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Original Article

Prognostic value of MRP, LRP and P-gp in patients with non-small cell lung cancer

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Abstract: The aim of this study was to investigate the prognostic value of glutathione-S-transferase p1 (GST-π), multidrug resistance proteins (MRP), lung resistance-related protein (LRP), P-glycoprotein (P-gp) and topoisomerase 2 expression (Topo IIα) in patients with non-small cell lung cancer (NSCLC) treated with platinum-based chemotherapy. Expression of GST-π, MRP, LRP, P-gp and Topo IIα were conducted using immunohistochemistry. Chi-Square, Kaplan-Meier, Log-rank test and multivariate Cox's regression analysis were used to detect the correlation of proteins, clinicopathological characteristics and survivals. MRP and P-gp were associated with tumor differentiation, respectively (MRP: P=0.020; P-gp: P=0.030), LRP was associated with tumor type (P<0.001), regional lymph node metastasis (P=0.014) and tumor stage (P=0.032). No significant association was observed between clinical features and GST-π and Topo IIα (P>0.05). Poor survivals were markedly correlated with higher expression of MRP, LRP or P-gp, respectively. Besides, combination of MRP, LRP and P-gp had a better predictive value compare with single marker. No significant difference was observed between GST-π or Topo IIα and survival time. MRP and P-gp were observed as independent prognostic factors. This is the first report P-gp overexpression is associated with poor survival in NSCLC, furthermore, this is the first study to evaluate the combination predictive value of MRP, LRP and P-gp in NSCLC. Our findings suggest combination of MRP, LRP and P-gp are more useful predictors in NSCLC patients treated with platinum-based chemotherapy.

Keywords: Biomarkers, personalized treatment, prognosis, outcome, chemotherapy

Introduction

Lung cancer remains the first leading cause of cancer-associated mortality worldwide. About 85% of the patients were diagnosed as non-small cell lung cancer (NSCLC), the rest 15% were classified as small cell lung carcinoma (SCLC) [1]. Till now, surgical resection combination with adjuvant chemotherapy remains to be the main therapeutic method for NSCLC, while for those with unresectable, recurrent, or metastatic tumors, chemotherapy (combination of two platinum drugs) is the predominant treatment method [2, 3]. However, the outcome is greatly limited in the majority of NSCLC patients since the existence of drug resistance, about 30-60% of patients underwent chemotherapy undergo tumor recurrence or distant metastasis. More predictive biomarkers are still needed to improve prognosis in NSCLC patients [4, 5].

Drug resistance takes form of unresponsiveness of tumor cells to anticancer drugs. The impact of drug resistance is correlated with host themselves and tumor microenvironment. During past decades, a lot of studies focused the relationship between chemotherapy resistance and various transporter proteins. Among these proteins, we chose glutathione-s-transferase-π (GST-π), multidrug resistance-associated protein (MRP), lung resistance-related protein (LRP), P-glycoprotein (P-gp) and topoisom-

erase IIα (Topo IIα) as research subjects, since the selected proteins have key role in drug influx, drug inactivation and alterations in the drug targets, resulting in chemotherapy resistance. Glutathione S-transferases (GSTs) are a family of enzymes that play an important role in detoxification by catalyze the conjugation reaction between glutathione (GSH) and anticancer drugs, resulting in glutathione S-conjugates [6]. The soluble GSTs are categorized into 4 main classes: alpha, mu, pi (also known as π), and theta. P-gp and MRP, two important ATP binding cassette transporter proteins in cells, could alter the intracellular drug concentration by regulating drug influx or efflux, resulting in drug resistance [7]. LRP, the main component of vaults, was considered as a major vault protein (MVP) which could mediate drug resistance. LRP can pump drugs away from intracellular drug targets by exocytotic vesicles or pump molecules. Topoisomerases belong to isomerase enzymes which exert their roles by acting on the topology of DNA. Topo IIα, a member of topoisomerases, is the primary target for anticancer drugs such as anthracyclines, amsacrine and epipodophy. Drug resistance to Topo II will occur when activity and sensitivity of Topo II is declined [8]. Taken together, the proteins mentioned above play many roles including increase in drug influx, decrease in drug influx, drug inactivation and alterations in the drug targets, leading to chemotherapeutical resistance.

Although some reports have shown that GST-π, MRP, LRP, and Topo IIα play key roles in drug resistance, while the association studies in patients with lung cancer varied in different studies. Besides, most of the previous studies only focused on one or two of the five drug resistance-related proteins, few studies involved all of the proteins. Moreover, whether there's co-expression between them remains unknown. Our present study aimed to determine the relationship between the protein levels of GST-π, MRP, LRP, P-gp and Topo IIα, survival and drug resistance to platinum-based chemotherapy.

