Cancer Chemother Pharmacol (1997) 39: 192–198

ORIGINAL ARTICLE

Kohichi Yamazaki · Hiroshi Isobe · Tarou Hanada
Tomoko Betsuyaku · Atsushi Hasegawa
Nobuyuki Hizawa · Shigeaki Ogura
Yoshikazu Kawakami

Topoisomerase IIα content and topoisomerase II catalytic activity cannot explain drug sensitivities to topoisomerase II inhibitors in lung cancer cell lines

Received: 11 July 1995 / Accepted: 18 May 1996

Abstract *Purpose*: Topoisomerase IIα content, topoisomerase II catalytic activity and drug sensitivities to the topoisomerase II inhibitors, doxorubicin and etoposide, were examined in a panel of 14 unselected human lung cancer cell lines in order to determine the relationship between topoisomerase II and drug sensitivities to the topoisomerase II inhibitors. *Methods*: Drug sensitivities were determined using a microculture tetrazolium assay. The topoisomerase IIα levels were determined by Western blot analysis and the topoisomerase II catalytic activity was determined using a decatenation assay of kinetoplast DNA, using nuclear protein from cells of each cell line. *Results*: Drug sensitivity tests revealed that small-cell lung cancer (SCLC) cell lines were more sensitive to drugs than non-small-cell lung cancer (NSCLC) cell lines. The relative topoisomerase IIα levels and relative topoisomerase II catalytic activity from SCLC cell lines (mean ± SD 0.89 ± 0.54 and 5.3 ± 3.4, respectively) were slightly higher than those from NSCLC cell lines (0.78 ± 0.56 and 4.0 ± 2.8, respectively), but the differences were not statistically significant, and not sufficient to account for the variation in drug sensitivities. Moreover, no clear association was observed between the topoisomerase IIα levels or the topoisomerase II catalytic activity and drug sensitivities in the cell lines studied. *Conclusions*: These findings suggest that the difference in drug sensitivities to doxorubicin and etoposide in human lung cancer cell lines might not be explainable by the topoisomerase IIα levels and topoisomerase II catalytic activity. Moreover, our results suggest that the topoisomerase IIα levels and topoisomerase II catalytic activity may play a minor role in the determination of clinical drug resistance of human lung cancers.

K. Yamazaki (✉) · H. Isobe · T. Hanada · T. Betsuyaku
A. Hasegawa · N. Hizawa · S. Ogura · Y. Kawakami
First Department of Medicine, School of Medicine, Hokkaido University, Kita 15, Nishi 7, Kitaku, Sapporo 060, Japan
Tel. 011-716-1161 (ext. 5911); Fax 011-727-6724

Key words Topoisomerase II · Drug sensitivity · Lung cancer

Introduction

The development of multidrug resistance remains a primary obstacle to the success of chemotherapy for many human tumors. A variety of biochemical mechanisms are responsible for imparting multidrug resistance [4]. One mechanism that confers multidrug resistance is the overproduction of P-glycoprotein, an integral membrane protein responsible for the active efflux of a broad spectrum of antitumor agents [36]. The overexpression of P-glycoprotein has been shown to be important in the clinical drug resistance of a variety of human tumor types [16, 37]. However, the expression of P-glycoprotein is relatively low in most lung cancers and lung cancer cell lines, and is not correlated with the clinical response or in vitro cytotoxicity [1, 23]. This indicates that P-glycoprotein is unlikely to play an important role in the clinical drug resistance of human lung cancers [37].

In addition to the overexpression of P-glycoprotein, topoisomerase II has been shown to be important in drug resistance. Topoisomerase II, a component of the nuclear matrix [25], influences nuclear structure and function and, in particular, affects replication, transcription, and chromosome segregation [2, 3]. Topoisomerase II can be classified into two isoforms [10]. Topoisomerase IIα is the target enzyme of anticancer drugs such as epipodophyllotoxins, amsacrine and anthracyclines, so-called topoisomerase II inhibitors, which are thought to be among the most efficacious agents against lung cancer [25, 40]. Increased expression of topoisomerase IIα protein is correlated with drug sensitivities to intercalating agents and epipodophyllotoxins in a mutant rodent cell line [8].

Analysis of topoisomerase II inhibitor-resistant lines has suggested that a relative decrease in the amount of or a change in the characteristics of topoisomerase IIα is one of the mechanisms of drug resistance to topoisomerase II inhibitors [24, 28, 33]. Moreover, several studies have analyzed the relationship between topoisomerase II and drug sensitivities to topoisomerase II inhibitors in lung cancer cell lines [13, 18]. However, the role of topoisomerase II in the clinical drug resistance of human lung cancer is not precisely understood.

