Multidrug Sensitivity Phenotype of Human Lung Cancer Cells Associated with Topoisomerase II Expression

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ABSTRACT

Patterns of drug sensitivities in relation to topoisomerase II gene expression and activity were studied in eight human lung cancer cell lines not selected in vitro for drug resistance. The cytotoxicities of doxorubicin, etoposide, teniposide, cisplatin, camptothecin, and 5-fluorouracil were measured and, remarkably, these unselected cell lines were shown to have a common pattern of multidrug sensitivity, i.e., a multidrug sensitivity phenotype. In fact, drug sensitivities were significantly correlated with each other in the studied cell lines, the correlation being best for the topoisomerase II-targeted agents and cisplatin, less strong with camptothecin, and weak with 5-fluorouracil. Almost 1-log range difference of topoisomerase II gene expression was found in these cell lines, and this was not explained by the cell-doubling time or cell cycle distribution. The level of topoisomerase II gene expression was positively and highly correlated with the cell sensitivity to epidophyllotoxins, doxorubicin, and cisplatin in seven cell lines. Although weak, an association was also observed between topoisomerase II gene expression and camptothecin cytotoxicity, while no association was observed with 5-fluorouracil. However, a non-small cell lung cancer cell line with neuroendocrine properties had very low levels of expression of the topoisomerase II gene, despite being highly sensitive to all drugs tested. The levels of topoisomerase II gene expression were not found to be correlated with the cytotoxicity of any drug tested. A specific enzymatic activity assay and a teniposide-stimulated DNA cleavage assay showed that the extent of active topoisomerase II present in nuclear extracts paralleled the level of topoisomerase II gene expression. Furthermore, in addition to the normal transcript, an abnormally sized topoisomerase II message and a rearrangement of the topoisomerase II gene were detected in a poorly sensitive small cell lung cancer cell line. Therefore, low levels of topoisomerase II gene expression, and possibly mutations, may predict a reduced sensitivity of unselected human lung cancer cell lines to several drugs, including agents with a cellular target other than topoisomerase II. It is hypothesized that topoisomerase II might be involved in a common pathway of cell death induced by drugs in tumor cell lines which present a multidrug sensitivity phenotype.

INTRODUCTION

The failure of chemotherapy in human lung cancers is thought to be mainly due to the development of cellular drug resistance either existing prior to or arising during treatment (1). The overexpression of the P glycoprotein, encoded by the MDR1 gene, has been studied as a mechanism of drug resistance (2). However, a common observation in patients with lung cancer is that clinical resistance is much broader than that observed in cell lines selected to be resistant in vitro (1). In particular, SCLC, which is initially highly responsive to chemotherapy, is virtually resistant to most drugs tested in second-line chemotherapy (1). Recently, an analogous observation came from an in vitro chemosensitivity study of a panel of unselected human lung cancer cell lines, in which the cytotoxicities of most, if not all, drugs tested were tightly correlated with one another (18). Although, the molecular basis for such a broad cellular sensitivity/resistance is not understood yet, this observation may suggest that unselected lung cancer cell lines could provide a cellular model of drug sensitivity/resistance more representative of human lung cancers than cell lines selected to be resistant in vitro.

A second mechanism of drug resistance has been suggested to involve DNA topoisomerase II, which is the target of several compounds, including doxorubicin and VP-16 (4–6), widely used active drugs in human lung cancer (1). Topoisomerase II is a nuclear enzyme involved in important steps of DNA metabolism, and the topoisomerase poisons stabilize the cleavable enzyme-DNA complexes, detectable as DNA strand breaks (4, 7). Topoisomerase II-mediated cellular effects were shown to be the basis of the drug sensitivity in drug-selected SCLC cell lines and two human SCLC xenografts (8–13). Indeed, a 2- to 4-fold reduction of topoisomerase II activity and a markedly decreased drug-induced topoisomerase II-mediated DNA cleavage were reported in several drug-resistant cultured cells, including human SCLC lines (9, 14–17). These results indicated that the topoisomerase II content in tumor cells might be a crucial determinant for drug cytotoxic activity.

