Differential Expression of DNA Topoisomerase IIα and IIβ Genes between Small Cell and Non-small Cell Lung Cancer

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DNA topoisomerase II (Topo II) inhibitors are widely used in lung cancer chemotherapy, but small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) show different sensitivity to them. In this study, we examined the gene expression levels of both isoforms of Topo II (IIα and IIβ) in lung cancer specimens to investigate the differential expression between SCLC and NSCLC. The expression levels of the Topo IIα and Topo IIβ genes were assessed in 80 autopsy samples (40 primary tumors and 40 corresponding normal lung tissues) by using the reverse transcription polymerase chain reaction. We found that the expression levels of the Topo IIα gene in tumors were significantly higher than those in normal lung tissues, and that those in SCLC were significantly higher than those in NSCLC. There were no significant differences in Topo IIβ gene expression between tumors and normal lung tissues and between SCLC and NSCLC. Furthermore, correlation analysis revealed that Topo IIα expression was correlated with Topo IIβ expression in both tumor and normal lung tissues. These results indicate that a difference exists in the regulation of the Topo II gene between lung tumors and normal lung tissues. Our finding of differential expression of Topo IIα between SCLC and NSCLC also suggests that the Topo IIα expression level is associated with sensitivity to Topo II inhibitors.

Key words: DNA topoisomerase IIα — DNA topoisomerase IIβ — Small cell lung cancer — Non-small cell lung cancer — Drug resistance

DNA topoisomerase II (Topo II) is a nuclear enzyme that plays roles in replication, transcription and recombination of DNA, and alters DNA topology. There are two isoforms of Topo II, a 170-kDa isoform (Topo IIα) and a 180-kDa isoform (Topo IIβ), which may have different functions due to the difference in their expression during cell cycle progression and their differential distribution. Topo II is a target for several anticancer drugs, but their effectiveness is limited because of constitutive and/or acquired resistance to them. Several mechanisms of resistance to Topo II inhibitors, including decreased drug accumulation and drug target alteration, have been suggested. Several studies have demonstrated that decreased levels of Topo II gene expression are associated with resistance to Topo II inhibitors in some cancer cell lines. Topo II inhibitors, such as etoposide, are widely used in lung cancer chemotherapy, but small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) show different sensitivity to them. Previously we have demonstrated that sensitivity to etoposide, etoposide uptake, and the activity and content of Topo II are higher in SCLC cell lines than in NSCLC cell lines. Although there have been a few reports describing differences in Topo II gene expression levels between lung tumors and normal lung tissues in clinical specimens, no study has compared Topo II gene expression levels between SCLC and NSCLC using clinical samples. Therefore, in the present study we examined Topo II mRNA expression levels (both Topo IIα and Topo IIβ) in both SCLC and NSCLC in addition to normal lung tissues, using human autopsy samples.

MATERIALS AND METHODS

Patients and samples Eighty autopsy samples (40 primary tumors and 40 corresponding normal lung tissues) from 40 patients with lung cancer admitted to Hiroshima University Hospital and Chugoku Rousai General Hospital between June 1992 and March 1997 were studied. Fresh specimens of primary lung tumors and normal lung tissues were obtained during autopsy after written informed consent had been obtained. When we obtained tumor tissues, we macroscopically discarded necrotic parts and normal tissues. Then, one slice of the tissue was subjected to hematoxylin and eosin staining, and we con-
firmed that there was no contamination with necrotic or normal tissue. The tissues were frozen in liquid nitrogen and stored at −80°C until analyzed.

**Reverse transcriptase polymerase chain reaction (RT-PCR)** Total cellular RNA was extracted using the guanidine isothiocyanate-phenol method, and cDNA was synthesized using random hexamer (Amersham, Buckinghamshire, UK) with Superscript RNase H− reverse transcriptase ( GibCO-BRL, Bethesda, MD), as described previously.18 Then reaction mixtures were tested in a titration experiment as amplification cycles, the accuracy of the quantitative PCR procedure was confirmed that there was no contamination with necrotic or normal tissue. The tissues were frozen in liquid nitrogen and stored at −80°C until analyzed.