Progress in the understanding of drug resistance has come from the study of cell lines made resistant by continuous in vitro drug exposures. Although these cell lines are useful for investigations of drug resistance mechanisms, they may not adequately represent the clinical drug resistance (intrinsic or acquired) encountered in lung cancer patients. Thus, it may be important to use a panel of cell lines unselected for resistance by in vitro drug exposures for the analysis of clinical drug resistance [13]. In this study, in order to evaluate the role of topoisomerase II in the clinical drug resistance of human lung cancer, we examined the relationship between topoisomerase IIα content, topoisomerase II catalytic activity and drug sensitivities to the topoisomerase II inhibitors, doxorubicin and etoposide, in a panel of unselected human lung cancer cell lines.

Materials and methods

Cell lines and cell culture

A total of 14 human lung cancer cell lines, including six human lung adenocarcinoma cell lines (A549, RERF-LC-MS, PC-3, RERF-LC-OK, ABC-1 and VMRC-LCD), four human lung squamous cell carcinoma cell lines (LC-1 Sq, PC-10, LK-2 and EBC-1) and four human lung small-cell carcinoma cell lines (PC-6, SBC-3, SBC-5 and RERF-LC-MA) were kindly provided by the Japanese Cancer Research Resources Bank, Tokyo. They were derived from patients who had not received previous chemotherapy and they were unselected for resistance by in vitro exposures [14, 15, 17, 21, 22]. All cell lines were cultured in RPMI-1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum (FBS) under an atmosphere of air containing 5% CO_2 at 37 °C, and the passage numbers at the time of the experiments were from 50 to 100 in all the cell lines. In every experiment DNA content was measured by flow cytometry after staining of cells with propidium iodide to confirm that the cells were in the exponentially growing phase.

Drug sensitivity assay

Drug sensitivities of exponentially growing cells were determined by a microculture tetrazolium assay, as described previously [6, 32]. Each cell line was plated at an optimum density, such that the cells were still growing exponentially after 5 days in culture. First, the plates were incubated for 24 h under standard conditions. Doxorubicin or etoposide was then added, and the plates were incubated for a further 4 days. Following drug incubation, 0.05 mg/well of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt, sodium salt (XTT; Sigma, St. Louis, MO, USA) with 0.4 μg of Phenazine methosulfate (PMS; Sigma) was added, and the plates were incubated for an additional 4 h. Absorbance was determined using an ImmunoReader NJ-2000 (Nippon InterMed, Tokyo, Japan) at a wavelength of 450 nm. Background absorbance was subtracted from each row, and the mean reduction was calculated as the proportion of the absorbance of the untreated controls. The IC_{50} was determined graphically as the drug concentration showing 50% of the absorbance of the untreated controls in three or more independent experiments, each performed in triplicate with 14 cell lines.

Preparation of nuclear protein

Crude nuclear protein from cells of each cell line, which was used for determination of topoisomerase IIα content and topoisomerase II catalytic activity as described previously [13, 18], was prepared as previously reported by Deffie et al. [9]. Briefly, exponentially growing cells ($20–30 \times 10^6$ cells) were collected, and washed twice with ice-cold nucleus buffer which consisted of 2 m*M* K_2HPO_4, 5 m*M* $MgCl_2$, 150 m*M* NaCl, 1 m*M* ethylene glycol-bis(β-aminoethylether) *N*,*N*,*N*′,*N*′-tetraacetic acid, and 0.1 m*M* dithiothreitol, adjusted to pH 6.5. The cells were resuspended in 1 ml nucleus buffer and 9 ml nucleus buffer containing 0.35% Triton X-100, and 1 m*M* phenylmethylsulfonyl fluoride was added. The cell suspension was placed on ice for 10 min, and then washed three times with nucleus buffer that was free of Triton X-100. Nuclear protein was extracted for 1 h at 4 °C with nucleus buffer containing 0.35 *M* NaCl. DNA and nuclear debris were pelleted by centrifugation at 17 000 *g* for 20 min. All procedures were performed on ice. The protein concentration was determined using a BCA protein assay kit (Pierce Chemical Co., Rockford, Ill.).

Topoisomerase IIα content

The topoisomerase IIα content of nuclear protein from cells of each cell line was determined by Western blot analysis, using an anti-topoisomerase II monoclonal antibody. This antibody, which recognized the α-isoform of topoisomerase II, was provided by Cambridge Research Biochemicals, Wilmington, DE, USA. The procedure for the Western blot analysis was essentially the same as described previously [18]. In brief, 30 μg nuclear protein was electrophoresed on a 7.5% sodium dodecyl sulfate-polyacrylamide slab gel. Proteins on the gel were electrically transferred to a nitrocellulose membrane then incubated with topoisomerase II monoclonal antibody, diluted 1:200, for 1 h. After incubation, the membranes were rinsed and incubated with horseradish peroxidase-conjugated donkey antirabbit IgG (Amersham International, Buckinghamshire, UK). After rinsing, blots were visualized by enhanced chemiluminescence detection (Amersham International). The topoisomerase IIα content was then determined densitometrically.

