Expression of P-gp, MRP, LRP, GST-π and TopoIIα and intrinsic resistance in human lung cancer cell lines

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Abstract. This study aimed to determine the relationship between the endogenous levels of P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), lung resistance-related protein (LRP), glutathione-s-transferase-π (GST-π) and topoisomerase IIα (TopoIIα) and intrinsic drug resistance in four human lung cancer cell lines, SK-MES-1, SPCA-1, NCI-H-460 and NCI-H-446, of different histological types. The expression of P-gp, MRP, LRP, GST-π and TopoIIα was measured by immunofluorescence, Western blotting and RT-PCR. Drug resistance to cisplatin, doxorubicin and VP-16 was determined using MTT assays. The correlation between expression of the resistance-related proteins and their roles in the resistance to drugs in these cancer cell lines was analyzed. We found that the endogenous levels of P-gp, MRP, LRP, GST-π and TopoIIα in the four cell lines varied. The level of GST-π in the SK-MES-1 cells was the highest, whereas the level of P-gp in the SPCA-1 cells was the lowest. The chemoresistance to cisplatin, doxorubicin and VP-16 in the four cell lines was different. The SPCA-1 cell line was most resistance to cisplatin; SK-MES-1 was most resistance to VP-16; whereas SK-MES-1 was most sensitive to doxorubicin. There was a positive correlation between GST-π expression and resistance to cisplatin, between TopoIIα expression and resistance to VP-16; and a negative correlation was noted between TopoIIα expression and resistance to doxorubicin. In summary, the endogenous expression of P-gp, MRP, LRP, GST-π and TopoIIα was different in the four human lung cancer cell lines of different histological types, and this variance may be associated with the variation in chemosensitivity to cisplatin, doxorubicin and VP-16. Among the related proteins, GST-π may be useful for the prediction of the intrinsic resistance to cisplatin, whereas TopoIIα may be useful to predict resistance to doxorubicin and VP-16 in human lung cancer cell lines.

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Introduction

Lung cancer is one of the most prevalent cancers in the world, and 1.2 million new cases are diagnosed annually and globally. Thus, it remains the leading cause of cancer-related death (1,2). Approximately 65% of lung cancer patients present with inoperable advanced-stage (IIIB/IV) disease at diagnosis (3). Therefore, chemotherapy plays an important role in the treatment of lung cancer. However, the efficacy of chemotherapy is not satisfactory due to the existence of drug resistance, particularly intrinsic resistance, which is frequently observed in both non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) (4,5).

Intrinsic resistance is the unresponsiveness of tumor cells to anticancer drugs from the beginning of treatment (6). It is related to many factors including the hosts themselves and the environment around the tumor. In the past decade, a number of studies on intrinsic chemotherapy resistance have focused on various transporter proteins inside tumor cells. Of these proteins, P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), lung resistance-related protein (LRP), glutathione-s-transferase-π (GST-π) and topoisomerase IIα (TopoIIα) have gained considerable attention. These proteins are involved in chemotherapeutical resistance via many mechanisms, including the increase in drug efflux, the decrease in drug influx, drug inactivation and alterations in the drug target (7).

P-gp and MRP, two important ATP binding cassette transporter proteins, affect the intracellular drug concentration

through the alteration of drug influx or efflux. The expression of these proteins has been demonstrated to correlate positively with the chemosensitivity of drugs such as anthracyclines and paclitaxel (8). LRP, the main component of vaults, was identified as a major vault protein (MVP). It mediates drug resistance by pumping drugs away from intracellular drug targets through exocytotic vesicles or pump molecules (9,10). GST-π, a member of the glutathione-s-transferase (GST) family of enzymes catalyzes the conjugation process between glutathione (GSH) and anticancer drugs, and then expels them from cells by the GSH-conjugate export pump (11-13). TopoIIα is the primary target for anticancer drugs such as anthracyclines, epipodophy and amsacrine. Drug resistance to TopoII occurs when the activity and sensitivity of the target enzyme TopoII are decreased by down-regulation or mutation (14).

Many reports have shown that P-gp, MRP, LRP, GST-π and TopoIIα play important roles in the intrinsic or acquired resistance of lung cancer. Expression of P-gp and/or MRP-1 has been found to contribute to the chemoresistant nature of NSCLC (15). Endogenous levels of both P-gp and LRP were found to be much higher in A549 than in NCI-H-460 cells (16). Arai *et al* stated that GST-π is associated with cisplatin and etoposide combination chemotherapy in NSCLC tissues (17). Guinee *et al* found that expression of TopoIIα in SCLC was significantly higher than that in NSCLC, and the expression of TopoIIα was positively correlated with the chemosensitivity to doxorubicin and etoposide (18). However, most of these studies mainly focused on one or two of these five drug resistance-related proteins; few reports involved all of the proteins, much less the comparison among them in different histological types of human lung cancer cell lines. Since chemotherapeutical resistance is a complicated process, which may be regulated by multiple resistance-related proteins, our study aimed to determine the relationship between the endogenous levels of the drug resistance-related proteins, P-gp, MRP, LRP, GST-π and TopoIIα, and intrinsic drug resistance to cisplatin, doxorubicin and VP-16 in four human lung cancer cell lines SK-MES-1, SPCA-1, NCI-H-460 and NCI-H-446 of different histological types.

