Multidrug resistance-associated biomarkers PGP, GST-π, Topo-II and LRP as prognostic factors in primary ovarian carcinoma

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ABSTRACT

This study aims to investigate the expression of P-glycoprotein (PGP), glutathione S-transferase π (GST-π), DNA topoisomerase II (Topo-II) and lung resistance-related protein (LRP) in ovarian carcinoma, thus providing better chemotherapy choice and post-operative prognosis for ovarian carcinoma patients. A total of 80 primary ovarian carcinoma, 16 benign ovarian epithelial neoplasm, and 12 normal ovarian tissue samples were collected. Immunohistochemistry was used to detect the expression of PGP, GST-π, Topo-II and LRP, and the results were analysed by correlation with clinicopathological parameters. Positive expression rates of PGP, GST-π, Topo-II and LRP in patients with ovarian carcinoma (57.5%, 58.8%, 76.3% and 73.8%, respectively) were all higher than those found in normal and benign tissue ($P<0.05$). In clinical stages I/II *vs.* III/IV, the expression rates of PGP, GST-π, Topo-II and LRP were 40.7% *vs.* 66% ($P<0.05$), 40.7% *vs.* 67.9% ($P<0.05$), 66.7% *vs.* 81.1% ($P>0.05$) and 55.6% *vs.* 83.0% ($P<0.05$), respectively. Carcinoma differentiation ranged from well to poor, and expression levels of each marker were as follows: PGP, 57.9%, 62.1% and 53.1% ($P>0.05$); GST-π, 36.8%, 55.2% and 75.0% ($P<0.05$); Topo-II, 52.6%, 79.3% and 87.5% ($P<0.05$); and LRP, 84.2%, 69.0% and 71.9% ($P>0.05$). Ovarian carcinoma patients with PGP-, GST-π-, Topo-II- and LRP-positive expression had a shorter median survival time than those who were negative for these markers (PGP: 36 months *vs.* 48 months [$P=0.0017$]; GST-π: 36 months *vs.* 41 months [$P=0.0103$]; Topo-II: 37 months *vs.* 39 months [$P=0.3811$]; LRP: 37 months *vs.* 55 months [$P=0.002$]). COX regression analysis demonstrated that the clinical stage of the tumour, and the expression of PGP, GST-π or LRP, may influence patient survival time after surgery. The relative death risk for patients with clinical stage III/IV tumours increased 9.46-fold compared to those with stage I/II tumours. The relative death risk in the PGP-, GST-π- and LRP-positive groups increased by 2.049-, 2.452- or 2.609-fold, respectively, compared with the corresponding negative groups. PGP, GST-π, Topo-II and LRP are all expressed in primary ovarian carcinoma, indicating the presence of multidrug resistance in this disease. Combined evaluation of PGP, GST-π, Topo-II and LRP expression may enable better chemotherapeutic choice and provide an accurate prognosis for ovarian carcinoma patients.

KEY WORDS: DNA topoisomerase, type II.
Drug resistance, multiple.
Glutathione S-transferase pi.
Lung resistance-related protein.
Ovarian neoplasms. P-Glycoprotein. Prognosis

Introduction

Chemotherapy is a valuable form of combined therapy for ovarian carcinoma. However, multidrug resistance means that no significant improvement in survival for ovarian carcinoma patients is afforded by this treatment option. Parker *et al.*[1] have reported that the first chemotherapy treatment for ovarian carcinoma achieves 76% effectiveness, but this reduces to 20% after relapse. In the present study, immunohistochemistry was used to investigate the expression of four multidrug resistance marker proteins (P-glycoprotein [PGP], glutathione S-transferase π [GST-π], DNA topoisomerase II [Topo-II] and lung resistance-related protein [LRP]) in primary ovarian carcinoma. It is hoped that these data will enable more effective decision-making in the choice of post-operative chemotherapeutic strategy, and provide a better prognosis for ovarian carcinoma patients.

Materials and methods

Eighty ovarian carcinoma patients (age range: 19–78 years, mean: 52.4 years) were enrolled from the Department of Gynaecology and Obstetrics, Yangzhou University School of Clinical Medicine, from January 2000 to December 2005. None had received treatment prior to surgery. The lesions included serous cystadenocarcinoma ($n=48$), mucinous cystadenocarcinoma ($n=19$) and endometrioid carcinoma ($n=13$). Histological grading was assessed using Broder's method (grade I [$n=19$], II [$n=29$), III [$n=32$]]. Clinical stage was defined according to FIGO stage criteria, with 27 cases being stage I/II and 53 cases being stage III/IV. Sixteen specimens of benign ovarian epithelial neoplasm and 12 specimens of normal ovarian tissue were collected simultaneously to serve as the benign control group and the normal control group, respectively.