Topoisomerase II catalytic activity

Topoisomerase II catalytic activity of nuclear protein from cells of each cell line was assayed by decatenation of kinetoplast DNA (kDNA) to minicircle DNA as described previously [29]. Briefly, serial 1:2 dilutions of nuclear protein were made and the same volume (1 μl) of nuclear protein or of dilutions was added to reaction mixtures containing 2 μg kDNA (TopoGEN, Columbus, OH, USA) and 2 μl 10× reaction buffer (1× reaction buffer = 50 m*M* Tris-Cl (pH 8.0), 120 m*M* KCl, 10 m*M* $MgCl_2$, 0.5 m*M* ATP, 0.5 m*M* dithiothreitol, and 30 μg bovine serum albumin/ml; total volume 20 μl). Reaction mixtures were incubated at 37 °C for 10 min and the

reaction was stopped by the addition of 4 μl stop buffer (5% Sarkosyl, 0.0025% bromophenol blue, and 25% glycerol). The samples were electrophoresed through a 1% agarose gel in 45 m*M* Tris-borate-EDTA (pH 8.0) and a final concentration of 0.5 μg/ml ethidium bromide at 50 V for 2 h. After destaining in water, the gel was photographed under UV light. Catalytic activity was detected as the lowest amount of nuclear protein which made the topological change of kDNA into minicircle DNA. The topoisomerase II catalytic activities are expressed as the relative levels compared to RERF-LC-MA, which had the lowest topoisomerase II catalytic activity.

Statistical methods

Wilcoxon's rank-sum test was used to assess differences between the non-small-cell lung cancer (NSCLC) or small-cell lung cancer (SCLC) and each biological property. The linear correlation coefficient (r) was used to evaluate the relationships between doubling times, the IC_{50} values, the levels of topoisomerase IIα and the levels of topoisomerase II catalytic activity.

Results

Characteristics of the human lung cancer cell lines studied

The biological properties of the human lung cancer cell lines studied are summarized in Table 1. They consisted of six adenocarcinoma cell lines, four squamous cell carcinoma cell lines and four small-cell carcinoma cell lines. Doubling times of exponentially growing cells ranged from 14.8 to 55.2 h. P-glycoprotein expression was not detected by flow cytometry utilizing monoclonal antibody MRK16 (Kyowa Medix, Tokyo, Japan) [36] in any cell line, as reported in previous studies [1, 23].

Drug sensitivities of the cell lines

The topoisomerase II inhibitors, doxorubicin and etoposide, were tested for their cell-killing effects on lung cancer cell lines during 4 days of treatment (Table 1). The SCLC cell lines were more sensitive to doxorubicin and etoposide than the NSCLC cell lines, and the difference was statistically significant for the sensitivity to etoposide (Table 2). Marked differences in drug sensitivities among the cell lines studied were observed. The ratio of drug sensitivities to doxorubicin between PC-10, which showed the highest IC_{50}, and PC-6, which showed the lowest, was about 15. The ratio of drug sensitivities to etoposide between PC-3, which showed the highest IC_{50}, and PC-6, which showed the lowest, was about 100. Drug sensitivity to doxorubicin was significantly correlated with that to etoposide in the cell lines studied (Fig. 1, $r = 0.687$, $P = 0.0066$). No clear association between doubling times and drug sensitivities was seen in the cell lines studied (Table 1).

Topoisomerase IIα content

The topoisomerase IIα content of nuclear protein from each cell line was determined by Western blot analysis (Fig. 2). The 170-kDa topoisomerase IIα was detected in nuclear protein of all the cell lines studied. The ratio of the levels of topoisomerase IIα between LC-1 Sq, which showed the highest level, and RERF-LC-MA, which showed the lowest, was about 10 (Table 1). No clear association was observed between doubling times and the levels of topoisomerase IIα in the cell lines studied (Table 1). This indicated that the differences in

Table 1 Characteristics of human lung cancer cell lines. The values for topoisomerase II content and activity are relative levels compared with the PC-6 and RERF-LC-MA cells lines, respectively

Cell line	Doubling time (h)	IC_{50} (μ*M*)		Topoisomerase II	
		Doxorubicin	Etoposide	Content	Activity
Adenocarcinoma					
A549	16.7	0.0330	0.409	1.17	8
RERF-LC-MS	31.6	0.0260	0.232	0.14	2
PC-3	38.4	0.204	12.8	0.40	4
RERF-LC-OK	30.6	0.0307	0.503	0.25	2
ABC-1	35.3	0.127	0.453	0.25	2
VMRC-LCD	28.5	0.232	1.04	0.89	2
Squamous cell carcinoma					
LC-1Sq	55.2	0.180	1.97	1.69	8
PC-10	22.1	0.315	0.590	1.56	8
LK-2	30.2	0.0443	0.897	0.92	2
EBC-1	35.8	0.138	1.43	0.50	2
Small-cell carcinoma					
PC-6	19.5	0.0201	0.134	1	4
SBC-3	17.9	0.0241	0.176	1.46	8
SBC-5	14.8	0.0645	0.224	0.95	8
RERF-LC-MA	32.3	0.0410	0.422	0.16	1