Materials and methods

Patients

All the eligible patients with NSCLC (either de novo or relapsed) who was undertaken platinum based chemotherapy were recruited from Shaanxi Provincial Tumor Hospital between October 2007 and February 2011. In total, 166 NSCLC patients were enrolled in the present retrospective study. All of tissue samples were obtained by surgery or biopsy. Then the tissue samples were fixed in 10% formaldehyde and made into formalin-fixed and paraffin-embedded (FFPE) tissue. The patients were diagnosed based on the cytological or histological findings and histological types were valued according to the World Health Organization criteria. The pathological stage was identified according to the 7th edition of the Union for International Cancer Control Tumor, Node, Metastasis (TNM) Classification for lung cancer. The clinical characteristics of the patients are listed in Supplementary Table 1.

Tumor response to chemotherapy was evaluated after two cycles by clinical test, imaging examination, and serum CA-125 according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria. The follow-up information of all the patients was obtained through telephone interviews, either with the patient or with a relative. Patients were followed-up from chemotherapy to February 2011 for clinical outcome. All of the tissue samples were collected with the approval of the Ethics Committee of Health Science Center of Xi'an Jiaotong University and the informed consent was waived since the retrospective study.

Immunohistochemistry

GST-π, MRP, LRP, P-gp and Topo IIα protein expression levels were evaluated by a standard protocol of immunohistochemistry staining. Briefly, FFPE tissue block from each patient was sliced into 4 μm sections and baked at 65°C for 1 h. All the sections were deparaffinized in xylene and rehydrated with a graded ethanol series. In order to do antigen retrieval, the sections were exposed to 10 mM citrate buffer (PH=6.0) for 10 minutes in an autoclave. Then, the sections were treated with 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity, rinsed in 150 mM PBS (PH=7.6) three times. Sequentially, slides were then incubated with the primary antibodies at 4°C overnight. The primary antibodies used in this study were as follows, mouse anti-human GST-π monoclonal antibody, mouse anti-human MRP monoclonal antibody, mouse anti-human LRP monoclonal antibody,

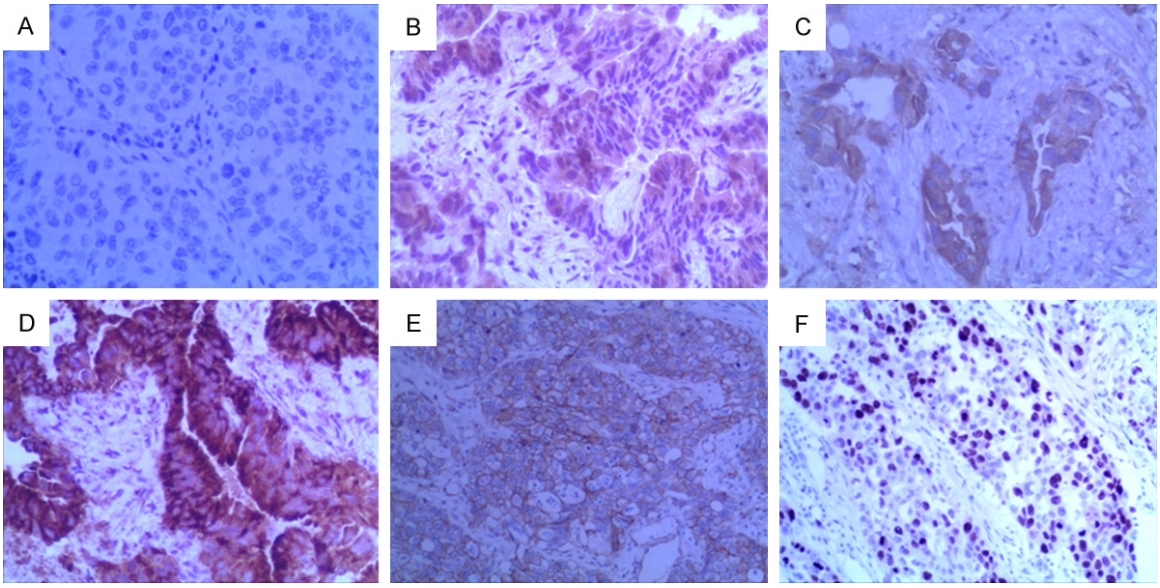

Figure 1. HE staining of NSCLC as control (A), positive immunostaining of NSCLC tumors for GST-π (B), MRP (C), LRP (D), P-gp (E), Topo IIα (F).