A comprehensive understanding of drug resistance has come in the past two decades from the study of cell lines selected for resistance by continuous in vitro drug exposures, and patterns of cross-resistance between drugs structurally unrelated were discovered (2). However, a common observation in patients with lung cancer is that clinical resistance is much broader than that observed in cell lines selected to be resistant in vitro (1). In particular, SCLC, which is initially highly responsive to chemotherapy, is virtually resistant to most drugs tested in second-line chemotherapy (1). Recently, an analogous observation came from an in vitro chemosensitivity study of a panel of unselected human lung cancer cell lines, in which the cytotoxicities of most, if not all, drugs tested were tightly correlated with one another (18). Although, the molecular basis for such a broad cellular sensitivity/resistance is not understood yet, this observation may suggest that unselected lung cancer cell lines could provide a cellular model of drug sensitivity/resistance more representative of human lung cancers than cell lines selected to be resistant in vitro.

Because DNA topoisomerase II might play a critical role in the drug sensitivity/resistance of human lung cancer lines (8–13), in this study topoisomerase II gene expression and activity were studied in relation to drug cytotoxicities in a panel of eight human lung cancer cell lines. Cell sensitivities to three topoisomerase II-targeted drugs (doxorubicin, VP-16, and VM-26) and to three drugs having cellular targets other than topoisomerase II (cisplatin, camptothecin, and 5-FU) were investigated. The cell lines were not selected in vitro for drug resistance but, nevertheless, showed marked differences in drug sensitivity, thus allowing us to investigate the molecular basis of the capacity of a drug to kill cells and resistance of the cell in a system probably closer to the human tumors (1, 18).

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* The abbreviations used are: VP-16, etoposide; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; PBS, phosphate-buffered saline; VM-26, teniposide; 5-FU, 5-fluorouracil; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GADPH, glyceraldehyde-3-phosphate dehydrogenase; IC50, drug concentration that inhibited 50% of cell growth; cDNA, complementary DNA; MSP, multidrug sensitivity phenotype.
MATERIALS AND METHODS

Cell Lines and Cytotoxicity Assay. The origin and maintenance of the human lung cancer cell lines studied have been previously described (19, 20). All cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C and 7% CO2. Drug cytotoxicity on exponentially growing cells was determined by the semiautomated MTT assay, as described previously (18). Drug treatments were for 4 days. VP-16, VM-26, cisplatin (Bristol-Myers, Syracuse, NY), and doxorubicin (Adria Laboratories, Columbus, OH) were used as formulated for clinical use; camptothecin (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethyl sulfoxide and 5-FU (Sigma) was dissolved in PBS. Following drug incubation, 0.1 mg/well of MTT (Sigma) was added, and the plates were incubated for an additional 4 h. Plates were then centrifuged, supernatant fluids were removed, and formazan crystals were dissolved in 150 μl dimethyl sulfoxide (Sigma). Absorbance at 570 nm was added, and the plates were incubated for an additional 4 h. Plates were then centrifuged, supernatant fluids were removed, and formazan crystals were dissolved in 150 μl dimethyl sulfoxide (Sigma). Absorbance at 570 nm was measured using a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) at a wavelength of 540 nm. The IC50 values relative to untreated control cells were the means of three or more independent experiments, each performed in replicates of eight.

Flow Cytometry. Exponentially growing cells were harvested, washed twice with PBS, and centrifuged at room temperature. Cells were rapidly fixed with cold 70% ethanol by vigorous vortexing. Cells were maintained at −20°C until stained for DNA with propidium iodide (21). Cell analysis was performed using a cytofluorograph 50H (Ortho Instruments, Inc., Winnocki, VT) at a wavelength of 540 nm. The IC50 values relative to untreated control cells were the means of three or more independent experiments, each performed in replicates of eight.

