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Original Research Article

Effect of expression levels of multidrug resistance gene-related protein 1, P-glycoprotein and topoisomerase II on paclitaxel, gemcitabine and vinorelbine sensitivity in pulmonary cancer

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Abstract

Purpose: *To investigate the possible correlation between drug resistance gene expression and development of drug sensitivity, and the possible clinical significance of this relationship.*
Methods: *A total of 100 cancer samples were surgically obtained. MTT assay was employed to determine drug sensitivity. The expression levels of drug resistance genes, multidrug resistance gene-related protein 1 (MRP1), P-glycoprotein (P-gp), and topoisomerase II (Topo II) were measured by immunohistochemistry.*
Results: *The expression levels of MRP1, P-gp, and Topo II genes in lung cancer were 70.0, 65.0, and 50.0 %, respectively. No significant statistical differences were observed in the expressions of MRP1, P-gp, and Topo II between human adenocarcinoma and squamous cell carcinoma ($p > 0.05$), but a significant difference was found in MRP1 and Topo II expressions between human adenocarcinoma or squamous carcinoma cell and small-cell lung cancer ($p < 0.05$). A significant positive correlation was observed between P-gp expression and resistance cisplatin, gemcitabine, vinorelbine, and paclitaxel ($p < 0.05$). A significant positive correlation was also found between MRP1 expression and the development of resistance to cisplatin, gemcitabine, and vinorelbine ($p < 0.05$), but no significant correlation was observed between MRP1 expression and the development of resistance to paclitaxel and ifosfamide ($p > 0.05$).*
Conclusion: *The up-regulated expression of MRP1 and P-gp, and the down-regulated expression of Topo II may be positively correlated with drug resistance in lung cancer patients. Thus, gene tests are recommended to guide the administration of chemotherapy.*

Keywords: *Lung carcinoma, Drug resistance gene, Drug sensitivity, Chemotherapy, Multidrug resistance gene-related protein*

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INTRODUCTION

Lung cancer is a leading cause of cancer-related morbidity and mortality. With the development of targeted therapy drugs and multidisciplinary combination treatment, the life expectancy of lung cancer patients has been prolonged. However, five-year survival cannot be increased because 62 – 83 % of patients with lung cancer are already at the late stage of the

disease during diagnosis. Most lung cancer patients prefer chemotherapy as their major treatment of lung cancer. The phenomenon of this therapeutic intervention is closely related to the multidrug resistance (MDR) of lung cancer [1-3]. The present study aimed to determine whether the expression of MDR gene-related protein 1 (MRP1), P- P-gp, and Topo II affects the response of cancer cells to some commonly used drugs in chemotherapy.

EXPERIMENTAL

Baseline data for patients

A total of 100 patients with pathologically confirmed lung cancer were selected from Huashan Hospital between January 2016 and December 2017. Thirty-six of the samples were surgical specimens, while the remaining samples were obtained via bronchoscopy. There were 72 males and 28 females, and the average age was (55.2 ± 7.2) years. There were 40, 42, and 18 patients were at clinical stages II, III, and VI, respectively. A total of 58, 24, and 18 patients presented with squamous carcinoma, glandular cancer, and small-cell carcinoma, respectively. This research was authorized by the Ethics Committee of Huashan Hospital (approval no. 20185236), and all the patients gave their informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards [4].

Specimen preparation

Exairesis and bronchoscopy microscopic tumor tissues were cut into 0.5 – 1.0 mm diameter fragments, processed by D-Hanks, and digested by type-1 collagenase. These fragments were cultured in PRMI 1640.

Medicine administration and drug-sensitivity test

The drug concentration (cisplatinum, 340 mg/L gemcitabine, 3400 mg/L navelbine, 100 mg/L ifosfamide, 600 mg/L and paclitaxel, 100 mg/L) in the experiment was selected as the peak plasma concentration in the human body. Cisplatinum, gemcitabine, navelbine, ifosfamide, and paclitaxel were admixed with normal saline 10 times as the peak plasma concentration of these drugs used in clinical application and then stored at −20 °C. The specific formula is as follows: drug concentration in experiment (mg/mL) = $60 \times D/5000 \div 50\ \% \times 10^3$, where D is the clinical dosage (mg/kg/d), 5000 stands for the blood volume (mL) of an adult, 70 is the average weight of adults (kg), and 50 % is the specific hemocyte volume [2]. Moreover, experimental drug concentrations were: cisplatinum, 340 mg/L gemcitabine, 3400 mg/L navelbine, 100 mg/L ifosfamide, 600 mg/L and paclitaxel, 100 mg/L. After digestion of tumor tissues, samples were combined into a single-cell suspension, in which the tumor cells of experimental and control groups (without anticancer drugs) were placed in CO_2 incubator for 24 h. MTT liquid was added to the tumor cells, and cells were cultured for additional 4 h, dimethyl sulfoxide was added at the end of the procedure. The absorbance per well (A) was measured at 570 and 690 nm was used as reference wavelength. Antitumor rate was calculated using the formula: antitumor rate (%) = (1 − A value of tested well/A value of control hole) × 100 %. A rate of ≥ 50 % was considered sensitive, whereas < 30% implied drug resistance; medium sensitivity was then found. The total sensitivity rate equaled to the sum of sensitivity and medium sensitivity rates.