mouse anti-human P-gp monoclonal antibody, mouse anti-human Topo IIα monoclonal antibody. All the primary antibodies were provided by Fuzhou Maixin Biotechnology Development Co. Ltd (Fuzhou, China). PBS was used instead of primary antibody as negative control. Next, the slides were incubated with appropriate biotin-streptavidin-peroxidase secondary antibodies (Cell Signaling Technology, USA) for 30 minutes at room temperature, and 3,3'-diaminobenzidine (DAB) was used as a chromogen substrate. Finally, all the slides were counterstained with hematoxylin, dehydrated with ethanol, cleaned with xylene and mounted by cover slips. Two independent pathologists (ZJ and DX) who were blinded to clinicopathologic features, reviewed the slides and performed the evaluation under the light microscope, 5 random fields were selected to assess the expression levels of these proteins. Staining was considered positive expression if tumor cells presented focal, patchy, or diffuse staining intracellularly. All sections were scored in a semi-quantitative manner on the basis of both the percentage and intensity of stained cells. The percentage was categorized as 0 points, 0-1%; 1 point, 2-25%; 2 points, 26-50%; 3 points, 51-75%; and 4 points, >75%. Tumor intensity was recorded as follows: 0 point, no staining, 1 point, weak staining; 2 points, moderate staining; and 3 points, strong staining. The final score was calculated as high expression/positive or low expression/negative by evaluating the percentage and intensity together.

Statistical analysis

Data were presented as numbers of subjects (percentage) for categorical variables. The correlations of the expression of biomarkers and the clinicopathological characteristics of the patients were assessed by Pearson chi-square test or Fisher's exact test. Multivariate Cox regression analysis was conducted to assess the association between each potential prognostic factors including all the clinicopathological factors and overall survival (OS). Survival time was defined as the time from the date of chemotherapy to the date of mortality of any cause, and patients who were survived at the last contact were censored. The Kaplan-Meier method and log-rank test were used for survival comparisons between different subgroups. Statistical significance was defined as $P<0.05$. Data analysis was performed using the SPSS 11.7 software package (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of NSCLC patients

The baseline demographic and clinical features of the 166 enrolled patients with NSCLC in the

Table 1. Associations among MRP, LRP, P-gp, GST-π, Topo IIα and clinical features of non-small cell lung cancer

Characteristic	MRP Low (n, %)	MRP High (n, %)	chi-square value	P	LRP Low (n, %)	LRP High (n, %)	chi-square value	P	P-gp Low (n, %)	P-gp High (n, %)	chi-square value	P	GST-π Negative (n, %)	GST-π Positive (n, %)	chi-square value	P	Topo IIα Negative (n, %)	Topo IIα Positive (n, %)	chi-square value	*P*
Age (range), y																				
<60	27 (42.9%)	54 (47.4%)	0.333	0.564	13 (44.8%)	73 (47.4%)	0.065	0.799	36 (48.6%)	48 (44.9%)	0.252	0.615	18 (42.9%)	65 (46.8%)	0.198	0.656	4 (44.4%)	80 (46.5%)	0.015	0.904
≥60	36 (57.1%)	60 (52.6%)			16 (55.2%)	81 (52.6%)			38 (51.4%)	59 (55.1%)			24 (57.1%)	74 (53.2%)			5 (55.6%)	92 (53.5%)		
Gender																				
Male	39 (68.4%)	62 (61.4%)	0.782	0.377	22 (88.0%)	83 (60.1%)	7.165	*0.007*	40 (61.5%)	63 (65.6%)	0.281	0.596	17 (48.6%)	86 (68.3%)	4.604	*0.032*	2 (22.2%)	101 (66.4%)	7.211	*0.011*
Female	18 (31.6%)	39 (38.6%)			3 (12.0%)	55 (39.9%)			25 (38.5%)	33 (34.4%)			18 (51.4%)	40 (31.7%)			7 (77.8%)	51 (33.6%)		
Pathological type																				
Squamous cell carcinoma	25 (43.9%)	40 (39.6%)	0.273	0.602	24 (96.0%)	42 (30.4%)	37.761	*0.000*	30 (46.2%)	36 (37.5%)	1.200	0.273	12 (34.3%)	54 (42.9%)	0.832	0.362	1 (11.1%)	64 (42.1%)	3.391	0.062
Adenocarcinoma	32 (56.1%)	61 (60.4%)			1 (4.0%)	96 (69.6%)			35 (53.8%)	60 (62.5%)			23 (65.7%)	72 (57.1%)			8 (88.9%)	88 (57.9%)		
Tumor differentiation																				
Well + moderate	46 (83.6%)	62 (66.0%)	5.437	*0.020*	20 (80%)	92 (71.3%)	0.796	0.264	51 (82.3%)	59 (66.3%)	4.709	*0.030*	23 (65.7%)	87 (74.4%)	1.007	0.316	8 (88.9%)	101 (71.1%)	1.330	0.229
Poor	9 (16.4%)	32 (34.0%)			5 (20%)	37 (28.7%)			11 (17.7%)	30 (33.7%)			12 (34.3%)	30 (25.6%)			1 (11.1%)	41 (28.9%)		
Regional lymph node metastasis																				
Negative	34 (59.6%)	52 (51.5%)	0.979	0.322	19 (76.0%)	68 (49.0%)	6.074	*0.014*	36 (55.4%)	50 (52.1%)	0.170	0.680	19 (54.3%)	67 (53.2%)	0.014	0.907	4 (44.4%)	83 (54.6%)	0.353	0.552
Positive	23 (40.4%)	49 (48.5%)			6 (24.0%)	70 (50.7%)			29 (44.6%)	46 (47.9%)			16 (45.7%)	59 (46.8%)			5 (55.6%)	69 (45.4%)		
Stage																				
I + II	36 (63.2%)	55 (54.5%)	1.130	0.288	19 (76.0%)	73 (52.9%)	4.595	*0.032*	38 (58.5%)	53 (55.2%)	0.167	0.683	21 (60.0%)	70 (55.6%)	0.220	0.639	4 (44.4%)	88 (57.9%)	0.628	0.325
III + IV	21 (36.8%)	46 (45.5%)			6 (24.0%)	65 (47.1%)			27 (41.5%)	43 (44.8%)			14 (40.0%)	56 (44.4%)			5 (55.6%)	64 (42.1%)		