Preparation of Nuclear Extracts and DNA-unknotting Assay. Nuclear proteins were extracted from exponentially growing cells by cell lysis with guanidine isothiocyanate and centrifugation in a cesium chloride gradient (22). Total RNA (10 μg) was electrophoresed in a 1% agarose-formaldehyde gel and then transferred to a Nytran membrane (Schleicher and Schuell, Keene, NH). Prehybridization and hybridization conditions were as suggested by the supplier. A 1.8-kilobase human p710 topoisomerase II cDNA fragment (Z11-1.8), cloned into the EcoRI site of Bluescript SK(+) (23), and a 0.7-kilobase human topoisomerase I cDNA fragment, cloned into the EcoRI site of pGEM-4 (24), were kindly provided by Dr. Leroy Liu (Baltimore, MD). A 1.3-kilobase fragment of rat GAPDH cDNA, cloned into the PstI site of a PSP vector, was kindly provided by Dr. Carol Thiele (Bethesda, MD) (25). The probes were 32P labeled with a random primer kit (Bethesda Research Laboratory, Rockville, MD). The final wash of Northern blots was at 62°C for 40 min in 0.1x SSPE (buffer 20x: NaCl, 3 mM; K2HPO4, 0.2 mM; EDTA, 0.02 mM)-0.1% SDS. DNA (10 μg) was digested with the indicated restriction enzymes and then run on a 0.8% agarose gel. Normal human liver or spleen DNA was used as a control. The DNA was then transferred to a nitrocellulose membrane. Hybridization was conducted with 32P-labeled cDNA probes. The final wash of Northern blots was at 60°C for 40 min in 0.2x SSC (buffer 20x: NaCl, 3 mM; Na2citrate, 2H2O, 0.3 mM)-0.1% SDS.

Densitometry of Northern blots was performed to determine the levels of topoisomerase gene expression which were normalized for RNA loading by dividing the absorbance of the topoisomerase transcript by that of the GAPDH gene. Correlations between the IC50 values and the level of expression of the topoisomease I and II genes were performed using the Pearson product-moment test.

RESULTS

Characteristics of the Studied Human Lung Cancer Cell Lines. The biological properties of the human lung cancer cell lines studied are summarized in Table 1. They consisted of three NSCLC and five SCLC cell lines. Only two cell lines were derived from patients who had received chemotherapy. The NCI-H69 line was derived from an SCLC patient treated with cyclophosphamide, methotrexate, and lomustine, alternating with vincristine, doxorubicin, and procarbazine (18). The NCI-H322 line was derived from a patient with a bronchioloalveolar carcinoma developed 3 years after the apparent successful treatment of an SCLC with cyclophosphamide, methotrexate, and lomustine, alternating with vincristine, doxorubicin, and procarbazine (18). Doubling times of exponentially growing cells ranged from 20 to 72 h, and the cell distribution through the cell cycle was rather similar in all the studied cell lines (Table 1). These data may suggest that doubling time differences were likely due to a slower progression of cells through the cycle rather than differences in the length of a particular cell cycle phase.

Correlations among Drug Sensitivities of the Eight Cell Lines. Three topoisomerase II inhibitors (VP-16, VM-26, and doxorubicin), a topoisomerase I inhibitor (camptothecin), an anti-metabolite (5-FU), and cisplatin were tested for their cell-killing
effects on these cell lines during 4 day of treatment. Although NSCLC cell lines were expected to be less sensitive to drugs than SCLC cell lines (18), the cell lines studied were selected for their extreme drug sensitivities; therefore, the difference between the histological types could not be appreciated. Marked differences in drug sensitivities were observed; the largest were for VP-16 and camptothecin (Table 2). Camptothecin was generally the most active drug, while 5-FU, cisplatin, and VP-16 were the least active ones. No clear association between doubling times and cytotoxicity was seen (Tables 1 and 2).

NCI-H69, NCI-H322, and NCI-N417 were, in general, the most resistant cell lines to the topoisomerase II-targeted drugs and cisplatin, while NCI-H522 and NCI-H69 were the most resistant cell lines to camptothecin and 5-FU, respectively (Table 2). Moreover, the patterns of drug sensitivities were analyzed, and the results indicated striking correlations among drug cytotoxicities (Fig. 1). The best correlation was found between the epipodophyllotoxins VP-16 and VM-26, but, in addition, the cell sensitivity to epipodophyllotoxins was correlated with the cell sensitivity to doxorubicin and cisplatin (Fig. 1A; Pearson product-moment correlation values of 0.87–0.98, P2 <0.001–0.005). Cell sensitivity to camptothecin showed a lower degree of association, but still significant, with cell sensitivities to cisplatin and VP-16 (Fig. 1B), VM-26, and doxorubicin (not shown) (Pearson product-moment correlation values >0.78). This result was unexpected because the mechanisms of action of doxorubicin and epipodophyllotoxins are different from those of cisplatin and camptothecin. The cytotoxicity of 5-FU was only weakly associated with the cytotoxicity of VP-16 and cisplatin (Fig. 1B) or any other drug (not shown) (Pearson product-moment correlation values <0.59). It is noteworthy that NCI-H460 and NCI-H187 were the most sensitive cell lines to all the drugs tested, including 5-FU (Table 2).