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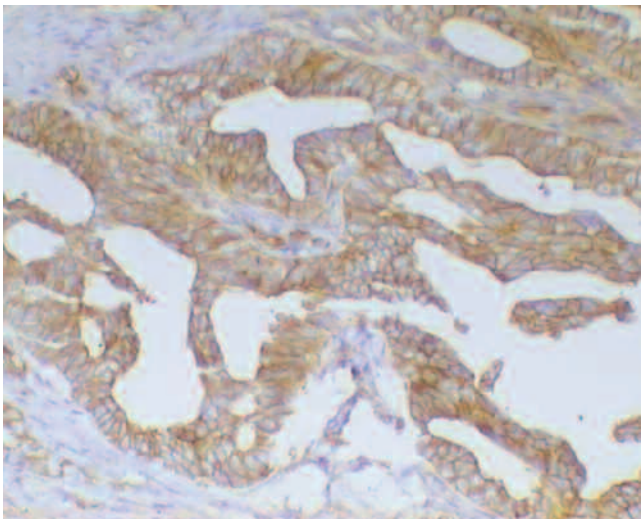

Fig. 1a. Immunohistochemical staining of PGP identified in the cytomembrane of malignant cells (original magnification x200).

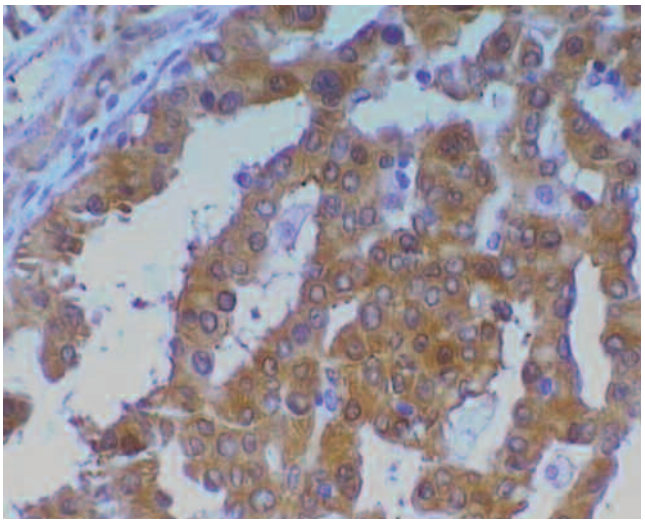

Fig. 1b. Immunohistochemical staining of GST-π identified in the cytoplasm of malignant cells (original magnification x200).

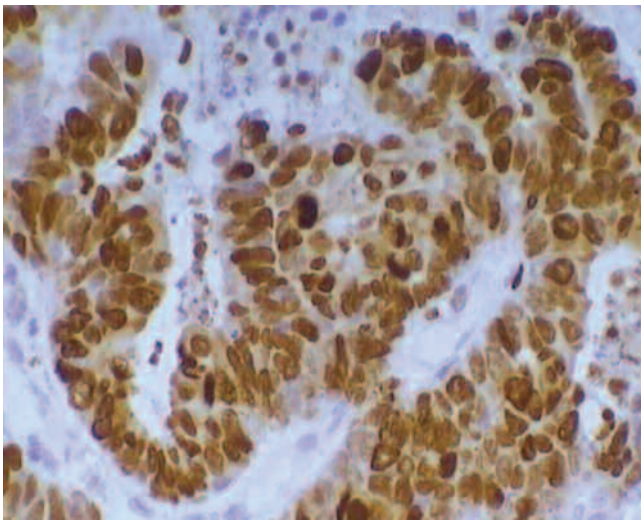

Fig. 1c. Immunohistochemical staining of Topo-II recognised to be expressed in the nuclei of malignant cells (original magnification x200