Table 2. The correlations between clinical features and combination expression of MRP, LRP and P-gp in non-small cell lung cancer

Characteristics	LRP and MRP Low (n, %)	LRP or MRP High (n, %)	LRP and MRP High (n, %)	chi-square value	P	P-gP and MRP Low (n, %)	P-gp or MRP High (n, %)	P-gp and MRP High (n, %)	chi-square value	P	P-gP and LRP Low (n, %)	P-gp or LRP High (n, %)	P-gp and LRP High (n, %)	chi-square value	P
Age (range), y															
<60	5 (50.0%)	26 (42.6%)	41 (47.7%)	0.441	0.802	13 (38.2%)	37 (53.6%)	31 (42.5%)	2.806	0.246	5 (35.7%)	33 (53.2%)	36 (42.9%)	2.228	0.328
≥60	5 (50.0%)	35 (57.4%)	45 (52.3%)			21 (61.8%)	32 (46.4%)	42 (57.5%)			9 (64.3%)	29 (46.8%)	48 (57.1%)		
Gender															
Male	10 (100.0%)	41 (67.2%)	50 (58.1%)	7.202	0.005	20 (66.7%)	39 (62.9%)	41 (63.1%)	0.142	0.931	13 (92.9%)	36 (58.1%)	54 (64.3%)	6.029	0.025
Female	0	20 (32.8%)	36 (41.9%)			10 (33.3%)	23 (37.1%)	24 (36.9%)			1 (7.1%)	26 (41.9%)	30 (35.7%)		
Pathological type															
Squamous cell carcinoma	10 (100.0%)	29 (47.5%)	26 (30.2%)	19.524	0.000	12 (40.0%)	31 (50.0%)	22 (33.8%)	3.443	0.179	13 (92.9%)	28 (45.2%)	25 (29.8%)	20.352	0.000
Adenocarcinoma	0	32 (53.5%)	60 (69.8%)			18 (60.0%)	31 (50.0%)	43 (66.2%)			1 (7.1%)	34 (54.8%)	59 (70.2%)		
Tumor differentiation															
Well + moderate	9 (90.0%)	48 (80.0%)	51 (64.6%)	5.727	*0.049*	27 (93.1%)	43 (72.9%)	38 (63.3%)	8.786	*0.006*	13 (92.9%)	45 (76.3%)	52 (66.7%)	4.69	0.066
Poor	1 (10.0%)	12 (20.0%)	28 (35.4%			2 (6.9%)	16 (27.1%)	22 (36.7%)			1 (7.1%)	14 (23.7%)	26 (33.3%)		
Regional lymph node metastasis															
Negative	8 (80.0%)	36 (59.0%)	41 (47.7%)	4.726	0.084	18 (60.0%)	34 (54.8%)	33 (50.8%)	0.725	0.696	13 (92.9%)	29 (46.8%)	43 (51.2%)	10.005	*0.003*
Positive	2 (20.0%)	25 (41.0%)	45 (53.3%)			12 (40.0%)	28 (45.2%)	32 (49.2%)			1 (7.1%)	33 (53.2%)	41 (48.8%)		
Stage															
I + II	8 (80.0%)	38 (62.3%)	44 (51.2%)	4.053	0.120	19 (63.3%)	36 (58.1%)	35 (53.8%)	0.778	0.678	13 (92.9%)	31 (50.0%)	46 (54.8%)	8.683	*0.005*
III + IV	2 (20.0%)	23 (37.7%)	42 (48.8%)			11 (36.7%)	26 (41.9%)	30 (46.2%)			1 (7.1%)	31 (50.0%)	38 (45.2%)		