Expression of Topoisomerase II and I Genes. The expression of topoisomerase II and I genes was analyzed in exponentially proliferating cells (Fig. 2). Differences in topoisomerase II expression may not be explained by different fractions of cells in G2-M and S phases or doubling times, although NCI-H322 and NCI-H69 were the most slowly growing cell lines and had the lowest level of expression. The topoisomerase II and I transcripts were detected in all the studied cell lines and, as expected (23, 24), their sizes were 6.2 and 4 kilobases, respectively. However, the cell line NCI-H69 expressed a topoisomerase II transcript of 7.4 kilobases in addition to the normal 4-kilobase transcript (Fig. 2). This higher molecular weight transcript was about one-eighth the intensity of the normal 4-kilobase transcript. Moreover, the variability of the expression of the topoisomerase II gene was higher than that of the topoisomerase I gene among these cell lines (Table 3). The ratio of topoisomerase II gene expression levels between the cell line NCI-H187, which showed the highest level, and NCI-H322, which showed the lowest one, was about 8.1. In the case of the topoisomerase I gene, the ratio between the expression levels of the extreme cell lines (NCI-H322 and NCI-H460) was 3.2 (Table 3). The levels of expression of the two topoisomerase genes were not different between SCLC and NSCLC cell lines (Tables 1 and 3); however, this result cannot be conclusive because the studied cell lines were not representative of the general sensitivity of their histological types since they were selected based on their very different drug sensitivities.

Association between Cell Sensitivity to Drugs and Topoisomerase II Expression. The relationships between drug cytotoxic activity and the level of topoisomerase gene expression were analyzed. With the important exception of one cell line, NCI-H460, cell sensitivity to doxorubicin, VP-16, VM-26, and cisplatin was positively and highly correlated with the level of expression of topoisomerase II. Surprisingly, the NCI-H460 cell line presented the distinct property of having a remarkably low level of topoisomerase II expression associated with a high drug sensitivity (Tables 2 and 3). When this cell line was excluded from the analysis, the correlation became highly significant for all of these four drugs, being strongest for the epipodophyllotoxins (Fig. 3). The Pearson product-moment correlation values were −0.92, −0.97, −0.87, and −0.78 for VP-16, VM-26, doxorubicin, and cisplatin, respectively (P2 <0.0001–0.038). Strikingly, an association between topoisomerase II expression and camptothecin was also observed, but it was weaker (correlation value, −0.51). On the other hand, a clear correlation was not observed between cell sensitivity to 5-FU and topoisomerase II expression levels (not shown). Moreover, there was no correlation between cell sensitivity to any drug and the level of topoisomerase I gene expression, even by excluding NCI-H460 or any other cell line from the analysis (Fig. 3 and data not shown). Although the range of levels of topoisomerase I expression was narrow, it has to be noted that the most resistant line to camptothecin had a high level of topoisomerase I expression (Tables 2 and 3).

Topoisomerase II Activities in Nuclear Extracts. In order to further confirm the correlation observed between topoisomerase II expression levels and drug cytotoxicity, the topoisomerase II activity present in nuclear extracts of the studied cell lines was determined by using the P4 DNA-unknotting assay and a drug-stimulated DNA fragmentation test (Table 4). The differences in the P4-unknotting activity were modest among the studied cell lines. Although the strand-passing activity assay does not discriminate between different isoforms of topoisomerase.
Fig. 1. Each point, one cell line. A, correlation of IC₅₀ values of doxorubicin, VP-16, VM-26, and cisplatin. Cytotoxicity experiments were performed using the MTT assay and drug treatments were for 4 days, as described in "Materials and Methods." The values for all four drugs were strongly correlated with each other (Pearson product-moment correlation values, 0.87-0.98; P2 < 0.001-0.005). B, correlation of IC₅₀ values of camptothecin and 5-FU with cisplatin and VP-16. Correlation was observed with camptothecin (correlation values, >0.78) but not with 5-FU (correlation values, <0.59).
TOPOISOMERASE II AND DRUG SENSITIVITY IN HUMAN LUNG CANCER CELLS

