstly we analyzed topo I mRNA expression in *in vivo* primary lung cancer tissues and non-cancerous lung tissues of the same patients. As shown in Figure 1, we could detect 4.0 kb mRNA in almost all samples. The intensity of the band was not so much different between lung cancer tissues and non-cancerous lung tissues.

Next we carried out Northern hybridization to see the expression of topo II gene. As shown in Figure 1, we could detect 6.1 kb mRNA in all lung cancer tissues except one sample (Pt 10). We could hardly detect this mRNA in all non-cancerous tissues. The level of expression was considered to be below the detection threshold.

Analysis of genomic DNA of topo I and topo II genes. There are some reports that DNA changes may be involved in resistance to anti-cancer drugs. We performed Southern blot analysis in ten lung cancer patients. Genomic DNA was digested with EcoRI and hybridized with topo I or topo II probe. As shown in Figure 2, no significant DNA changes in both topo I and topo II were observed.

DISCUSSION

In advanced inoperable cancer patients, the treatment of choice is usually chemotherapy. Nowadays camptothecin and VP 16 are likely becoming the most popular and effective anti-cancer drugs. These drugs inhibit topo I or topo II. DNA-topo complex formation and reactivity of cancer cells against these drugs are considered to be higher than those of normal cells. However, precise mechanisms of topo-DNA complex formation and tumor death are not clearly understood.

It is very important to elucidate drug resistant mechanisms by using parental cell lines and established drug resistant cell lines. In the clinical situation, however, it is