Table 2 Characteristics of NSCLC and SCLC cell lines. Values are means ± SD (median, range)

Characteristic	NSCLC ($n = 10$)	SCLC ($n = 4$)	P-value
Doubling time (h)	32.4 ± 10.3 (30.6, 16.7–55.2)	21.1 ± 7.7 (17.9, 14.8–32.3)	0.897
IC_{50} (μM) Doxorubicin	0.133 ± 0.100 (0.127, 0.0260–0.315)	0.037 ± 0.020 (0.0241, 0.0201–0.0643)	0.066
Etoposide	2.031 ± 3.821 (0.590, 0.232–12.8)	0.239 ± 0.127 (0.176, 0.134–0.422)	0.011
Relative topoisomerase IIα content	0.78 ± 0.56 (0.50, 0.14–1.69)	0.89 ± 0.54 (0.95, 0.16–1.46)	0.671
Relative topoisomerase II activity	4.0 ± 2.8 (2, 2–8)	5.3 ± 3.4 (4, 1–8)	0.597

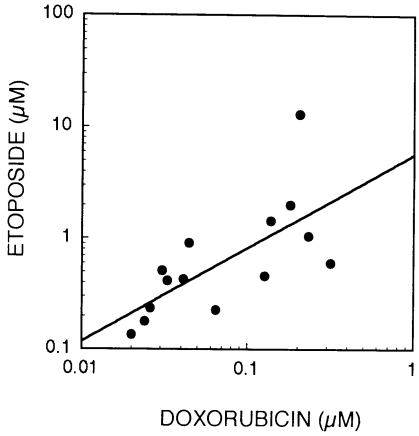

Fig. 1 Correlation of IC_{50} values of doxorubicin and etoposide. Drug sensitivity tests were determined using a microculture tetrazolium assay. The logarithms of the IC_{50} values for doxorubicin and etoposide were strongly correlated with each other ($r = 0.687$, $P = 0.0066$)

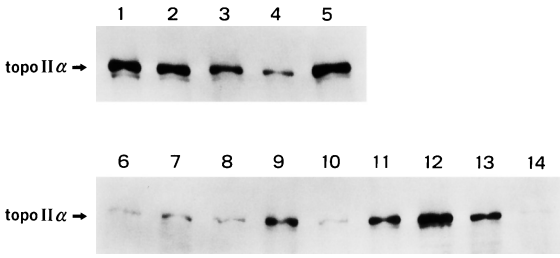

Fig. 2 Topoisomerase IIα content by Western blot analysis in the studied human lung cancer cell lines. *Lane* 1 LC-1Sq, *lane* 2 PC-10, *lane* 3 LK-2, *lane* 4 EBC-1, *lane* 5 A549, *lane* 6 RERF-LC-MS, *lane* 7 PC-3, *lane* 8 RERF-LC-OK, *lane* 9 ABC-1, *lane* 10 VMRC-LCD, *lane* 11 PC-6, *lane* 12 SBC-3, *lane* 13 SBC-5, *lane* 14 RERF-LC-MA

topoisomerase IIα content might not be explainable by doubling times. Moreover, although SCLC cell lines had slightly higher relative levels of topoisomerase IIα (mean ± SD 0.89 ± 0.54) than NSCLC cell lines (0.78 ± 0.56), the difference was not statistically significant (Table 2).

Association between drug sensitivities to doxorubicin and etoposide and topoisomerase IIα content

The relationships between drug sensitivities and the levels of topoisomerase IIα were analysed. The drug sensitivities to doxorubicin and etoposide were almost the same in RERF-LC-MS and SBC-3, but the levels of topoisomerase IIα were entirely different: SBC-3 had an approximately ten times higher topoisomerase IIα content than RERF-LC-MS. Moreover, the levels of topoisomerase IIα were almost the same in LC-1 Sq and SBC-3, but the drug sensitivities to doxorubicin and etoposide were entirely different: SBC-3 was approximately 7.5 times more sensitive to doxorubicin, and approximately 11 times more sensitive to etoposide than LC-1 Sq. No clear association was observed between drug sensitivities to doxorubicin and etoposide and the levels of topoisomerase IIα in any of the 14 cell lines, particularly in the ten NSCLC cell lines. In the four SCLC cell lines, those with higher levels of topoisomerase IIα had a tendency to be more sensitive to drugs, but this was not statistically significant.