Table 3 Relative levels of expression of topoisomerase I and II genes

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Topoisomerase I</th>
<th>Topoisomerase II</th>
</tr>
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<tbody>
<tr>
<td>NCI-H460</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NCI-H187</td>
<td>2.2</td>
<td>6.6</td>
</tr>
<tr>
<td>NCI-H209</td>
<td>1.1</td>
<td>4.8</td>
</tr>
<tr>
<td>NCI-H522</td>
<td>2.8</td>
<td>4.4</td>
</tr>
<tr>
<td>NCI-H1284</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>NCI-N417</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>NCI-H322</td>
<td>3.2</td>
<td>0.8</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>1.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

While NCI-H322, NCI-H522, and NCI-N417 cell lines showed was compared to that present in the NCI-H187 cell line. NCI-4). The topoisomerase II DNA-cleaving activity in each cell line cleavage was topoisomerase II mediated. The VM-26-induced activity, even at the highest VM-26 concentration used (Fig. 4), indicating that the observed DNA addition of VM-26 (Fig. 4), showing normal and abnormally sized transcripts, have been calculated only for the normally sized message.

For the DNA cleavage assay, VM-26 was used because it is one of the most active and specific compounds in inducing topoisomerase II-mediated DNA cleavage (4, 5). The extent of the cleavage of the plasmid DNA in the presence of a fixed amount of nuclear proteins (2 µg) was greatly increased by the addition of VM-26 (Fig. 4), indicating that the observed DNA cleavage was topoisomerase II mediated. The VM-26-induced DNA cleavage patterns were very similar among cell lines (Fig. 4). The topoisomerase II DNA-cleaving activity in each cell line was compared to that present in the NCI-H187 cell line. NCI-H209 and NCI-H1284 cell lines showed a lower DNA-cleaving activity than the NCI-H187 cell line (Fig. 4A, Table 4). In contrast, the NCI-H69 and the drug-sensitive NCI-H460 cell lines showed very little or no cleavage activity, even at the highest VM-26 concentration used (Fig. 4A, Table 4). Thus, the VM-26-stimulated topoisomerase II-mediated DNA-cleaving activity present in 0.35 M NaCl nuclear extracts matched the level of topoisomerase II gene expression.

Topoisomerase Gene Rearrangements. DNA restriction patterns of the topoisomerase II and I genes were also analyzed in all eight cell lines with at least three restriction enzymes. The topoisomerase II gene copy number was not increased in these cell lines as compared to normal human DNA (Fig. 5 and not shown), suggesting that the observed differences in gene expression were likely due to the regulation of the transcription and/or the translation processes. Furthermore, the cell line NCI-H69 showed topoisomerase II DNA restriction patterns differ-

Fig. 2. Topoisomerase II and I gene expressions in the studied human lung cancer cell lines. Lane A, NCI-N417; lane B, NCI-H187; lane C, NCI-H69; lane D, NCI-H522; lane E, NCI-H322; lane F, NCI-H460; lane G, NCI-H209; lane H, NCI-H1284. Total RNA (10 µg) was fractionated in a 1% agarose-formaldehyde gel, transferred to a nylon filter, and hybridized to a 32P-labeled topoisomerase II (TOPO II) probe. The same filter was stripped and sequentially rehybridized with topoisomerase I (TOPO I) and GAPDH probes. Kb, kilobases.