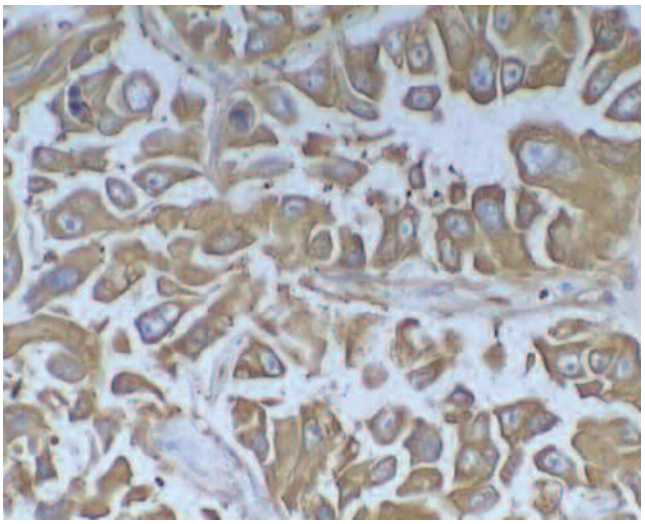

Fig. 1d. Immunohistochemical staining of LRP was located in the cytoplasm of malignant cells (original magnification x200)

All specimens were fixed in 10% formalin, embedded in paraffin wax and cut at 4 μm. Sections were stained with haematoxylin and eosin (H&E) and with mouse anti-human antibodies to PGP, GST-π, Topo-II or LRP (Beijing Zhong Shan-Golden Bridge Biological Technology, China). Staining procedures were performed according to the instructions provided with the primary antibodies, and signal detection was performed using streptavidin peroxidase. Normal tissues or tumour tissue sections positive for marker expression were used as positive controls, while phosphate-buffered saline (PBS) was used instead of primary antibody in the negative controls.

Scoring was performed according to the percentage of marker-positive cells per total cell number in each section and graded as follows:[2] (–) <10% marker-positive cancer cells, (+) 10–25% marker-positive cancer cells, (++) 25–75% marker-positive cancer cells, and (+++) >75% marker-positive cancer cells.

All patients were included in follow-up visits until 31 December 2007. Survival time was calculated from the day of surgery to either the day of the last follow-up visit or the day of death due to relapse or metastasis.

Statistical analysis

Data management was performed using SPSS 10.0 software. Clinical and pathological data were analysed in correlation with marker protein expression and ovarian carcinoma stage using the χ^2 test and Fisher's definite probability method. Survival curves were plotted according to the Kaplan-Meier method. A Log-Rank test was used to compare survival times. Prognosis analysis was conducted using the multifactorial COX risk regression model.

Results

Results for expression of PGP, GST-π, Topo-II and LRP are shown in Table 1. Positive staining was observed in the plasma membrane (Fig. 1a), cytoplasm (Fig. 1b), nucleus (Fig. 1c) or cytoplasm (Fig. 1d). Positive expression rates were

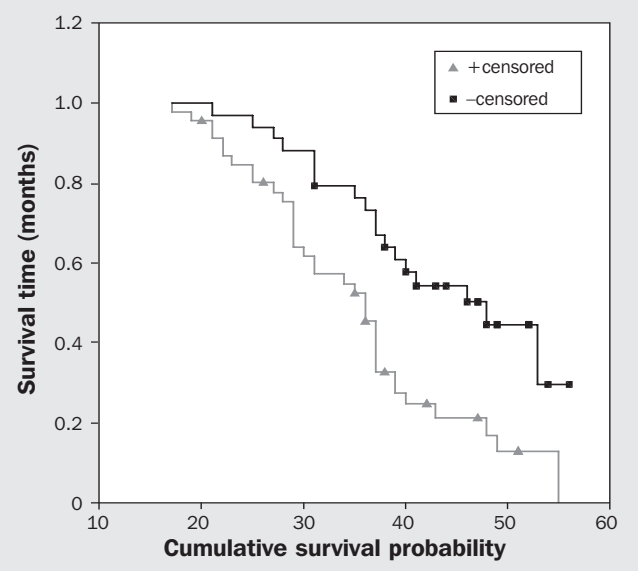

Fig. 2a. Patients with PGP-positive expression had a significantly shorter survival time than those with negative expression (χ^2=9.81, P=0.0017).