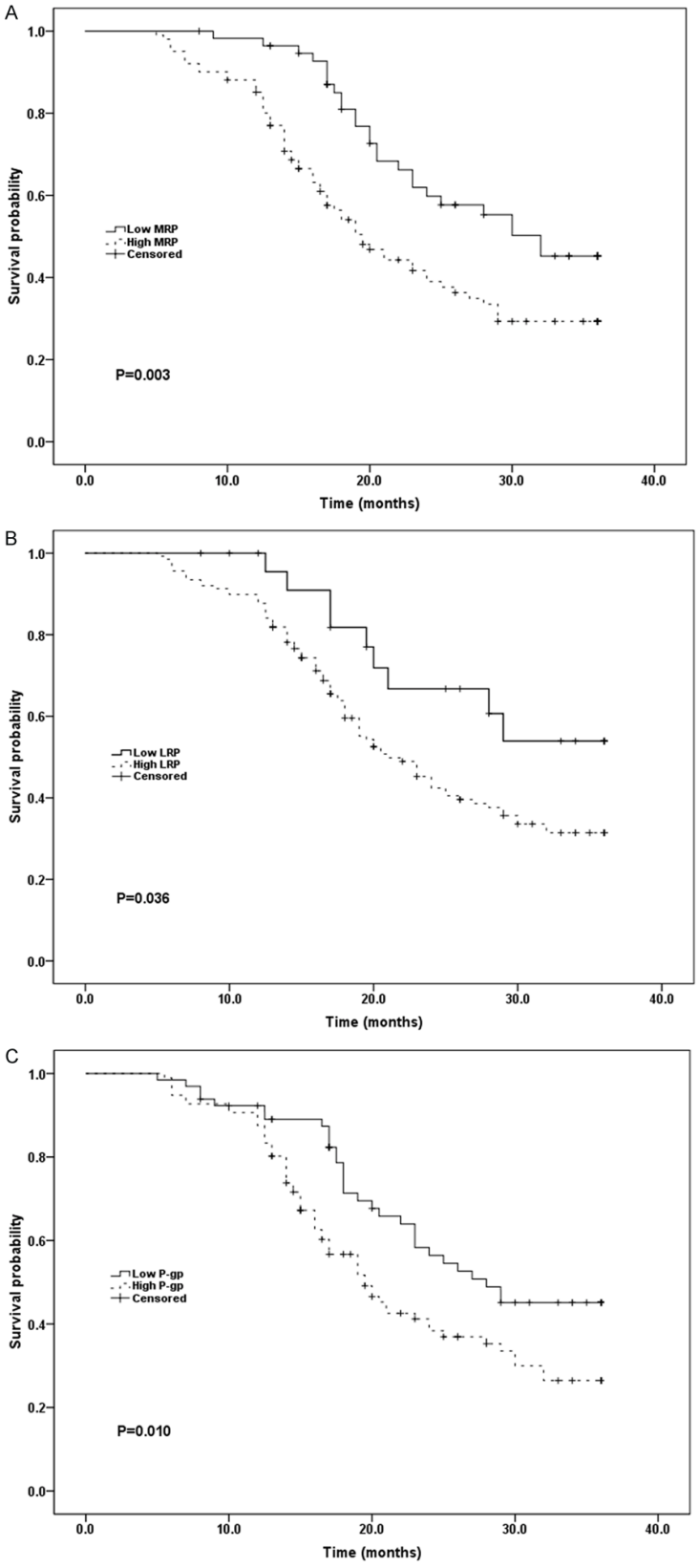

Figure 2. Kaplan-Meier curves for overall survival according to MRP, LRP and P-gp expression in patients with NSCLC that treated with platinum-based chemotherapy.

present study are summarized in Supplementary Table 1. The expression and cell location of these biomarkers were shown in **Figure 1**.

Correlation between these biomarkers' expression and clinicopathological parameters in NSCLC

Clinicopathological features of NSCLC are classified as negative/low expression or positive/high expression according to the protein expression status, respectively (**Table 1**). Compare to negative expression of MRP, positive expression of MRP was associated with poor differentiation (P=0.020), similar result was found in P-gp (P=0.030). AS for LRP, overexpression of LRP was found to be correlated with adenocarcinoma, regional lymph node metastasis (P=0.014) and advanced stages (P=0.032). No significant difference was found between varied expression levels of GST-π and Topo IIα and clinicopathological features (P> 0.05) (**Table 1**).

In the present study, we also carried out the association studies between combination proteins (combination of MRP and LRP, combination of MRP and P-gp, combination of LRP and P-gp) and clinical parameters in NSCLC (**Table 2**).