Gene expression assay

The experiment was conducted using Elivision Plus KIT [3] as follows. The cell is defined positive for MRP_1 and P-gp if claybank particles appeared in the cell membrane and cytoplasm and the cell is defined as positive for Topo II if claybank particles appeared in the cell nucleus and cytoplasm. Five high-power fields were randomly selected and counted by 1000 cells. The result was judged in terms of the number and color intensity of positive cells [4]. If the number of positive cells was ≤10 %, it would be considered negative. MRP_1 and P-gp are localized in the cytomembrane or cytoplasm, and Topo II is localized in the nucleus.

Statistical analysis

Statistical analysis was carried out using SPSS 19.0 database software (SPSS Inc., Chicago, USA). Statistical comparison among groups was analyzed by using χ^2 test and Fisher exact probability method. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of drug resistance-related genes and lung cancer drug resistance

The positive part of MRP_1 presented with claybank and was positioned on cytomembrane or cytoplasm (Figure 1 A). Among the 80 tested

samples, 24 were negative (30.0 %), and 56 were positive (70.0 %). No significant difference was found among the squamous carcinoma, adenocarcinoma, and small-cell lung cancer in terms of MRP_1 expression ($p > 0.05$) (Table 1). For the five chemotherapeutics, MRP_1 expression revealed a significant positive correlation ($p < 0.05$) with resistance to cisplatinum, gemcitabine, and navelbine but showed no significant correlation with resistance to paclitaxel and ifosfamide ($p > 0.05$, Table 2).

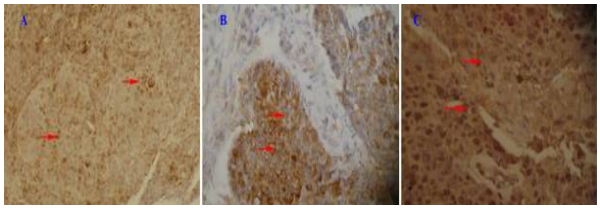

Figure 1: Immunohistochemical staining (×400), A: MRP1 in adenocarcinoma cells; B: P-gp in squamous cell carcinoma and C: Topo II in small-cell lung cancer as the arrows indicate

Expression of P-gp in lung cancer and lung cancer drug resistance

The positive part of P-gp presented with claybank and was located on the cytomembrane or cytoplasm (Figure 1 B). Among the 80 tested samples, 28 were negative (35.0 %), and 52 were positive (65.0 %). No significant difference was observed between squamous carcinoma and adenocarcinoma in the P-gp expression ($p < 0.05$), but a significant difference was found between squamous carcinoma and small-cell lung cancer/adenocarcinoma ($p < 0.05$) (Table 2). P-gp expression showed a significant positive correlation ($p < 0.05$) with resistance to cisplatinum, gemcitabine, paclitaxel, and navelbine but revealed no significant correlation with ifosfamide resistance ($p < 0.05$) (Table 2).

Expression of Topo II in lung cancer and lung cancer drug resistance

The positive part of Topo II presented with claybank and was positioned in the cell nucleus (Figure 1 C). Among the 80 tested samples, 40 were negative (50 %), and 40 were positive (50 %). No significant difference ($p > 0.05$) (Table 1) was observed among the three types of lung cancer (squamous carcinoma, adenocarcinoma, and small-cell lung cancer) on Topo II

expression. Topo II expression revealed a significant positive correlation ($p < 0.05$) with the resistance to cisplatinum, gemcitabine, and navelbine but showed no significant correlation with resistance to paclitaxel and ifosfamide ($p > 0.05$). For the five strategies of drug selection in chemotherapy, Topo II expression showed a significant positive correlation ($p < 0.05$) with the resistance to chemotherapeutics, cisplatinum, gemcitabine, paclitaxel, and ifosfamide but revealed no significant correlation with navelbine resistance ($p > 0.05$, Table 2).

DISCUSSION

Lung cancer drug resistance and its mechanism remain unclear. Tumor MDR-related genes include P-gp, MRP1, LRP, GST-π, and Topo II. In this study, the expression levels of MRP1, P-gp, and Topo II in lung cancers were measured, and their resistances to *in vitro* chemotherapy were compared. The correlation between the expressions of these genes and drug resistance were measured.

The findings in this study may provide reference for the clinical application of chemotherapy for lung cancer. As a classic MDR protein, MDR-associated protein (MRP) was first separated from doxorubicin-resistant human small-cell lung cancer cell line. Multidrug resistance-associated protein (MRP) is a trans-membrane protein, and the mechanism of associated drug resistance is related to its drug pump function. MRP recognizes and transports glutathione (GSH)-bound substrates. This pump increases drug outflow and decreases the drug concentration inside the cell, leading to drug resistance [5].