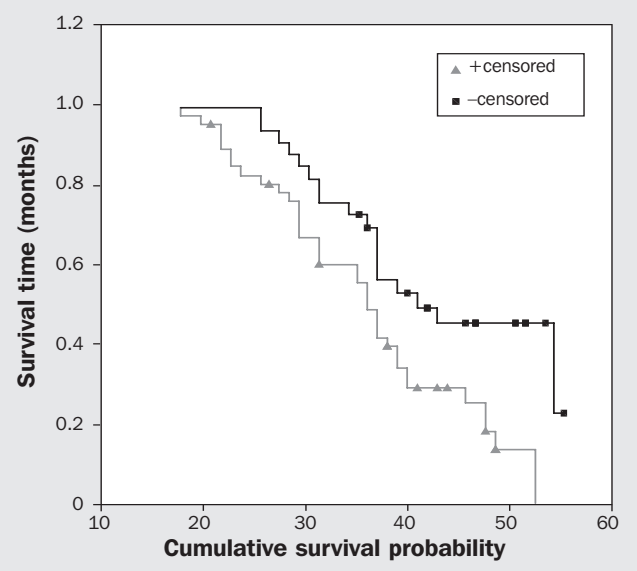

Fig. 2b. Patients with GST-π-positive expression had a significantly shorter survival time than those with negative expression (χ^2=6.58, P=0.0103).

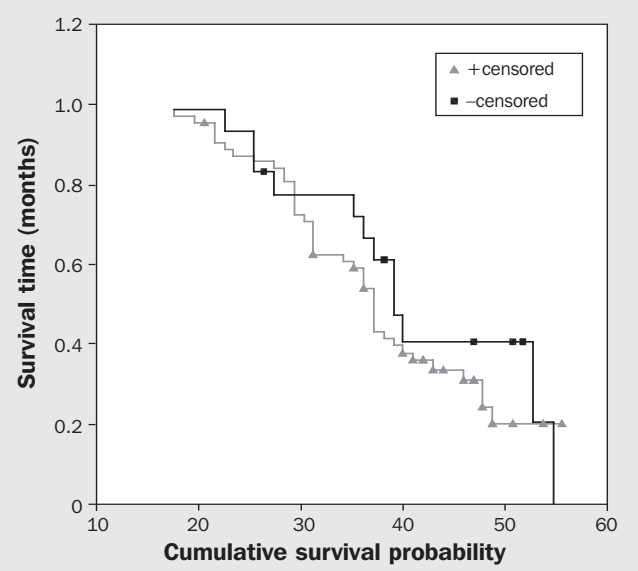

Fig. 2c. No significant correlation was observed between the expression of Topo-II and median survival time (χ^2 = 0.77, P=0.3811).

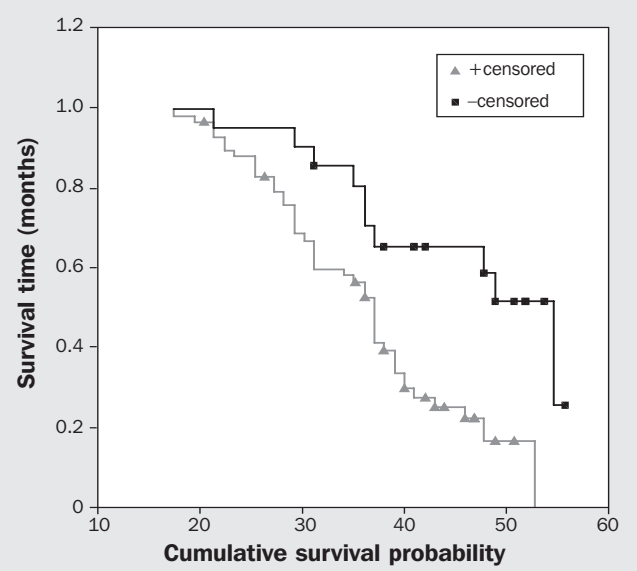

Fig. 2d. Patients with LRP-positive expression had a significantly shorter survival time than those with negative expression (χ^2=9.48, P=0.0021).

higher than those in benign tumour and normal tissues (P<0.05).