Fig. 3. Each point, one cell line. Regression analysis between the logarithms of IC50 values for VP-16 and doxorubicin (A) and for VM-26 and cisplatin (B) and the expression level of the topoisomerase II (top) and topoisomerase I (bottom) genes. The regression lines are drawn after exclusion of the NCI-H460 cell line data (datum point in the left lower corner of each graph) for topoisomerase II. Correlations including NCI-H460 are all nonsignificant, although of borderline significance for VM-26 (r = -0.655, P2 = 0.08). Correlations excluding NCI-H460 are all significant (r and P2, respectively: -0.92, 0.003 for VP-16; -0.97, <0.0001 for VM-26; -0.87, 0.01 for doxorubicin; -0.78, 0.038 for cisplatin). The regression lines for topoisomerase I include all eight cell lines (no improvement was found by excluding any cell line).
of at least two independent experiments: —¿, no activity observed; nd, not done.

amount able to unknot completely 0.2 fig of knotted P4 DNA under the conditions described in “Materials and Methods.” The values reported represent the mean

ent from the normal DNA and the other cell lines (Fig. 5 and not shown). This result, together with the presence of the abnormal topoisomerase II message (Fig. 2) suggested the presence of a rearrangement of the topoisomerase II gene in the NCI-H69 cell line. The DNA analysis with the human topoisomerase I probe revealed a normal pattern in seven cell lines, while the cell line NCI-H322 had an additional band of higher molecular weight only in the BamH1 digest (not shown). Because the additional band was observed only with one restriction enzyme, it may represent a polymorphism rather than a rearrangement of the topoisomerase II gene.

In order to determine the frequency of the mutational events of the topoisomerase II gene in human lung cancer cell lines, the DNAs and total RNAs from an additional 15 human lung cancer cell lines were tested. No other cell line had abnormal RNA transcripts (not shown), and only one cell line, NCI-H661, exhibited abnormal DNA restriction patterns (Fig. 5 and not shown). The cell line NCI-H661 is a large cell lung carcinoma and was derived from a patient treated with cisplatin and vinblastine. The DNA modifications observed in the case of NCI-H661 were much less striking than in NCI-H69 (Fig. 5), although they were consistently found in several DNA digests. The study of RNAs of 22 additional human lung cancer cell lines and DNAs of 14 of them did not show any topoisomerase II gene alteration (not shown).

**DISCUSSION**

Unlike what is generally seen in *in vitro* selected drug-resistant cells, the human lung cancer cell lines studied were either relatively sensitive or resistant to the tested drugs. Therefore, these unselected human lung cancer cell lines presented a previously unrecognized MSP. This MSP is characterized by being either sensitive or resistant at the same time to a wide range of drugs, including topoisomerase II- and I-targeted drugs, cisplatin, and, to a lesser extent, 5-FU. Noticeably, NCI-H460 and NCI-H187 were the most sensitive cell lines to all these drugs. Indeed, striking associations among cytotoxic effects of many unrelated drugs have been described in a larger panel of human lung cancer cell lines (18). A tight correlation of cytotoxicities was particularly noted between cisplatin and VP-16 in long-term SCLC cell lines derived from untreated patients (Fig. 4 in Ref. 27). Moreover, in SCLC patients with extensive disease, to whom the best *in vitro* regimen was given based on short- and intermediate-term culture results, the clinical response to cisplatin-VP-16 combination chemotherapy was associated not only with *in vitro* sensitivity to VP-16 but also with those to nitrogen mustard, doxorubicin, and vincristine (28). Thus, the MSP might include drugs with very different mechanisms of action. It is noteworthy that Volm *et al.* (29) successfully used *in vitro* sensitivity to doxorubicin to predict survival in stage III adenocarcinoma patients who received several different chemotherapy regimens after resection. It has to be pointed out that the MSP of unselected human lung cancer cell lines corresponds to the common clinical observation that untreated SCLC is responsive to most active drugs, while relapsed SCLC and newly diagnosed NSCLC patients are highly refractory to many active drugs (1).

The molecular basis of MSP is not yet known. However, the significant correlation of sensitivity/resistance between many unrelated drugs in human lung cancer cell lines may suggest that different drug actions might ultimately lead to a common final pathway of cell death (30, 31). A common cellular effect of many cytotoxic agents is a G2 arrest; prolongation of this phase may permit repair of damaged DNA before entering mitosis, but a much longer G2 phase in the presence of high levels of DNA damage may lead to cell death (32). Therefore, cell sensitivity or resistance to a certain drug can be determined by molecular events or alterations at the level of the specific target of the drug and at the level of the final common pathway of cell death. The observation of the MSP in unselected human tumor cell lines might suggest that a low degree of drug sensitivity in these cells could be due to altered regulations of the drug-induced final common pathway of cell death. Interestingly, the correlation between topoisomerase II gene expression and cytotoxicity was observed not only for doxorubicin, VP-16, and VM-26, which are topoisomerase II inhibitors (4, 7), but also for cisplatin. Furthermore, topoisomerase II expression levels were associated also with campothecin cytotoxicity, although to a lesser extent, probably because the cytotoxicity of campothecin was less strongly associated with the cytotoxicity of the other drugs. In addition, no association at all was observed with 5-FU cytotoxicity. Because topoisomerase II expression was associated with the MSP, one might speculate that topoisomerase II could be involved in critical steps, as yet unknown, of nuclear metabolism during the process of cell death.