Materials and methods

Cell lines and culture conditions. Four human lung cancer lines of different histological types, SK-MES-1 (squamous-cell carcinoma), SPCA-1 (adenocarcinoma), NCI-H-460 (large-cell carcinoma) and NCI-H-446 (small-cell carcinoma), were obtained from the American Type Culture Collection (Manassas, VA, USA). SK-MES-1 was cultured in MEM medium (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS and 0.11 g/l sodium pyruvate, and the other three cell lines, SPCA-1, NCI-H-460 and NCI-H-446, were cultured in RPMI-1640 medium (HyClone, Logan, UT, USA) supplemented with 10% FBS, at 37˚C in a humidified atmosphere and 5% CO_2. The medium was routinely changed 2-3 days after seeding. All experiments were performed 3 times.

Immunofluorescence. Cells in the exponential phase were dispensed onto the cover glass in 6-well plates at a density of $1x10^5$ cells per well. After 2-3 days of culture, the cells were washed three times with PBS, fixed with 4% paraformaldehyde in PBS for 15 min, permeablized in PBS containing 0.1% Triton X-100 and 5% bovine serum albumin for 1 h, prior to the detection of P-gp, MRP, LRP, GST-π and TopoIIα with immunofluorescence. The four cell lines were incubated with the primary antibodies against P-gp, MRP, LRP, GST-π and TopoIIα, respectively (all purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA; diluted at 1:200 for each) at 4˚C overnight. After being washed with PBS three times, the cells were stained with FITC green-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.) at a dilution of 1:200 at 37˚C for 1 h. Cell images were subsequently captured with fluorescence microscopy and analyzed using Northern exposure image analysis/archival software (Mississauga, Ontario, Canada).

Western blot analysis. Protein extracts were prepared using a total protein extraction kit (KeyGen, Nanjing, China) according to the manufacturer's instructions. Protein (50 μg) was separated in 6-12% SDS-PAGE, and probed by goat or mouse primary antibodies (Santa Cruz Biotechnology, Inc.) against LRP, GST-π and TopoIIα at a dilution of 1:200, respectively, at 4˚C overnight, and then, with secondary antibodies (Santa Cruz Biotechnology, Inc.) at a dilution of 1:2000, respectively, at room temperature for 1.5 h. β-actin expression was examined to show the loading equality of the total protein in each well by using anti-β-actin polyclonal antibody (Santa Cruz Biotechnology, Inc.) at a 1:200 dilution. The horseradish peroxidase signal was detected using enhanced chemiluminescence (GE Healthcare, UK) determined by quantitative analysis of the digital images of gels using Labworks 4.6 software (UVP Products, Upland, CA, USA).

RNA isolation and RT-PCR. Total RNA was extracted from the cells using RNAiso Plus (Takara, Japan) according to the manufacturer's instructions. In brief, the lysis of the cells in TRIzol was centrifuged at 12,000 x g at 4˚C for 15 min in the presence of chloroform. The upper aqueous phase was collected, and the RNA was precipitated by the addition of 100% isopropanol and a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) and centrifuged at 12,000 x g at 4˚C for 10 min. RNA pellets were washed with ice-cold 75% ethanol, dried, resuspended in sterile water and quantified by spectrometry. RT-PCR was performed with an RNA PCR Kit (AMV, Ver.3.0, Takara) according to the manufacturer's instructions. The housekeeping gene, β-actin, was used as an internal control to confirm equal loading in each experiment and was amplified from the same cDNAs. The primers specific to P-gp, MRP, LRP, GST-π, TopoIIα, GAPDH and β-actin are shown in Table I. Reverse transcription was performed in a 10-μl volume mixture consisting of 1 μl (≤500 ng) of total RNA, 0.5 μl Oligo(dt)-Adaptor primer (2.5 pmol/μl), 1 μl of 10X Reverse-transcription buffer, 1 μl dNTP mixture (10 mM each), 0.5 μl AMV Reverse transcriptase XL (5 U/μl) and 0.25 μl RNA ribonuclease inhibitor (40 U/μl). Reverse transcription was performed at 50˚C for 50 min and terminated at 99˚C for 5 min. The cDNA generated from this experiment was then subjected to PCR to amplify the transcript of P-gp, MRP, LRP, GST-π and TopoIIα. The PCR reaction was performed in 10 μl PCR buffer, 0.25 μl Taq DNA polymerase (5 U/μl), 10 μl RT product and 0.5 μl of each primer (20 pmol/μl). The PCR cycle started with 1 cycle at 94˚C for 4 min, 30 cycles of 1 min

Table I. Characteristics of the primers used for conventional RT-PCR.