Topoisomerase II catalytic activity

The topoisomerase II catalytic activity of nuclear protein from each cell line was determined by a decatenation assay of kDNA. Figure 3 shows the decatenation of kDNA to minicircle DNA using nuclear protein extraction from A549, PC-3 and RERF-LC-MS. Formation of minicircle DNA was observed in the lanes that contained 0.25 μg nuclear protein extracted from A549, 0.5 μg from PC-3 and 1.0 μg from RERF-LC-MS. Thus, the topoisomerase II catalytic activity in the nuclear protein from A549 was fourfold higher than in that from RERF-LC-MS, and twofold higher than in that from PC-3. The ratio of the topoisomerase

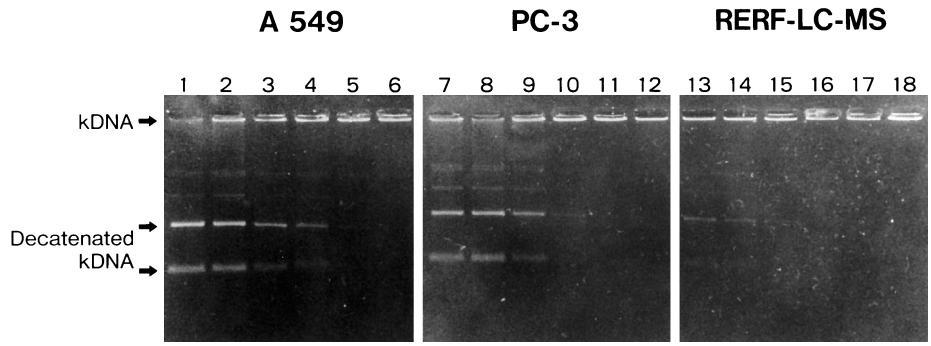

Fig. 3 Topoisomerase II catalytic activity in the human lung cancer cell lines studied and determined by decatenation of kinetoplast DNA. Amounts of nuclear protein were as follows: *lanes* 1, 7 *and* 13 2.0 μg, *lanes* 2, 8 *and* 14 1.0 μg, *lanes* 3, 9 *and* 15 0.5 μg, *lanes* 4, 10 *and* 16 0.25 μg, *lanes* 5, 11 *and* 17 0.125 μg, *lanes* 6, 12 *and* 18 0.0625 μg. Formation of minicircle DNA was observed in the lanes that contained 0.25 μg of nuclear protein extracted from A549, 0.5 μg from PC-3 and 1.0 μg from RERF-LC-MS

II catalytic activity between A549, LC-1 Sq, PC-10, SBC-3 and SBC-5, which showed the highest level, and RERF-LC-MA, which showed the lowest, was about 8 (Table 1). Although the topoisomerase II activity assay does not discriminate between topoisomerase IIα and β isozyme activities [38], a clear correlation was observed between the topoisomerase II catalytic activity and the levels of topoisomerase IIα in the cell lines studied (Fig. 4). SCLC cell lines had slightly higher relative levels of topoisomerase II catalytic activity (5.3 ± 3.4) than NSCLC cell lines (4.0 ± 2.8), but the difference was not statistically significant (Table 2).

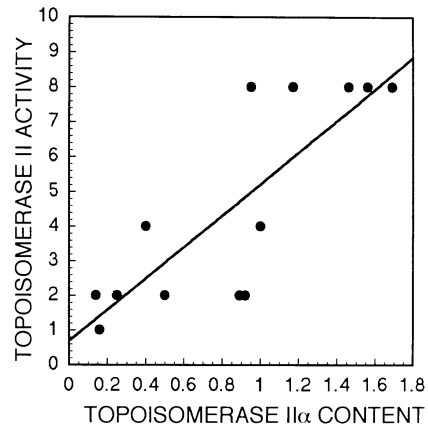

Fig. 4 Correlation of topoisomerase IIα content and topoisomerase II catalytic activity. The relative topoisomerase IIα content and the relative topoisomerase II catalytic activity were strongly correlated with each other ($r = 0.830$, $P = 0.0002$). The values of topoisomerase IIα content are the relative levels compared with PC-6 cell line. The values of topoisomerase II activity are the relative levels compared with the RERF-LC-MA cell line

Association between drug sensitivities to doxorubicin and etoposide, and topoisomerase II catalytic activity

The relationship between drug sensitivities and topoisomerase II catalytic activity was also analyzed. The drug sensitivities to doxorubicin and etoposide were almost the same in RERF-LC-MS and SBC-3, but the topoisomerase II catalytic activities were entirely different: SBC-3 had an approximately four times higher topoisomerase II catalytic activity than RERF-LC-MS. Moreover, the topoisomerase II catalytic activities were almost the same in LC-1 Sq and SBC-3, but the drug sensitivities to doxorubicin and etoposide were entirely different: SBC-3 was approximately 7.5 times more sensitive to doxorubicin, and approximately 11 times more sensitive to etoposide than LC-1 Sq. No clear association was observed between drug sensitivities to doxorubicin and etoposide and the topoisomerase II catalytic activity in any of the 14 cell lines, particularly in the ten NSCLC cell lines. In the four SCLC cell lines, those with higher topoisomerase II catalytic activities had a tendency to be more sensitive to drugs, but this was not statistically significant.