The reverse-transcribed cDNA from each sample was subjected to PCR amplification using primers based on the Topo IIα, Topo IIβ and β-actin (internal control) gene sequences. After pre-denaturation at 94°C for 5 min, the cDNA was added to 5 µl of PCR mixture, comprising 1 µl of 10× PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl), 1 µl of 15 mM MgCl2, 2 µl of distilled water, 0.2 µl of 20 mM dNTPs (Takara, Tokyo), 0.2 µl of 50 µM forward primer, 0.2 µl of 50 µM backward primer, and 0.4 µl (0.2 U) of Taq polymerase (Promega, Madison, WI). We used the Topo IIα and IIβ primers described previously.19 Their sequences were: Topo IIα forward 5′-ACATTTGACCCGTGTA-3′ and reverse 5′-GCTCTTCCTCATATTACCC3′; Topo IIβ forward 5′-ACAGGTGGTCCGTAATTCC3′ and reverse 5′-GTTTCAGCTGATA-CACC-3′. In order to determine the optimal number of amplification cycles, the accuracy of the quantitative PCR procedure was tested in a titration experiment as described previously.18 Then reaction mixtures were amplified for 25 cycles in a thermal cycler (Geneamp PCR System 2400; Perkin Elmer Applied Biosystems Division, Norwalk, CT) under the following conditions: denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 min, followed by a final incubation at 72°C for 7 min. The PCR products were 588 and 583 bp long, corresponding to Topo IIα and IIβ, respectively. We used the β-actin gene as an internal control, and the sequences of its primers, amplification cycles and the PCR products were as described previously.20

**Quantification of PCR products and analysis of mRNA expression** The PCR products were electrophoresed using 2% w/v agarose gels, transferred to nylon membranes (Hybond N+; Amersham), and subjected to hybridization analysis with 32P-labeled cDNA probes. After washing of each filter, the radioactivity level was measured with a laser imaging analyzer (BAS-2000; Fuji Photo Film, Tokyo). The PCR products of Topo IIα and IIβ described above were used as cDNA probes. The radioactivity associated with gene expression in each sample was expressed as the yield of the target gene relative to that of the β-actin gene.

**Statistical analysis** Contingency table analyses based on χ2 statistics were used to determine the significance of associations between categorical variables. Differences between the expression levels of each gene in tissue samples were analyzed using the Mann-Whitney U-test. All the gene expression levels were skewed toward higher expression, and were subjected to logarithmic transformation so that they approximated more closely a normal distribution, and then Pearson’s correlation analysis was performed. The statistical calculations and tests were performed using Stat View J4.11 Software (Abacus Co., CA) on a Macintosh computer. All the statistical tests were two-sided, the data were expressed as medians and ranges, and differences at P values of less than 0.05 were considered to be significant.

**RESULTS**

**Patient characteristics** We analyzed the levels of Topo IIα and IIβ gene expression in 80 autopsy samples (40 primary tumor tissues and 40 corresponding normal lung tissues) obtained from 40 patients, whose characteristics are presented in Table I. There were 32 males and 8 females, ranging in age from 44 to 86 years old (median, 68 years). Eleven had SCLC and 29 had NSCLC, almost all of them (37 of 40) had been smokers, and 16 had received etoposide administration for their tumors.

**Expression levels of Topo II genes** Both gene transcripts were detected in all the samples tested using the RT-PCR method. There was considerable variability among tumors or normal lung tissues in the level of expression of the two Topo II genes. The median levels of Topo IIα expression were 0.117 (range 0.024–2.285) in tumors and 0.066 (0.004–0.981) in normal lung tissues. The expression levels in tumors were significantly higher than those in normal lung tissues (P=0.0026, Fig. 1). On the other hand,

| Table I. Characteristics of Patients |
|-------------------------------------|
| All patients                        | 40 |
| Age (years)                         |    |
| median                              | 68 |
| range                               | 44–86 |
| Sex (male/female)                   | 32/8 |
| Smoker (yes/no)                     | 37/3 |
| Histology                           |    |
| small cell lung cancer              | 11 |
| non-small cell lung cancer          | 29 |
| Samples exposed to etoposide (yes/no) | 16/24 |
| Interval from the last etoposide administration to death (month) |    |
| median                              | 3.5 |
| range                               | 1–15 |
| Interval from death to autopsy (h)  |    |
| median                              | 2.3 |
| range                               | 1–16 |
the median Topo IIβ expression was 0.069 (range 0.008–1.783) in tumors and 0.055 (0.005–2.373) in normal lung tissues, and this difference was not significant ($P=0.2338$, Fig. 1).