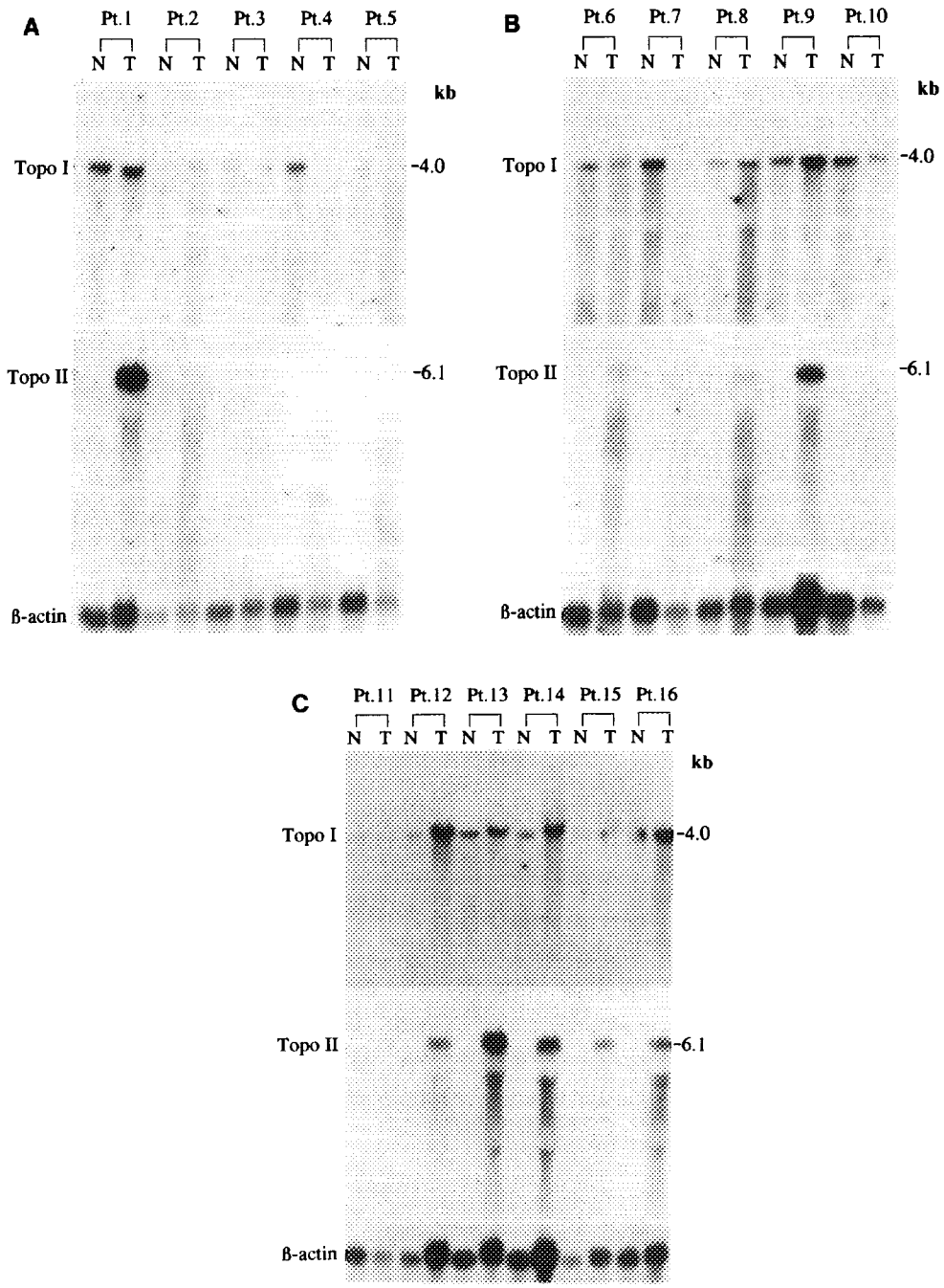

Fig.1.
Northern blot analysis of topo I and topo II mRNA expressions in lung cancer patient specimens. About 10 µg total RNA was electrophoresed on a formaldehyde-agarose gel, transferred to a nylon membrane and hybridized with the ^{32}P-labeled EcoRI-EcoRV fragment of phtopI and EcoRI-XbaI fragment of topo II cDNA. After hybridization, the filters were washed as described in the "Materials and Methods" section and autoradiographed. The same filters were rehybridized with human ß-actin probe and the results were shown at the bottom of the figure.

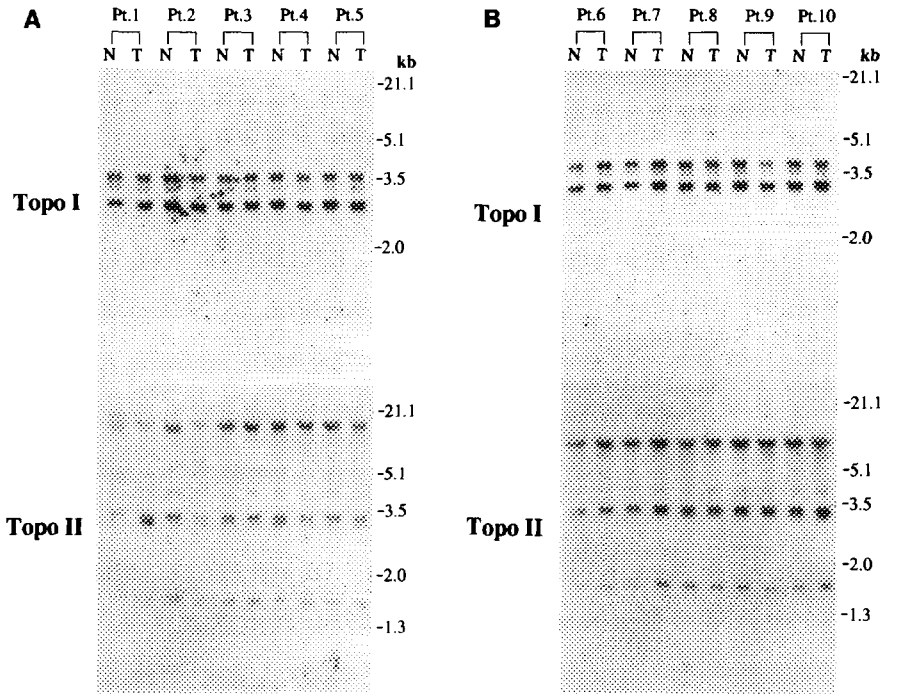

Fig.2.
Southern blot analysis of topo I and topo II DNA in lung cancer patient specimens. About 10 μg genomic DNA was digested with EcoRI and electrophoresed on a 0.8% agarose gel, transferred to a nylon membrane and hybridized with topo I and topo II cDNA.

also important to investigate the sensitivity of primary solid tumors against anti-cancer drugs. As far as we know, there were no reports about topo I or topo II expression in primary lung cancers. From these reasons, we analyzed topo I and topo II mRNA expressions in primary lung cancer specimens and non-cancerous lung tissues of the same patient. If the expression of either topo I or topo II is elevated in some patients, it may be possible to select anti-cancer drugs after surgery as adjuvant chemotherapy.