Correlation of these biomarkers' expression with survivals

All NSCLC patients were followed up until February 2011, during follow-up period, 72 (43.4%) patients were survived, while 94 (56.6%) died from disease progression. The OS using the Kaplan-Meier analysis revealed that the prognosis of NSCLC patients with high MRP, LRP or P-gp expression was significant-

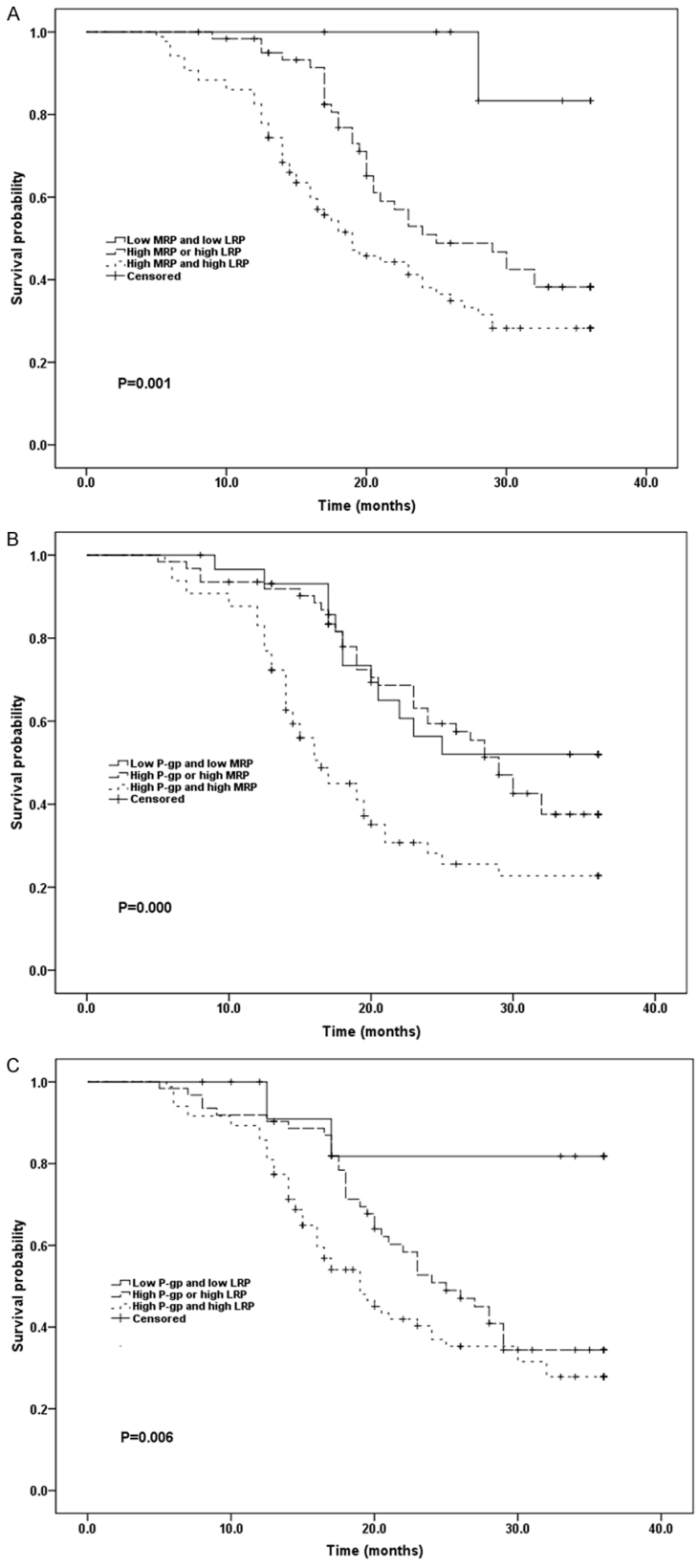

Figure 3. Kaplan-Meier curves for overall survival according to MRP, LRP or P-gp combination expression in patients with NSCLC that treated with platinum-based chemotherapy.

ly poorer than those with low MRP, LRP or P-gp expression, respectively (MRP: log-rank P= 0.003; LRP: log-rank P=0.036; P-gp: log-rank P=0.010) (**Figure 2**). As for GST-π and Topo IIα, Kaplan-Meier plots showed that neither GST-π nor Topo IIα expression was associated with OS (Supplementary Figure 1).

Interestingly, when analysis was conducted by combination MRP and LRP, patients with double negative expression of the two proteins had the longest survival time, patients with single positive expression of two proteins had mediate survival time, while patients with double positive expression of two proteins had the shortest survival time, survival time in the subgroups were markedly significant (log-rank P= 0.001) (**Figure 3A**). when analysis was conducted by combination MRP and P-gp, patients with double negative or single negative expression of the two proteins had longer survival time, while patients with double positive expression of the two proteins had shorter survival time, survival time in the subgroups were significantly different (log-rank P=0.000) (**Figure 3B**). when analysis was conducted by combination LRP and P-gp, patients with double negative expression of the two proteins had longer survival time, while patients with single positive or double positive expression of the two proteins had shorter survival time, survival time in the subgroups were significantly different (log-rank P=0.006) (**Figure 3C**).