A role has been suggested for topoisomerase II in DNA repair mechanisms (33, 34). Human cell lines resistant to nitrogen mustard expressed higher levels of topoisomerase II than parent cells, were hypersensitive to topoisomerase II-targeted drugs, and showed increased DNA interstrand crosslink repair (35, 36); moreover, recent work suggested that topoisomerase II may participate in the repair of DNA strand breaks induced by both drugs and radiation (37, 38). In contrast to the studies of the nitrogen mustard-resistant cell lines, among our cell lines we observed higher levels of topoisomerase II expression in the more chemosensitive cell lines. If topoisomerase II were a determinant enzyme for the DNA repair process in some instances, the observed correlation between gene expression and cytotoxicity would be weak or unexpected. Because we found that this correlation was less strong in the case of cisplatin, topoisomerase II might have an important role in different metabolic pathways and the relative importance might depend on the specific cell type being analyzed. It would then be also interesting to evaluate the DNA damage repair capacity of these cell lines.

In this study, we used a CDNA probe which can detect only the topoisomerase II p170 isoform (23). The level of topoisomerase II gene expression predicted the cell sensitivity to drugs
in seven of eight cell lines, indicating that low expression levels of topoisomerase II may be the basis of a reduced cell sensitivity to drugs in unselected human lung cancer cell lines. These findings were supported by the results of the topoisomerase II strand-passing and VM-26-induced DNA-cleaving activities present in nuclear extracts. Although we did not determine the protein content, the amount of catalytically active and VM-26-sensitive topoisomerase II was evaluated. The expression of topoisomerase II p170 gene paralleled the topoisomerase II-dependent action of VM-26, as measured by the DNA cleavage assay. This is interesting, because the study of these topoisomerase II activities in nuclear extracts might not reflect so accu-

Fig. 4. Topoisomerase II-mediated DNA cleavage activity in the nuclear extracts of human lung cancer cell lines. Linear pBR322 DNA (5 ng) was incubated with nuclear extract proteins (2 μg) in the presence of the indicated concentrations of VM-26 for 30 min at 37°C. Reactions were stopped with SDS and proteinase K, and DNA was fractioned with a 0.8% agarose gel electrophoresis, transferred to a nitrocellulose membrane, and hybridized with pBR322 32P-labeled DNA. NCI-H187 was kept as a reference cell line in the different experiments. bp, base pair.
some faint additional bands of low molecular weight can be seen in all three NCI-H69, in addition to the bands visible in the normal pattern, bands of higher molecular weight are in NCI-H661 human lung cancer cell lines and normal human spleen (lane N). For digests, while the minor band (approximately 6 kilobases (Kb)), visible in molecular weight band is visible in BamH1 digests (not shown). For NCI-H661, some faint additional bands of low molecular weight can be seen in all three digests, while the minor EcoRI band (approximately 6 kilobases (Kb)), visible in normal DNA, is not visible.

Fig. 5. DNA restriction patterns of the topoisomerase II gene in NCI-H69 and NCI-H661 human lung cancer cell lines and normal human spleen (lane N). For NCI-H661, in addition to the bands visible in the normal pattern, bands of higher molecular weight are seen in PstI and BglII restriction patterns. Similarly, a higher molecular weight band is visible in BamHI digests (not shown). For NCI-H661, some faint additional bands of low molecular weight can be seen in all three digests, while the minor EcoRI band (approximately 6 kilobases (Kb)), visible in normal DNA, is not visible.

rately the gene expression profile, since the extractable topoisomerase II can consist of different enzyme isoforms, which may play different roles in drug sensitivity (26). Indeed, the absence of marked differences of P4-unknotting activity among cell lines might depend on the presence of different enzyme isoforms.