Genes	Sequence 5'-3' (forward and reverse primers)	Products (bp)
GAPDH (for MDR-1)	GCC AAA AGG GTC ATC ATC TC GTA GAG GCA GGG ATG ATG TTC	287
MDR-1 (P-gp)	ATA TCA GCA GCC CAC ATC AT GAA GCA CTG GGA TGT CCG GT	154
MRP	TGA AGG ACT TCG TGT CAG CC GTC CAT GAT GGT GTT GAG CC	242
LRP	GTC TTC GGG CCT GAG CTG GTG TCG CTT GGC CGT CTC TTG GGG GTC CTT	240
GST-π	ACC TCC GCT GCA AAT ACA TC GGT TAG GAC CTC ATG GAT CA	206
TopoIIα	TGA CAG TGA AGA AGA CAG C GAG AGA CAC CAG AAT TCA A	117
β-actin	TCG TCA CCA ACT GGG ACG ACA TGG GAT CTT GAT CTT CAT TGT GCT GGG	750

at 94˚C, 30 sec at 58˚C, 30 sec at 72˚C; and a final incubation at 72˚C for 10 min. A total of 5 μl PCR products was separated on a 1-2% agarose gel and stained with ethidium bromide for visualization. The relative abundance of each PCR product was determined by quantitative analysis of digital images of gels using Labworks 4.6 software.

Cell viability assay. Cell viability was measured using the MTT assay. Cells at the exponential phase were dispensed in 96-well plates at a density of $1x10^4$ cells per well. The cells were incubated with different concentrations of cisplatin (0, 2, 4, 6 and 8 μM), doxorubicin (0, 2, 4, 6 and 8 μM) and VP-16 (0, 20, 40, 60 and 80 μM), respectively. After a 48-h incubation with the indicated drugs, 20 μl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, USA) reagent was added to each well for 4 h. The medium was discarded and 100 μl of dimethyl sulfoxide was added to each well, and incubated for 10 min. The optical density of each well was measured with Multiskan Ascent (Thermo, USA). The cell viability and IC_{50} were calculated by the following equations: Cell viability (%) = mean optical density of the experimental group/mean of the control x 100; IC_{50} = the concentration of the indicated drug at 50% cell viability.

Statistical analysis. All values are represented as the mean ± SEM. Statistical comparisons were carried out using the one-way ANOVA. Correlation between expression of the five drug resistance-related proteins and IC_{50} values of the chemotherapy drugs was assessed by Pearson correlate analysis. All analyses were performed with SPSS 16.0 software. A value of $P<0.05$ was regarded as statistically significant.

Results

The endogenous levels of P-gp, MRP, LRP, GST-π and TopoIIα in four cell lines of different histological types. To test the endogenous levels of P-gp, MRP, LRP, GST-π and TopoIIα in the four lung cancer cell lines, SK-MES-1, SPCA-1, NCI-H-460 and NCI-H-446, immunofluorescence and Western blot analysis were carried out. As shown in Fig. 1A, immunofluorescence analysis indicated that the expression of the five proteins in all four lung cancer cell lines varied. P-gp and MRP were distributed on the membrane of the cells; while LRP and GST-π were found in the plasma of the cells, and TopoIIα was noted in both the plasma and the nucleus.

Table II. Expression of GST-π, LRP and TopoIIα at the protein level in the lung cancer cell lines as determined by Western blot analysis.

Cell line	GST-π	LRP	TopoIIα
SK-MES-1	1.51±0.52	1.02±0.08[a]	0.39±0.06
SPCA-1	1.15±0.33	1.53±0.16[a]	1.26±0.05[b]
NCI-H-460	0.55±0.04	2.38±0.45	1.39±0.27[b,c]
NCI-H-446	0.41±0.10[b]	0.78±0.04[a]	0.57±0.11[c]

[a]$P<0.05$, compared with NCI-H-460. [b]$P<0.05$, compared with SK-MES-1. [c]$P<0.05$, compared with SPCA-1.

In order to determine the quantifiable levels of these proteins, Western blotting was performed on LRP, GST-π and TopoIIα. Similarly, positive expression of LRP, GST-π and TopoIIα was observed in all four lung cancer cell lines. However, their expression levels were different. Expression of GST-π was 3-fold higher in the SK-MES-1 than that in the NCI-H-446 cells (1.51±0.52 vs. 0.41±0.10). Whereas, LRP expression was 1.5-, 2- and 3-fold higher in the NCI-H-460 cells compared to that in the SPCA-1, SK-MES-1 and NCI-H-446 cells, respectively (2.38±0.45 vs. 1.53±0.16, 2.38±0.45 vs. 1.02±0.08, 2.38±0.45 vs. 0.78±0.04; $P<0.05$) (Fig. 1B). In contrast, TopoIIα expression was ~3-fold lower in the SK-MES-1 cell line than that in the SPCA-1 and NCI-H-460