The predictive impact of clinicopathologic parameters is shown in Supplementary Table 2. A multivariate analysis was performed using Cox regression model, the results showed that MRP (P=

0.001), P-gp (P=0.009) were independent factors associated with OS.

Discussions

Although the mechanisms of MDR remains to be elucidate, it may be possibly associated with changes in the expression levels of a variety of MDR-related proteins. In the present study, we reported that overexpression of MRP, LRP or P-gp in human NSCLC patients were markedly correlated with platinum-based chemotherapy resistance and shorter survival time, respectively. Interestingly, patients with double negative expression of the three proteins had the longest survival time, while patients with double positive expression of the three proteins had the shortest survival time. To our knowledge, this is the first report to evaluate the association between P-gp and NSCLC survival, furthermore, this is the first study to evaluate the combination predictive value of MRP, LRP and P-gp in NSCLC patients.

Generally, MRP is widely distributed in NSCLCs. In our present study, 63.9% patients showed MRP positivity. Compare to negative expression of MRP, positive expression of MRP was associated with poor differentiation. Overexpression of MRP was associated with worse survival compare to lower expression of MRP. MRP is an independent predictive factor by multivariate analysis. Our data was consistent with some previous studies [9, 10]. However, results from some other studies were inconsistent with ours. No correlation was seen between MRP expression and chemotherapy response or survival with platinum-based combinations in some other NSCLC studies [11-13]. Meta-analysis including all the published papers related with MRP and NSCLC survival are warranted.

In our study, the positive rate of LRP was 84.7%, which was similar with previous study [14]. Our data also demonstrated that overexpression of LRP had more patients with adenocarcinoma, more regional lymph node metastasis and advanced stages. Of note, the positive LRP protein expression rate in lung adenocarcinoma was 99.0% (96/97), markedly higher compared with that of squamous cell carcinoma 63.6% (42/66), which was markedly different (P< 0.001). This finding suggests that LRP may be treated as an adjuvant marker to distinguish lung adenocarcinoma from lung squamous cell carcinoma. Larger population analyses are needed to confirm the finding. Our results also showed that higher expression level of LRP was significantly associated with shorter survival of NSCLC patients, which was similar with the previous studies [10, 14]. However, another previous study did not detect any correlation between the expression of LRP and the response to chemotherapy in patients with advanced NSCLC [15]. Overall, the predictive value of LRP in NSCLC needs to be confirmed in a larger population later.

The positivity rate of P-gp in our results is 59.6%, which was consistent with previous studies [16]. Positive expression of P-gp was associated with poor differentiation. Our data also indicated that overexpression of P-gp was associated with shorter survival and served as a independent factor in NSCLC patients, which is the first report for the predictive value of P-gp in NSCLC till now.

Moreover, we evaluated the prognostic value combined MRP and LRP, MRP and P-gp, LRP and P-gp, respectively. To best of our knowledge, this is the first study to assess prognostic implications of combined MRP, LRP and P-gp expression in patients with NSCLC treated with platinum-based chemotherapy. As expected, patients with double negative expression of the three proteins had the longest survival time, while patients with double positive expression of the three proteins had the shortest survival time. These findings suggest that combination of any two of the three biomarkers including MRP, LRP and P-gp is a better options for prognosis evaluation when expression level of single biomarker is opposite in patients with NSCLC.

Recently, one study has demonstrated that overexpression of Topo IIα (with a positive rate of 37.8%) was significantly correlated with brain metastatic features in NSCLC [17]. While in our current study, the positive rate of Topo IIα is as high as 95%, this may be partly due to the different definition of Topo IIα positive and partly due to population diversity in different area. Standard diagnosis criterion and larger scale of population study is needed to clarify the association between survival time and expression level of Topo IIα.

As for GST-π, Our GST-π results showed that 78.6% of samples were GST-π positive. Moreover, there's no significant association between GST-π and clinicopathological features, and no significant correlation between GST-π and OS. Our results were consistent with some others [18, 19]. However, some results were inconsistent with ours. Shi et al. [20] showed that GST-π was higher in poorly differentiated tumor cells than in moderately and well-differentiated cells. Overexpression of GST-π was associated with decreased response to various platinum-based regimens [21]. Results from Allen et al. showed that higher expression of GST-π was associated with decreased survival of NSCLCs [22]. Further studies related with GST-π and NSCLC survival should be conducted later.

Two important limitations must be recognized. Firstly, this study is conducted retrospectively. A prospective study is needed to determine the combination prognostic value of MRP, LRP and P-gp. Secondly, the size of the studied population was relatively small and follow-up time is relatively short. Therefore, our results should be interpreted conservatively. Future studies with larger population and longer follow-up time are warranted to confirm our results.