We then compared the levels of expression of both genes between SCLC and NSCLC. There were no significant differences in age, sex, smoking history, radiotherapy history, etoposide dose received, interval between the last etoposide administration and death, or interval from death to autopsy between SCLC and NSCLC (data not shown). The median expression levels of Topo IIα were 0.284 (range 0.102–2.285) in SCLC and 0.092 (0.024–2.098) in NSCLC, the difference between the two groups being significant ($P=0.0064$, Fig. 2). On the other hand, the median expression levels of Topo IIβ were 0.109 (range 0.047–1.452) in SCLC and 0.061 (range 0.008–1.783) in NSCLC, the difference between the two groups being non-significant ($P=0.0870$, Fig. 2).

We also compared the levels of expression of both genes among SCLC and normal lung tissues and between
NSCLC and normal lung tissues. The expression levels of both genes in SCLC were significantly higher than those in normal lung tissues (Topo IIα, \( P = 0.0002 \); Topo IIβ, \( P = 0.0295 \)). In contrast, there was a trend toward increased Topo IIα gene expression in NSCLC compared to that in normal lung tissues (\( P = 0.0700 \)), but this was not the case for Topo IIβ (\( P = 0.7224 \)).

To investigate the association between the expressions of both genes and previous etoposide exposure, we grouped the results according to tissues which had been unexposed or exposed to this agent antemortem. The median levels of Topo IIα expression in exposed normal lung tissues and lung tumors were 0.067 (0.007–0.981) and 0.216 (0.024–2.285), respectively, and the respective values for non-exposed samples were 0.066 (0.004–0.433) and 0.099 (0.031–2.098). On the other hand, the median levels of Topo IIβ expression in exposed normal lung tissues and lung tumors were 0.046 (0.012–1.508) and 0.113 (0.033–1.452), respectively, and the respective values for the non-exposed samples were 0.063 (0.005–2.373) and 0.057 (0.008–1.783). There was no significant difference between the two groups for either of the genes (Fig. 3).

Correlation analysis revealed that Topo IIα expression was correlated with Topo IIβ expression in both tumor and normal lung samples (normal lung, \( r = 0.892, P < 0.0001 \); tumor, \( r = 0.836, P < 0.0001 \), Fig. 4).

**DISCUSSION**

Since Topo II is one of the most commonly employed targets for anticancer drugs in lung cancer chemotherapy, and the sensitivity to Topo II inhibitors differs between SCLC and NSCLC,13,14 it is important to examine the expression levels of Topo IIα and IIβ mRNA in both SCLC and NSCLC. Our data showed that the level of Topo IIα expression in lung tumors was significantly higher than that in normal lung, and furthermore that the level of Topo IIα expression in SCLC was significantly higher than that in NSCLC, whereas no such difference was observed for Topo IIβ.

Giaccone et al.17 used an RNase protection assay to examine differences in expression of the Topo II genes between normal lung tissues and NSCLC tumors, using specimens obtained surgically. They showed that expression of the Topo IIα, but not Topo IIβ, gene in tumors was higher than that in normal lung, and our present data confirmed their findings. Hasegawa et al.16 also showed by northern blot analysis that expression of the Topo IIα gene in NSCLC tumors was higher than that in normal lung tissues using specimens obtained surgically, although they did not examine expression of the Topo IIβ gene. These results suggest that there is a difference in regulation of expression of the Topo II genes between lung tumors and normal lung.

Although a previous report has described differences in Topo IIα expression between SCLC and NSCLC using immunohistochemistry,21 the present report is the first to document such differences based on molecular biological methods. From our results, we think that Topo IIα plays an important role in determining the difference in *in vivo* sensitivity to Topo II inhibitors between SCLC and...
NSCLC. Previously, it was demonstrated that increased Topo II gene expression showed a strong positive correlation with cell sensitivity to Topo II inhibitors in lung cancer cell lines. In fact, SCLC is more sensitive to Topo II inhibitors than NSCLC in the clinical situation, and higher content levels and activity of Topo II have been observed in SCLC than in NSCLC cell lines. In contrast, decreased levels of Topo IIα gene expression are associated with resistance to Topo II inhibitors in lung cancer cell lines. These results suggest that Topo IIα gene expression plays an important role in the action of Topo II inhibitors.