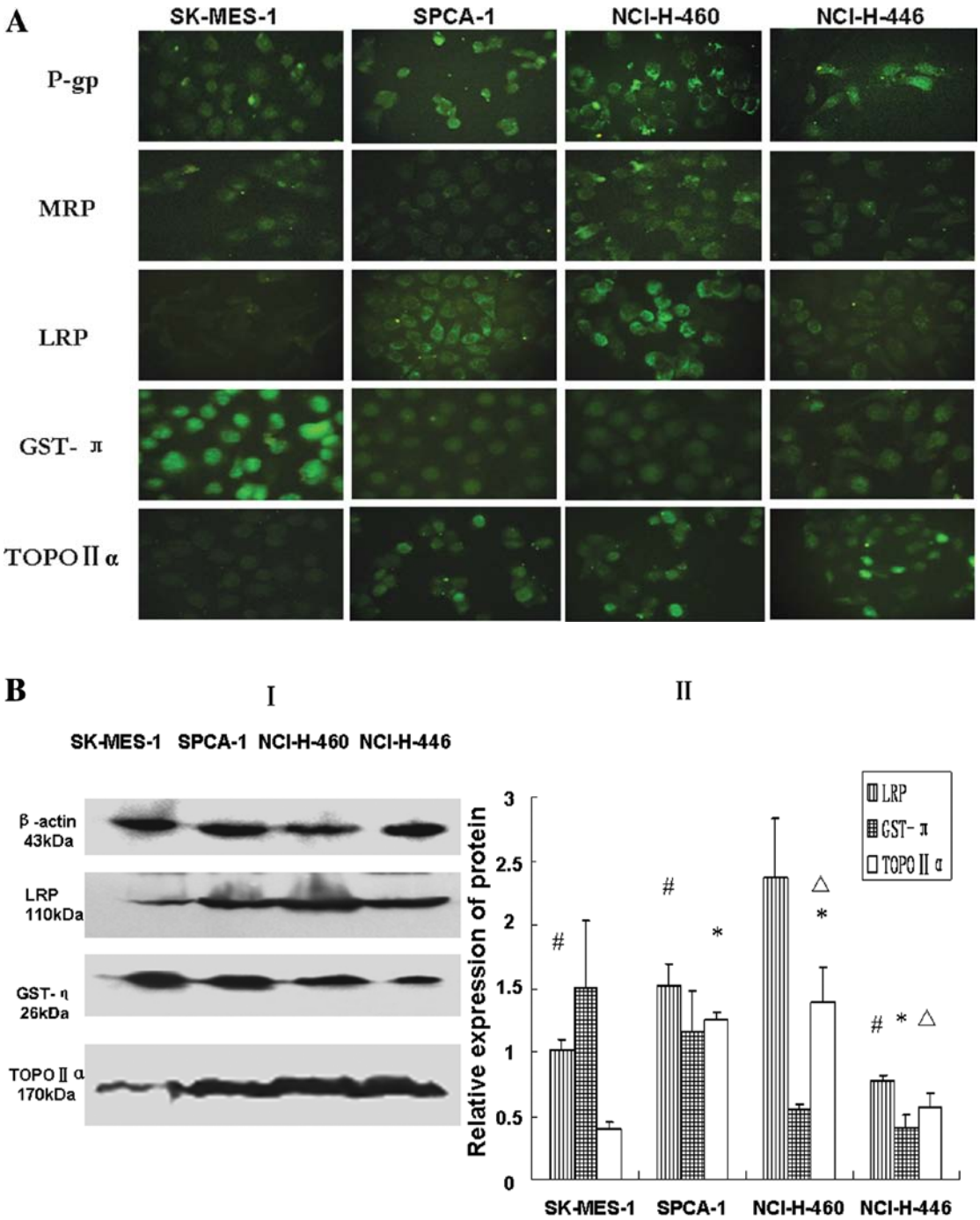

Figure 1. Expression of P-gp, MRP, LRP, GST-π and TopoIIα at the protein level examined by (A) immunofluorescence (magnification, x40) and (B) Western blot analysis in the four lung cancer cell lines (I, electrophoregram; II, bar graph). *P<0.05, compared to SK-MES-1. △P<0.05, compared to SPCA-1. #P<0.05, compared to NCI-H-460.

cells (0.39±0.06 vs. 1.26±0.05, 0.39±0.06 vs. 1.39±0.27; P<0.05). Detailed data are shown in Table II.