Conclusion

In conclusion, the present study found that overexpression of MRP, LRP or P-gp is significantly associated with poor survival of NSCLC patients treated by platinum-based chemotherapy, respectively. Besides, MRP and P-gp can be treated as independent factors for prognosis. Moreover, combination of MRP, LRP and P-gp are more useful predictive indicators than single marker in NSCLC patients treated with platinum-based chemotherapy.

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Disclosure of conflict of interest

None.

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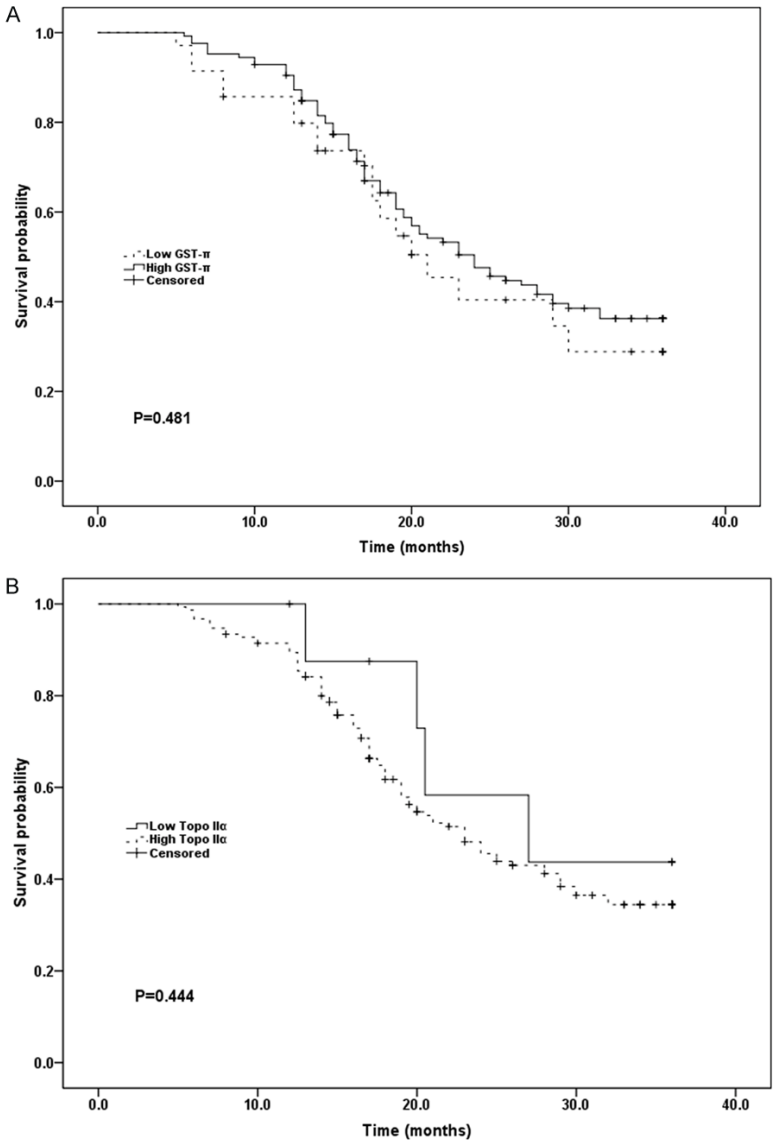
Supplementary Table 1. The clinical features of all the patients with non-small cell lung cancer

Characteristics	All patients	
	N	%
Age (range), y		
<60	78	47.0%
≥60	88	53.0%
Gender		
Male	106	63.9%
Female	60	36.1%
Pathological type		
Squamous cell carcinoma	66	39.8%
Adenocarcinoma	100	60.2%
Tumor differentiation		
Well + moderate	113	72.4%
Poor	43	27.6%
Regional lymph node metastasis		
Negative	88	53.7%
Positive	76	46.3%
Stage		
I + II	93	56.0%
III + IV	73	44.0%
GST-π		
Low	35	21.7%
High	126	78.3%
MRP		
Low	57	36.1%
High	101	63.9%
LRP		
Low	25	15.3%
High	138	84.7%
P-gp		
Low	65	40.4%
High	96	59.6%
Topo IIα		
Low	9	5.6%
High	152	94.4%



Supplementary Figure 1. Kaplan-Meier curves for overall survival according to GST-π and Topo IIα expression in patients with NSCLC that treated with platinum-based chemotherapy.

Supplementary Table 2. Multivariate cox regression analysis of the factors affecting the survival of the patients

Variables	*P*
Age	0.127
Pathological type	0.967
Tumor differentiation	0.504
Regional lymph node metastasis	0.305
Stage	0.096
GST-π	0.075
MRP	*0.001*
LRP	0.120
P-gp	*0.009*
Topo IIα	0.878