Discussion

Several studies have analyzed the relationship between topoisomerase II and in vitro drug sensitivities or clinical responses to topoisomerase II inhibitors. Fry et al. found that testis carcinoma cell lines, which were more sensitive to drugs, had higher topoisomerase II activities and higher levels of topoisomerase IIα protein than three bladder cancer cell lines [11]. In clinical materials, very low levels of topoisomerase IIα and mRNA expression have been reported in human chronic lymphocytic leukemia cells, which are relatively insensitive to topoisomerase II inhibitors, in contrast to some more undifferentiated lymphoproliferative disorders [12, 30]. Kim et al. reported that, in fresh tumor tissues obtained from 15 patients with breast, hepatocellular,

metastatic breast or gastric cancers, topoisomerase IIα mRNA expression is significantly correlated with the clinical response [20]. On the other hand, Kaufmann et al. found that the content of the two topoisomerase II isoenzymes does not correlate with drug sensitivity in vitro or in vivo in marrow samples from adult acute myelogenous leukemia patients [19]. Kasahara et al. reported that SCLC cell lines, which are more sensitive to drugs, have higher topoisomerase II activities and higher levels of topoisomerase IIα than NSCLC cell lines [18]. However, despite a 10- to 80-fold increased sensitivity to the topoisomerase II inhibitors, SCLC cell lines had only about twofold higher activity of topoisomerase II than NSCLC cell lines. This indicates that additional factors could be involved and could play a more critical role in the drug resistance of lung cancer.

Giaccone et al. found a correlation between steady-state levels of topoisomerase IIα mRNA expression, DNA-unknotting activities, DNA cleavage activities and drug sensitivities to doxorubicin, etoposide and teniposide in eight unselected lung cancer cell lines [13]. However, they excluded one cell line, which showed a low level of topoisomerase IIα mRNA expression despite being highly sensitive to all the drugs tested. When this cell line was excluded from the analysis, the correlation between the levels of topoisomerase IIα mRNA expression and drug sensitivity became significant. Thus, the relationship between the level of topoisomerase IIα content and drug sensitivities to topoisomerase II inhibitors is not precisely understood in lung cancer cell lines.

In this study, although we did not determine the topoisomerase II cleavable complex, we analyzed the relationship between the levels of topoisomerase IIα, the topoisomerase II catalytic activity and drug sensitivities to doxorubicin and etoposide in a panel of 14 unselected lung cancer cell lines, which showed no overexpression of P-glycoprotein. SCLC cell lines were more sensitive to doxorubicin and etoposide than NSCLC cell lines as described previously, and this is consistent with the clinical response of topoisomerase II inhibitors in human lung cancer chemotherapy [18]. The levels of the topoisomerase IIα and topoisomerase II activity of SCLC cell lines were slightly higher than those of NSCLC cell lines, but the differences were not statistically significant and were not sufficient to account for the variation in drug sensitivities of SCLC and NSCLC cell lines. Moreover, no clear association was observed between the levels of topoisomerase IIα or topoisomerase II catalytic activity and drug sensitivities in the cell lines studied.

Although our results came from only 14 cell lines and these cell lines might have great variability in multifactorial mechanisms of multidrug resistance other than topoisomerase II, our findings suggest that the differences in drug sensitivities to doxorubicin and etoposide are not explainable only by the levels of topoisomerase IIα and topoisomerase II catalytic activity in human lung cancer cell lines. The cell lines studied might not be representative of clinical drug sensitivity since they were selected by repeated passaging, based on their very different genotypes, but our results suggest that the levels of topoisomerase IIα and topoisomerase II catalytic activity may be a minor factor in the determination of clinical drug resistance of human lung cancers.

Recently, several in vitro studies have shown that, apart from decreased topoisomerase IIα levels, qualitative alterations in topoisomerase II, mainly induced by mutations in the topoisomerase II gene, might lead to insensitivity of this drug target [13, 34, 40]. However, topoisomerase II mutations have so far been very rare in clinical samples or unselected cell lines [19]. The relationship between the qualitative alterations in topoisomerase II and drug sensitivity should be further analyzed in human lung cancers.

In this study, nuclear protein of cells in each cell line was used to determine topoisomerase IIα content and topoisomerase II catalytic activity, as reported for most other studies [13, 18]. However, there is the possibility that differences in extractability of the topoisomerase II from cell nuclei might play some role in explaining the differences in topoisomerase IIα content and topoisomerase II catalytic activity. While we do not think it negates our findings, this possibility should be considered.