The mRNA level of P-gp, MRP, LRP, GST-π and TopoIIα in the four cell lines of different histological types. To test whether the mRNA levels of the P-gp, MRP, LRP, GST-π and TopoIIα genes demonstrate the same pattern as observed at the protein level in the four lung cancer cell lines, RT-PCR was performed. Similarly, the results from the mRNA analysis of the five genes confirmed the results of the immunofluorescence and Western blot analyses. Although mRNA levels of the five genes varied among the four cell lines as well, the difference was not as significant as that assayed by the Western blot analysis. As shown in Fig. 2, there was no significant difference in mRNA levels of P-gp, MRP and TopoIIα in the four cell lines. Similarly, the mRNA level of GST-π was ~1.4-fold higher in the SK-MES-1 than that in the NCI-H-446 cells (0.56±0.05 vs. 0.40±0.05; P<0.05), but no significant difference was observed in other two cell lines. The mRNA level of LRP showed a significant difference between SK-MES-1 and

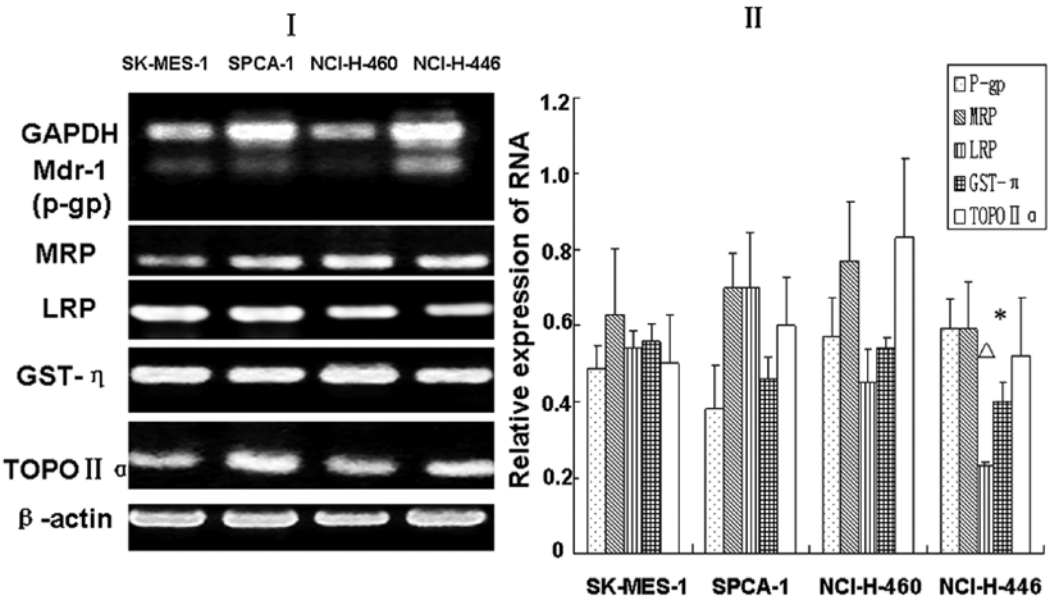

Figure 2. Expression of MDR-1 (P-gp), MRP, LRP, GST-π and TopoIIα at the mRNA level as determined by RT-PCR in the four lung cancer cell lines. Results are expressed as a ratio of target mRNAs to GAPDH (MDR-1) or β-actin mRNA (I, electrophoregram; II, bar graph). *P<0.05, compared to SK-MES-1. △P<0.05, compared to SPCA-1.

Table III. Expression of P-gp, MRP, LRP, GST-π and TopoIIα at the mRNA level in the lung cancer cell lines as determined by RT-PCR.

Cell line	P-gp	MRP	LRP	GST-π	TopoIIα
SK-MES-1	0.49±0.06	0.63±0.17	0.54±0.05	0.56±0.05	0.50±0.13
SPCA-1	0.38±0.11	0.70±0.09	0.70±0.14	0.45±0.06	0.60±0.13
NCI-H-460	0.57±0.10	0.77±0.16	0.45±0.09	0.53±0.03	0.83±0.21
NCI-H-446	0.59±0.08	0.59±0.13	0.23±0.01[a,b]	0.40±0.05[a]	0.52±0.16

[a]P<0.05, compared to SK-MES-1. [b]P<0.05, compared to SPCA-1.

NCI-H-446 cells (0.54±0.05 vs. 0.23±0.01; P<0.05). Detailed data are shown in Table III.

Chemoresistance to cisplatin, doxorubicin and VP-16 of the four lung cancer cell lines. To study whether there are differences in the response to chemotherapeutical agents in the four lung cancer cell lines of different histological types, the cells were treated with different concentrations of cisplatin, doxorubicin and VP-16 for 48 h, and the number of surviving cells was examined by MTT assays; IC_{50} values were determined as well. The results showed that all three drugs, cisplatin, doxorubicin and VP-16, inhibit the growth of SK-MES-1, SPCA-1, NCI-H-460 and NCI-H-446 cells in a dose-dependent manner. However, the chemosensitivity to the drugs among the four cell lines was different. The IC_{50} of cisplatin for SK-MES-1 was ~2- to 4-fold higher than that of the other three cell lines (SK-MES-1/SPCA-1: 9.15±1.54 vs. 2.05±0.34; SK-MES-1/NCI-H-460: 9.15±1.54 vs. 3.36±0.83; and SK-MES-1/NCI-H-446: 9.15±1.54 vs. 4.49±0.48; all P<0.05) (Fig. 3A). As for doxorubicin, its IC_{50} for SK-MES-1 was the highest when comparing the four cell lines (Fig. 3B; P<0.05). For VP-16, the lowest IC_{50} was observed in the SK-MES-1 cells when comparing the four cell lines (Fig. 3C; P<0.05), while there was no significant difference in IC_{50} values among the other three cell lines (Fig. 3C; P>0.05). Detailed data are shown in Table IV. These findings indicate that the chemosensitivity to cisplatin, doxorubicin and VP-16 of the four lung cancer cell lines of different histological types was different.