Table 1: Expression levels of MRP1, P-gp, and Topo II in lung cancer patients

Histological Type	Volume of example	*P-gp expression			#MRP1 expression			#Topo II expression		
		—	+/++	Positive rate (%)	—	+/++	Positive rate (%)	—	+/++	Positive rate (%)
Squamous carcinoma	42	10	32	76.2	14	28	66.7	24	18	42.9
Adenocarcinoma	20	4	16	80.0	4	16	80.0	8	12	60.0
Small-cell lung cancer	18	8	10	55.6	14	4	22.2	8	10	55.6

*Fisher exact probability method was used. Comparison between squamous carcinoma and adenocarcinoma, $p > 0.05$; comparisons between squamous carcinoma and small-cell lung cancer, and adenocarcinoma and small-cell lung cancer, $p < 0.05$. #Fisher exact probability method was utilized

Table 2: Relationship between expressions of MRP1, P-gp, and Topo II and lung cancer drug resistance

Chemotherapeutics	Sensitivity	Quantity of example	MRP1*			P-gp#			Topo Ⅱ☆		
			—	+	++	—	+	++	—	+	++
Cisplatinum	Sensitive	46	20	14	12	20	16	10	14	18	14
	Drug-resistant	34	2	10	22	6	6	22	26	6	2
Navelbine	Sensitive	36	18	12	6	22	10	4	12	16	8
	Drug-resistant	44	4	16	24	4	12	28	28	12	4
Gemcitabine	Sensitive	38	18	12	8	18	14	6	10	12	16
	Drug-resistant	42	4	14	24	8	12	22	30	8	4
Paclitaxel	Sensitive	28	12	10	6	16	8	4	8	16	4
	Drug-resistant	52	10	16	26	8	12	32	32	10	10
Ifosfamide	Sensitive	22	10	12		10	12		4	18	
	Drug-resistant	58	12	46		16	42		36	22	

* X^2 test was used on sensitive group and drug-resistant group. Cisplatinum group: $X^2 = 8.458$ and $p < 0.05$; Navelbine group: $X^2 = 9.839$ and $p < 0.01$; Gemcitabine: $X^2 = 8.453$ and $p < 0.05$; Paclitaxel group: $X^2 = 3.773$ and $p > 0.05$; Ifosfamide group: $P < 0.05$. As mentioned above, same statistical method was utilized. Cisplatinum group: $X^2 = 7.562$ and $p < 0.05$; Navelbine group: $X^2 = 15.072$ and $p < 0.01$; Gemcitabine: $X^2 = 6.488$ and $p < 0.05$; Paclitaxel group: $X^2 = 8.169$ and $p < 0.05$; Ifosfamide group: $p < 0.05$. ☆As mentioned above, same statistical method was utilized. Cisplatinum group: $X^2 = 8.593$ and $p < 0.05$; Navelbine group: $X^2 = 3.790$ and $p > 0.05$; Gemcitabine: $X^2 = 8.922$ and $p < 0.05$; Paclitaxel group: $X^2 = 6.130$ and $p < 0.05$; Ifosfamide group: $p < 0.05$

Similar to MRP, P-gp is a drug pump that relies on energy supply from ATP; it pumps insoluble substances out of the cell against the concentration gradient, hence weakening the effect of chemotherapeutic agents and affecting the treatment outcome. Currently, evaluation of new anti-cancer drugs is based on MDR cancer cell model [6].

Topo II is the most important ribozyme involved in DNA transcription, translation, replication, and chromosome separation. Topo II is mainly expressed during the SG2/M phase of cell cycle. Topo II is an indicator of cell proliferation and is a major target of anticancer drugs. Chemotherapy drugs targeting Topo II impede tumor cell proliferation, mainly by binding to Topo II, and causing abnormalities in DNA replication and transcription [7]. Previous studies on human Topo II reveal that the strand passage reaction of Topo II is the target of several major categories of anticancer drugs including Topo II poison and activator of cell cycle checkpoint control [8-10]. To date, non-smoking Asian female adenocarcinoma patients receiving TKI therapy have obviously longer overall survival compared with those receiving chemotherapy alone.

However, long-term exposure to TKI targeting EGFR and HER2 may induce resistance to doxorubicin, etoposide, and m-AMSA through Topo IIa down-regulation [11,12]. Consistent with these studies, the results showed that low Topo II level contributes to the resistance to platinum, gemcitabine, paclitaxel, and ifosfamide.

CONCLUSION

Multiple genes contribute to the drug resistance of lung cancer. In addition to lung cancer, these genes are involved in the drug resistance of other malignancies, such as breast cancer and colorectal cancer. Therefore, the development of new chemotherapy drugs through genetic analysis and proteomics to overcome drug resistance and achieve true tailored lung cancer therapy is needed.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hao Zhenhua did the study design. Tan Yulong and Na Di Er Yi Min processed the data and was responsible for data collection. Bai Yunbiao handled manuscript writing. Gao Kaiheng finalized the manuscript and makes proposals and corrections.

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