The present findings are consistent with previous studies of the enzyme activity in nuclear extracts (9) and drug-induced DNA cleavage (15, 16) in SCLC cells, supporting an important role of topoisomerase II in the drug sensitivity of tumor cells. Furthermore, a very low content of topoisomerase II has been described in human chronic lymphocytic leukemia cells, which are poorly sensitive to doxorubicin, other topoisomerase II inhibitors, and other cytotoxic drugs, in contrast to some more undifferentiated lymphoproliferative disorders (39). Because topoisomerase II gene amplification was not observed in our system, these studies indicate that the regulation of the expression of the topoisomerase II gene, and, possibly, posttranslational modifications of the protein, may be relevant for drug activity in human cancers, including lung and hematological malignancies. Altogether, these results suggest that topoisomerase II can be a marker of chemosensitivity of tumor cell lines and human cancers.

Strikingly, the NCI-H460 cell line, a NSCLC with neuroendocrine properties, was highly drug sensitive and the most rapidly proliferating cell line; nevertheless, its topoisomerase II gene expression level was very low, and the level of topoisomerase I was the lowest. Topoisomerase II strand-passing activity was undetectable and VM-26 did not induce appreciable DNA cleavage in nuclear extracts of these cells. Moreover, as in other NSCLC cell lines with neuroendocrine properties, which are mainly sensitive to chemotherapy (40), NCI-H460 cells had appreciable levels of expression of the MDR1 gene, whereas most SCLC and NSCLC do not express this gene (3). The chemosensitivity of NSCLC with neuroendocrine properties has also been reported in patients (41). Therefore, the NCI-H460 cell line may represent a relevant exception to the common finding of drug sensitivity corresponding directly to target enzyme level or inversely related to P-glycoprotein content. Although more studies are needed to understand the molecular basis of the remarkable drug sensitivity of NSCLCs with neuroendocrine properties, one might suspect that in this cell line the cellular response to drug-induced damage may be enhanced; thus, a low level of primary lesions might still result in cell death. The level of correlation between topoisomerase II expression and cytotoxic drug response of NCI-H460 emphasizes a role of multiple cellular factors in determining cell chemosensitivity. They may include DNA damage repair capacity and cell responses to primary DNA lesions.

In our study, we did not find any correlation between camptothecin activity and topoisomerase I expression. The narrow range of the levels of topoisomerase I expression contrast with the wide range (3-log difference) in sensitivity to camptothecin observed in these cell lines. Our results do not parallel the finding of lower levels of gene expression and catalytic activity of topoisomerase I in cell lines selected in vitro for resistance to camptothecin (42, 43). Specific topoisomerase I gene rearrangements and hypermethylation of the topoisomerase I gene have also been described during selection of P388 cells with camptothecin (42, 43) and have been suggested as responsible for the reduced expression and activity. No rearrangement of the topoisomerase I gene was observed in our study. Further studies are necessary to better understand the role of topoisomerase I in drug sensitivity of unselected tumor cells.

Mutations of the topoisomerase genes have been proposed as a possible mechanism of drug resistance in model tumor lines (14, 16, 43). However, the role of a possible mutation of the topoisomerase II gene which leads to a mutated enzyme and cellular resistance to topoisomerase II inhibitors remains to be established in unselected human tumor cells (16). In our study, one cell line (NCI-H69) expressed an abnormal topoisomerase II transcript and probably had a grossly rearranged allele of the topoisomerase II gene. One other cell line (NCI-H661) showed abnormal DNA restriction patterns for the topoisomerase II gene. The patients from whom both of these cell lines were derived had been treated with chemotherapy, and cell lines were poorly sensitive to drugs (18). Tumor cell exposure to drugs which act on DNA might have induced the mutations in these cells, although it remains to be elucidated whether these gene mutations may be important for cell sensitivity to drugs. However, the general relevance of this phenomenon remains uncertain, because topoisomerase II gene rearrangements were infrequent events among the human lung cancer cell lines tested.

REFERENCES

TOPOISOMERASE II AND DRUG SENSITIVITY IN HUMAN LUNG CANCER CELLS


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