Correlation between the endogenous levels of drug resistance-related proteins and intrinsic resistance in the lung cancer cells. To understand whether the endogenous levels of P-gp, MRP, LRP, GST-π and TopoIIα are related to the chemoresistance to cisplatin, doxorubicin and VP-16, in the four lung cancer cell lines, Pearson correlation coefficient analysis was performed using the endogenous mRNA and protein expression levels of the five drug resistance-related proteins and the IC_{50} values of the three chemotherapy drugs for the four of cell lines. Our data showed that not all of the five proteins were associated with the intrinsic resistance to cisplatin, doxorubicin and VP-16 in the cell lines tested. Pearson correlation analysis indicated there were positive correlations between

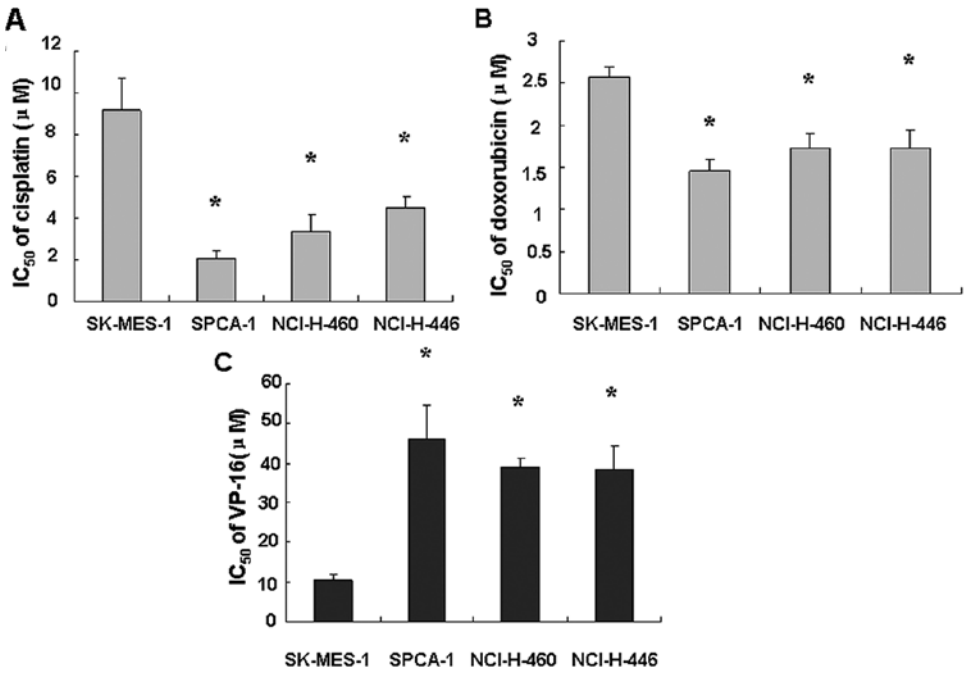

Figure 3. IC_{50} values of the chemotherapy drugs in the four lung cancer cell lines. After the cells were treated with (A) cisplatin, (B) doxorubicin and (C) VP-16 (C) at various concentrations for 48 h, the IC_{50} values were determined by MTT viability assay *P<0.05, compared to SK-MES-1.

Table IV. IC_{50} values of the chemotherapy drugs in the lung cancer cell lines.

Cell line	IC_{50} of cisplatin (μM)	IC_{50} of doxorubicin (μM)	IC_{50} of VP-16 (μM)
SK-MES-1	9.15±1.54	2.58±0.12	10.61±1.24
SPCA-1	2.05±0.34[a]	1.45±0.14[a]	46.27±8.27[a]
NCI-H-460	3.36±0.83[a]	1.74±0.17[a]	39.02±2.40[a]
NCI-H-446	4.49±0.48[a]	1.72±0.20[a]	38.44±5.92[a]

[a]P<0.05, compared to SK-MES-1.

Table V. Correlation between the IC_{50} of the chemotherapy drugs and the expression of P-gp, MRP, LRP, GST-π and TopoIIα at the mRNA level in the four lung cancer cell lines.