Giovanella et al.(4) reported that the amount of topo I in colon cancer tissues is more than that of adjacent non-cancerous colon tissues. However, in the present study, there was no difference of topo I mRNA expression between lung cancer tissues and non-cancerous lung tissues, while topo II mRNA expression in lung cancer specimens was much higher than that in non-cancerous lung tissues of the same patient. Therefore, topo II might be a good target for lung cancer treatment. However, the response rate of VP 16 against lung cancers is not so high as a single agent. Factors other than topo I and topo II expression, for example, influx and efflux of anti-cancer drugs may play an important role in sensitivity of anti cancer drugs.

Finally, we studied Southern blot analysis to see DNA change. No large DNA changes in lung cancers and non-cancerous lung tissue specimens in ten patients were observed. Although it is difficult to detect point mutation or small DNA deletions by

Southern blot analysis, it is suggested that large DNA changes may not be involved in regulation of topo I or topo II gene expression.

REFERENCES

1. Wall, M.E., Wani, M.C., Cook C.E., Palmar K.H., McPhail A.T., Sim G.A. (1966) J. Am .Chem. Soc. 88:3888-90.
2. Gallo R.C., Whang-Peng J., and Adamson R.H.(1971) J. Natl. Cancer Inst. 46:789-95.
3. Muggia F.M., Creaven P.J., Hansen H.H., Cohen M.H., and Selawry O.S. (1972) Cancer Chemother .Rep. 56:515-21.
4. Giovanella B.C., Stehlin J.S., Wall M.E., Wani M.C., Nicholas A.W., et al. (1989) Science 246:1046-8.
5. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, et al. (1992) J .Clin .Oncol. 10:16-20.
6. Chen G.L., Yang L, Rowe T.C., Halligan B.D., Tewey K.M., and Liu L.F., (1984). J. Biol. Chem. 259:13560-6.
7. Tewey K.M., Chen G.L., Nelson E.M., and Liu LF. (`1984)J. Biol. Chem. 259:9182-7.
8. Tewey K.M, Rowe T.C, Yang L., Halligan B.D., and Liu LF. (1984) Science 226:466-8.
9. Nelson E.M., Tewey K.M., and Liu L.F. (1984) Proc. Natl. Acad. Sci. USA 81:1361-5.
10. Hsiang Y-H, Hertzberg R., Hecht S., Liu L.F. (1985) J. Biol. Chem. 260:14873-8.
11. Andoh T., Ishii K., Suzuki Y., Ikegami Y., Kusunoki Y., Takemoto Y., et al. (1987) Proc .Natl. Acad. Sci. USA 84:5565-9.
12. Eng W.K., McCabe F.L., Tan K.B., Mattern M.R., Hofmann G.A., Woessner R.D., et al(1990) Mol .Pharmacol. 38:471-80.
13. Sugimoto Y., Tsukahara S., Oh-hara T., Liu L.F., and Tsuruo T.(1990) Cancer Res. 50:7962-5.
14. Pommier Y., Kerrigan D., Schwartz R.E., Swack J.A.,and McCurdy A. (1986) Cancer Res. 46:3075-81.
15. Beran M., and Andersson B.S. (1987)Cancer Res. 47:1897-904.
16. Danks M.K., Yalowich J.C., and Beck W.T. (1987) Cancer Res. 47:1297-301.
17. Danks M.K., Schmidt C.A., Cirtain M.C., Suttle D.P.,and Beck W.T. (1988)Biochemistry 27:8861-9.
18. Sinha B.K, Haim N., Dusre L., Kerrigan D., and Pommier Y. (1988) Cancer Res. 48:5096-100.
19. Beck W.T. (1989) J .Natl. Cancer Inst. 81:1683-5.
20. Deffie A.M, Batra J.K., and Goldenberg G.J. (1989)Cancer Res.49:58-62.
21. de Jong S., Zijlstra J.G., de Vries E.G.E., and Mulder N.H. (1990) Cancer Res. 50:304-9.
22. Tan K.B., Mattern M.R., Eng W-K, McCabe F.L., and Johnson R.K. (1989) J. Natl .Cancer Inst .81:1732-5.
23. Tamura H., Kohchi C., Yamada R., Ikeda T., Koiwai O., Patterson E., et al. (1991) Nucleic Acids Res. 19:69-75.
24. Tsai-Pflugfelder M., Liu L.F., Liu A.A., Tewey K.M., Whang-Peng J., Knutsen T., et al. (1988)Proc. Natl .Acad. Sci. USA 85:7177-81.
25. Nakajima-Iijima S., Hamada H., Reddy P., and Kakunaga T. (1985)Proc. Natl. Acad. Sci. USA 82:6133-7.
26. Chomczynski P.,and Sacchi N. (1987)Anal .Biochem .162:156-9.