In summary, our findings indicate that topoisomerase IIα content and topoisomerase II catalytic activity may play a minor role in determining drug sensitivities to topoisomerase II inhibitors, as well as P-glycoprotein. Other biochemical mechanisms are thought to exist in lung cancer, including the MRP gene [7, 39], decreased drug accumulation [35], subcellular drug distribution [31], c-*myc* expression [5, 26] and different susceptibility to apoptosis [27]. Further studies of the clinical drug resistance of human lung cancer are required to determine the mechanism by which it occurs.

Acknowledgement Many thanks are due to Dr. M. Tabata of Okayama University, Japan, for the development of the Western blot analysis.

References

1. Abe Y, Nakamura M, Ota E, Ozeki Y, Tamai S, Inoue H, Ueyama Y, Ogata T, Tamaoki N (1994) Expression of the multidrug resistance gene (MDR1) in non-small cell lung cancer. Jpn J Cancer Res 85: 536–541
2. Andoh T (1991) Biological functions of DNA topoisomerases (in Japanese). Jpn J Cancer Chemother 18: 1543–1549
3. Beck WT, Danks MK (1991) Mechanisms of resistance to drugs that inhibit DNA topoisomerases. Cancer Biol 2: 235–244
4. Biedler JL (1994) Drug resistance: genotype versus phenotype (32nd G.H.A. Clowes Memorial Award Lecture). Cancer Res 54: 666–678
5. Bordow SB, Haber M, Madafiglio J, Cheung B, Marshall GM, Norris MD (1994) Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-*myc* oncogene in childhood neuroblastoma. Cancer Res 54: 5036–5040

6. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47 : 936–942
7. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258 : 1650–1654
8. Davies SM, Robson CN, Davies SL, Hickson ID (1988) Nuclear topoisomerase II levels correlate with the sensitivity of mammalian cells to intercalating agents and epipodophyllotoxins. J Biol Chem 263 : 17724–17729
9. Deffie AM, Batra JK, Goldenberg GJ (1989) Direct correlation between DNA topoisomerase II activity and cytotoxicity in adriamycin-sensitive and -resistant P388 leukemia cell lines. Cancer Res 49 : 58–62
10. Drake FH, Hofmann GA, Bartus HF, Mattern MR, Crooke ST, Mirabelli CK (1989) Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. Biochemistry 28 : 8154–8160
11. Fry AM, Chresta CM, Davies SM, Walker MC, Harris AL, Hartley JA, Masters JRW, Hickson ID (1991) Relationship between topoisomerse II level and chemosensitivity in human tumor cell lines. Cancer Res 51 : 6592–6595
12. Gekeler V, Frese G, Noller A, Handgretinger R, Wilisch A, Schmidt H, Muller CP, Dopfer R, Klingebiel T, Diddens H, Probst H, Niethammer D (1992) Mdr1/P-glycoprotein, topoisomerase, and glutathion-S-transferase π gene expression in primary and relapsed state adult and childhood leukaemias. Br J Cancer 66 : 507–517
13. Giaccone G, Gazdar AF, Beck H, Zunino F, Capranico G (1992) Multidrug sensitivity phenotype of human lung cancer cells associated with topoisomerase II expression. Cancer Res 52 : 1666–1674
14. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP (1973) In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 51 : 1417–1423
15. Hiraki S, Miyai M, Seto T, Tamura T, Watanabe Y, Ozawa S, Ikeda H, Nakata Y, Ohnoshi T, Kimura I (1982) Establishment of human continuous cell lines from squamous cell, adeno- and small cell carcinoma of the lung and the results of heterotransplantation (in Japanese). Lung Cancer 22 : 53–58
16. Isobe H, Wellham L, Sauerteig A, Sridhar KS, Ramachandran C, Krishan A (1994) Doxorubicin retention and chemoresistance in human mesothelioma cell lines. Int J Cancer 57 : 581–585
17. Itoh H, Kataoka H, Koita H, Nabeshima K, Inoue T, Kangawa K, Koono M (1991) Establishment of a new human cancer cell line secreting protease nexin-II/amyloid β protein precursor derived from squamous-cell carcinoma of lung. Int J Cancer 49 : 436–443
18. Kasahara K, Fujiwara Y, Sugimoto Y, Nishio K, Tamura T, Matsuda T, Saijo N (1992) Determinants of response to the DNA topoisomerase II inhibitors doxorubicin and etoposide in human lung cancer cell lines. J Natl Cancer Inst 84 : 113–118
19. Kaufmann SH, Karp JE, Jones RJ, Miller CB, Schneider E, Zwelling LA, Cowan K, Wendel K, Burke PJ (1994) Topoisomerase II levels and drug sensitivity in adult acute myelogenous leukemia. Blood 83 : 517–530
20. Kim R, Hirabayashi N, Nishiyama M, Saeki S, Toge T, Okada K (1991) Expression of MDR1, GST-π and topoisomerase II as an indicator of clinical response to adriamycin. Anticancer Res 11 : 429–432
21. Kinjo M, Oka K, Naito S, Kohga S, Tanaka K, Oboshi S, Hayata Y, Yasumoto (1979) Thromboplastic and fibrinolytic activities of cultured human cancer cell lines. Br J Cancer 39 : 15–23
22. Kishimoto N (1992) Studies on cell biology and chemotherapy of lung cancer using tissue culture techniques. Part 2. Biological characteristics of five newly established small cell lung cancer cell lines (in Japanese). Okayama-Igakukai-Zasshi 104 : 905–913
23. Lai S, Goldstein LJ, Gottesman MM, Pastan I, Tsai C, Johnson BE, Mulshine JL, Ihde DC, Kayser K, Gazdar AF (1989) MDR1 gene expression in lung cancer. J Natl Cancer Inst 81 : 1144–1150
24. Lee M, Wang JC, Beran M (1992) Two independent amsacrine-resistant human myeloid leukemia cell lines share an identical point mutation in the 170 kDa form of human topoisomerase II. J Mol Biol 223 : 837–843
25. Liu LF (1989) DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem 58 : 351–375
26. Lotem J, Sachs L (1993) Regulation by *bcl*-2, c-*myc*, and p53 of susceptibility to induction of apoptosis by heat shock and cancer chemotherapy compounds in differentiation-competent and -defective myeloid leukemic cells. Cell Growth Differ 4 : 41–47
27. Lowe SW, Ruley HE, Jacks T, Housman DE (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74 : 957–967
28. Mirski SEL, Evans CD, Almquist KC, Slovak ML, Cole SPC (1993) Altered topoisomerase IIα in a drug-resistant small cell lung cancer cell line selected in VP-16. Cancer Res 53 : 4866–4873
29. Muller MT, Helal K, Soisson S, Spitzner JR (1989) A rapid and quantitative microtiter assay for eukaryotic topoisomerase II. Nucleic Acids Res 17 : 9499
30. Potmesil M, Hsiang Y, Liu LF, Bank B, Grossberg H, Kirschenbaum S, Forlenzar TJ, Penziner A, Kanganis D, Knowles D, Traganos F, Silber R (1988) Resistance of human leukemic and normal lymphocytes to drug-induced DNA cleavage and low levels of DNA topoisomerase II. Cancer Res 48 : 3537–3543
31. Schuurhuis GJ, Broxterman HJ, Cervantes A, Heijningen THM van, Lange JHM de, Baak JPA, Pinedo HM, Lankelma J (1989) Quantitative determination of factors contributing to doxorubicin resistance in multidrug-resistant cells. J Natl Cancer Inst 81 : 1887–1892
32. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 48 : 4827–4833
33. Takano H, Kohno K, Ono M, Uchida Y, Kuwano M (1991) Increased phosphorylation of DNA topoisomerase II in etoposide-resistant mutants of human cancer KB cells. Cancer Res 51 : 3951–3957
34. Takano H, Kohno K, Matsuo K, Matsuda T, Kuwano M (1992) DNA topoisomerase-targeting antitumor agents and drug resistance. Anticancer Drugs 3 : 323–330
35. Tanaka S, Aizawa K, Katayanagi N, Tanaka O (1994) Flow cytometric analysis of early steps in development of adriamycin resistance in a human gastric cancer cell line. Jpn J Cancer Res 85 : 86–92
36. Tsuruo T (1988) Mechanisms of multidrug resistance and implications for therapy. Jpn J Cancer Res 79 : 285–296
37. Twentyman PR (1992) MDR1 (P-glycoprotein) gene expression – implications for resistance modifier trials. J Natl Cancer Inst 84 : 1458–1460
38. Woessner RD, Chung TDY, Hofmann GA, Mattern MR, Mirabelli CK, Drake FH, Johnson RK (1990) Differences between normal and *ras*-transformed NIH-3T3 cells in expression of the 170 kD and 180 kD forms of topoisomerase II. Cancer Res 50 : 2901–2908
39. Zaman GJR, Versantvoort CHM, Smit JJM, Eijdems EWHM, Haas M de, Smith AJ, Broxterman HJ, Mulder NH, Vries EGE de, Baas F, Borst P (1993) Analysis of the expression of MRP, the gene for a new putative transmembrane drug transporter, in human multidrug resistant lung cancer cell lines. Cancer Res 53 : 1747–1750
40. Zee AGJ van der, Jong S de, Keith WN, Hollema H, Boonstra H, Vries EGE de (1994) Quantitative and qualitative aspects of topoisomerase I and IIα and β in untreated and platinum/cyclophosphamide treated malignant ovarian tumors. Cancer Res 54 : 749–755