Chemotherapy drugs	P-gp	MRP	LRP	GST-π	TopoIIα
Cisplatin					
r-value	0.088	0.046	0.220	0.444	-0.402
P-value	0.787	0.888	0.492	0.149	0.167
Doxorubicin					
r-value	-0.026	-0.134	-0.063	0.456	-0.173
P-value	0.936	0.678	0.845	0.136	0.590
VP-16					
r-value	0.042	0.358	0.206	-0.391	0.033
P-value	0.898	0.254	0.520	0.209	0.920

the expression of GST-π and resistance to cisplatin (Fig. 4A; P<0.05, r=0.5946), between the expression of TopoIIα and resistance to VP-16 (Fig. 4C; P<0.05, r=0.6883); whereas there was a negative correlation between the expression of TopoIIα and resistance to doxorubicin (Fig. 4B; P<0.05, r=-0.5896). However, there were no correlations between the mRNA and

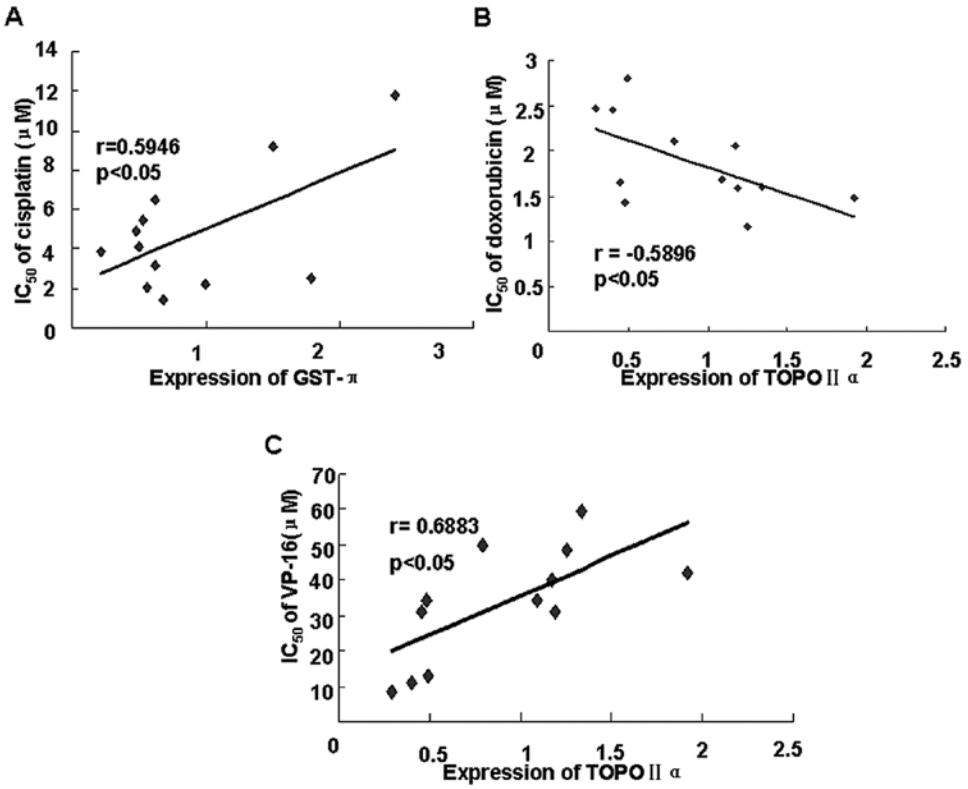

Figure 4. Significant correlation between the IC_{50} values of the chemotherapy drugs and the expression of the resistance proteins at the protein level in the lung cancer cells. A positive correlation was noted (A) between cisplatin and GST-π and (C) between between VP-16 and TopoIIα. (B) A negative correlation was noted between doxorubicin and TopoIIα.

Table VI. Correlation between the IC_{50} of the chemotherapy drugs and the expression of LRP, GST-π and TopoIIα at the protein level in the four lung cancer cell lines.

Chemotherapy drugs	LRP	GST-π	TopoIIα
Cisplatin			
r-value	-0.272	0.595	-0.562
P-value	0.393	0.041	0.057
Doxorubicin			
r-value	-0.351	0.549	-0.590
P-value	0.263	0.065	0.044
VP-16			
r-value	0.390	-0.278	0.688
P-value	0.210	0.382	0.013

protein expression of P-gp, MRP, and LRP and chemoresistance to cisplatin, doxorubicin and VP-16 in the cancer cell lines. Detailed data are shown in Tables V and VI.

Collectively, these findings suggest that GST-π is associated with chemoresistance to cisplatin, and TopoIIα is associated with chemoresistance to both doxorubicin and VP-16 in lung cancer cell lines. Thus, the levels of TopoIIα and GST-π may be useful predictors for the chemotherapy resistance to cisplatin, doxorubicin and VP-16 in human lung cancer cell lines.