Furthermore, levels of Topo IIα gene expression in tumors were higher than those in normal tissues in breast cancer and in ovarian cancer. It appears that Topo II inhibitors can be selective for tumors rather than normal tissues, assuming that Topo IIα is the principal target enzyme.

On the other hand, Yamazaki et al. have reported that the sensitivity of lung cancer cell lines to Topo II inhibitors can not be explained by the Topo IIα content levels or Topo II catalytic activity. Furthermore, Sandri et al. have indicated that Topo IIβ might play a significant role as a target for anti-tumor therapy, because Topo IIβ protein is more widely expressed than Topo IIα protein in breast cancer cells.

In the present study, we observed that some NSCLC tissues showed higher levels of Topo IIα gene expression levels than SCLC tissues. Although the precise reason for this remains to be elucidated, it is possible that not only Topo II gene expression, but also some other factors, such as bcl-2, may be determinants of the sensitivity of lung cancer cells to Topo II inhibitors. We think that the relationship between the level of Topo II gene expression and drug sensitivity is both interesting and important, and that further studies are required to determine whether the level of Topo IIα and/or Topo IIβ expression can be a good marker of sensitivity to Topo II inhibitors.

Recently it has been demonstrated that multidrug resistance-associated protein (MRP) is related to etoposide transport, and that its overexpression is associated with etoposide resistance. Previously, we examined MRP gene expression in the same samples as those used in the present study, and found that there was no significant association between MRP expression and history of etoposide exposure. Although it has been suggested that Topo II gene expression is related to etoposide resistance, we did not find any relationship between the level of Topo II gene expression and antemortem exposure to etoposide in this study. As etoposide has been reported to persist in tissues for several days, and our autopsy samples were taken several months after final administration of the anticancer drug, we think that the effect of etoposide on tumors or normal lung tissues could not have been assessed properly in the present study. Even though it is unclear whether MRP and/or Topo II plays a major role in etoposide resistance, we think that monitoring of the expression levels of both genes immediately after etoposide exposure may be needed.

As we had examined the levels of DNA topoisomerase I (Topo I) gene expression using the same samples as those employed in the present study, we analyzed the relationships between the levels of Topo I and Topo II gene expression. However, Topo I expression was found not to be correlated with the expression of either of the Topo II isoform genes (data not shown). These results suggest that there is a difference in regulation between the Topo I and Topo II genes.

We found that the expression of Topo IIβ was correlated with that of Topo IIβ in both tumors and normal lung tissues. A similar correlation was reported in acute myelogenous leukemia and lung cancer cell lines, although studies using clinical samples have failed to reveal such a correlation. Our results are somewhat confusing in that there was no significant difference in Topo IIβ expression between tumors and normal lung tissues, or between SCLC and NSCLC. Topo IIβ gene expression in SCLC was significantly higher than that in normal lung tissues (Figs. 1, 2), and therefore the lack of a statistically significant difference in Topo IIβ gene expression between SCLC and NSCLC may have been due to error resulting from the small sample size. Furthermore, there was considerable variability among tumors and normal lung tissues in the level of expression of the two Topo II genes. Our results suggest that the two Topo II isoforms show coordinated variation of their expression in both lung cancer and normal lung tissues.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan, a Fellowship for Study at a Center of Excellence Abroad from the Japan Society for the Promotion of Science and grants from Osaka Cancer Research Foundation, and Chugai Seiyaku Co., Ltd. We are grateful to Drs. T. Tsuya, T. Ohune (Department of Respiratory Disease, Chugoku Rousai Hospital) for providing autopsy samples. Thanks are also extended to Drs. O. Katoh (Department of Environment and Mutation, Research Institute for Radiation Biology and Medicine, Hiroshima University), and N. Ohashi (Department of Respiratory Disease, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital) for their practical suggestions during the study.

(Received April 15, 1998/Revised June 1, 1998/Accepted June 5, 1998)
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