Discussion

Generally, lung cancer displays an intrinsic multidrug resistance, limiting the chance of successful chemotherapy (4). Although chemotherapeutic drugs such as cisplatin, doxorubicin and VP-16 are widely used in the cure and palliation of human lung cancer, intrinsic resistance is still one of the major drawbacks of lung cancer chemotherapy. Therefore, investigation of the mediators of intrinsic resistance may improve the efficacy of chemotherapy in lung cancer (19). In the present study, we investigated the relationship between the expression of P-gp, MRP, LRP, GST-π and TopoIIα and intrinsic resistance in four lung cancer cell lines. First, the endogenous levels of P-gp, MRP, LRP, GST-π and TopoIIα at both the protein and mRNA level varied in the four lung cancer cell lines of different histological types, but with some similarity of expression at the protein and mRNA level. Among the five proteins, GST-π expression in the SK-MES-1 cells was the highest, whereas P-gp expression in the SPCA-1 cell line was the lowest. Second, the chemoresistance to cisplatin, doxorubicin and VP-16 among the four cell lines was different. The SPCA-1 cell line was most resistance to cisplatin; SK-MES-1 was most resistance to VP-16; whereas SK-MES-1 was most sensitive to doxorubicin. Third, for the correlation between the endogenous levels of the five drug resistance-related proteins and intrinsic resistance in lung cancer cells, only GST-π and TopoIIα but not P-gp, MRP and LRP were associated with the chemotherapy resistance to cisplatin, doxorubicin and VP-16. There was a positive correlation between the expression of

GST-π and resistance to cisplatin, between the expression of TopoIIα and resistance to VP-16, whereas a negative correlation was noted between expression of TopoIIα and resistance to doxorubicin in the human lung cancer cells. These findings indicate that P-gp, MRP, LRP, GST-π and TopoIIα play different roles, respectively, in the chemotherapy resistance among heterogeneous subtypes of lung cancer cell lines, and up- or down-regulation of GST-π or TopoIIα may alter the chemoresistance to cisplatin, doxorubicin and VP-16 in lung cancer.

In agreement with our results, studies have demonstrated that the expression of the five drug resistance-related proteins varied and served different functions in chemotherapy resistance. It has been reported that the expression of P-gp, MRP, LRP was distinct in adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma cell lines, and also, the expression of these drug resistance-related proteins at the protein and mRNA level was not always consistent, suggesting that there may be a post-transcription regulation or the separation of protein and mRNA (15,20). Also, in line with our findings, studies have shown that the chemosensitivity to cisplatin, doxorubicin and VP-16 in adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma is different.

In the present study, we found that among the five drug resistance-related proteins only GST-π and TopoIIα but not P-gp, MRP and LRP were associated with the intrinsic chemotherapy resistance to cisplatin, doxorubicin and VP-16 in lung cancer cells. Similar to our results, Siddik found that a high level of GST-π significantly contributes to clinical cisplatin resistance in different types of human cancers (21). Kasahara *et al* found that the overexpression of TopoIIα was well correlated with the sensitivity of lung cancer cell lines to doxorubicin (22). Whereas, in colorectal cancer cells, there was a strong association between TopoIIα overexpression and resistance to VP-16 (23). In addition, no correlation was noted between P-gp, MRP or LRP expression and drug resistance to cisplatin and VP-16 in NSCLC cell lines or childhood leukemia (20,24,25).

Regarding the mechanism of cisplatin resistance due to GST-π, the following four possibilities can be hypothesized based on the physiological properties of GST-π: i) the involvement of GST-π in detoxification of cisplatin by GSH conjugation; ii) the sequestration of cisplatin as a result of binding with GST-π; iii) the chemical reduction of lipid peroxides by GST-π; iv) GST-π together with GSH functions to prevent DNA cross-linking by cisplatin (26,27). As for the important role of TopoII in the intrinsic resistance to doxorubicin as noted in our study, this may be due to the decrease in the activity and sensitivity of the target enzyme TopoII by down-regulation or mutation (14). The weak correlation between P-gp, MRP and LRP and resistance to the drugs in our study indicates that the increase in drug efflux by P-gp and MRP may not play a main role in the chemoresistance to cisplatin, doxorubicin and VP-16 in lung cancer cells. This finding may be significant for the selection of a proper anticancer drug in chemotherapy. Cisplatin, doxorubicin and VP-16 are common drugs currently used in the treatment of human lung cancer. Yet, resistance to chemotherapy is a huge obstacle to successful treatment. Therefore, further investigation of the possible mechanisms of drug-related proteins may be beneficial for overcoming intrinsic resistance and for developing more effective treatments for human lung cancer.

In conclusion, the expression of the chemotherapy resistance-related proteins, P-gp, MRP, LRP, GST-π and TopoIIα, in human lung cancer cell lines, SK-MES-1, SPCA-1, NCI-H-460 and NCI-H-446, was different. This varied expression may lead to different degrees of chemoresistance to cisplatin, doxorubicin and VP-16. GST-π was the most important mediator among the five proteins to predict the intrinsic resistance to cisplatin, TopoIIα also contributed, to certain degree, to the intrinsic resistance to doxorubicin and VP-16. Further investigation of the specific mechanisms involved is necessary to elucidate the causes of chemotherapy resistance in lung cancer.

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