Correlation between clinical pathological parameters and expression of PGP, GST-π, Topo-II and LRP is shown in Table 2. In Figure 2, during clinical stages I/II *vs.* III/IV, the expression rates of PGP, GST-π, Topo-II and LRP were 40.7% *vs.* 66% (P<0.05), 40.7% *vs.* 67.9% (P<0.05), 66.7% *vs.* 81.1% (P>0.05) and 55.6% *vs.* 83.0% (P<0.05), respectively. Carcinoma differentiation ranged from well to poor and marker expression levels were: PGP, 57.9%, 62.1% and 53.1% (P>0.05); GST-π, 36.8%, 55.2% and 75.0% (P<0.05); Topo-II, 52.6%, 79.3% and 87.5% (P<0.05) and LRP, 84.2%, 69.0% and 71.9% (P>0.05). Expression of PGP, GST-π, Topo-II and LRP showed no correlation with histological type of the ovarian carcinoma tissue, original tumour size, or size of the residual focus (P>0.05).

Correlation between survival time and expression of PGP, GST-π, Topo-II and LRP is shown in Figures 2a–d).

Patients with PGP-, GST-π- or LRP-positive expression had a significantly shorter survival time than those with negative expression (PGP: 36 months *vs.* 48 months [P=0.0017]; GST-π: 36 months *vs.* 41 months [P=0.0103]; LRP: 37 months *vs.* 55 months [P=0.0021]). However, no significant correlation was observed between the expression of Topo-II and median survival time (P=0.3811).

COX regression analysis of the factors influencing survival time is shown in Table 3. Regression analysis demonstrated that tumour clinical stage and expression of PGP, GST-π or LRP may influence survival time of ovarian carcinoma patients following surgery. These four indices were all

Table 1. Expression of PGP, GST-π, Topo-II and LRP in ovarian tissue.

Group	Cases	PGP (%)	GST-π (%)	Topo-II (%)	LRP (%)	*P* value
Normal tissue	12	2 (16.7)	1 (8.3)	2 (16.7)	1 (8.3)	<0.05
Benign tumour tissue	16	3 (18.8)	4 (25.0)	5 (31.3)	4 (25.0)	
Ovarian carcinoma tissue	80	46 (57.5)	47 (58.8)	61 (76.3)	59 (73.8)	

risk factors. Relative death risk for patients with clinical stage III/IV tumours increased 9.46-fold compared to those with stage I/II tumours. The relative death risk for patients in the PGP-, GSTπ- or LRP-positive groups increased 2.049-, 2.452- and 2.609-fold, respectively, compared with their corresponding negative groups.

Discussion

The multidrug resistance of tumours hinders the progressive advancement of chemotherapy for the treatment of ovarian carcinoma. Studies have demonstrated that the complete remission rate and five-year survival rate in ovarian carcinoma patients remains at 20–40% and 10–20%, respectively.[3,4] due to primary or acquired drug resistance to chemotherapeutics. However, combined chemotherapy based on Platinum (Pt) has improved the response rate and prognosis of chemotherapy after cytoreductive surgery.

P-glycoprotein, the product of multidrug resistance gene-1, can eliminate some natural drugs from cells and reduce cumulative drug concentration, thereby hindering the effects of chemotherapeutics. The expression of PGP is positively related to the observed drug resistance.[5] In the present study, the positive expression rate of PGP in normal tissues and benign tumour tissues was 16.7% and 18.8%, respectively. According to the findings of Holzamayer *et al*.[5] the ovary is not rich in PGP. From the data presented here, the positive expression rate of PGP was 57.5% (46/80 ovarian carcinoma patients), which suggests that PGP does not play a key role in mediating primary drug resistance in ovarian carcinoma. This is also consistent with the fact that most ovarian carcinoma patients are responsive to initial chemotherapy, while few exhibit primary drug resistance. In addition, the median survival times of patients in the PGP-positive and -negative expression groups were 36 months and 48 months, respectively. The relative death risk for patients with PGP-positive expression was 2.049-fold higher than in the negative expression group, which suggests that PGP expression has an effect on survival rate. Expression of PGP by tumour tissue has clinical value for predicting drug resistance to, and prognosis for, ovarian carcinoma chemotherapy.

Tissue distribution of GST-π *in vivo* is organ-specific. GST-π is a predominant marker of human ovary and ovarian carcinoma tissue, and its high expression may reflect embryonic expression levels.[7] Vanhoefer *et al*.[8] have shown that GST-π and PGP co-mediate the occurrence of early drug resistance via a mechanism in which GST-π assists drug

Table 2. Correlation between the expression of PGP, GST-π, Topo-II, LRP and clinical pathology data in ovarian carcinoma.

Group	Cases	PGP positive (%)	GST-π positive (%)	Topo-II positive (%)	LRP positive (%
Histological type					
Serous cystadenocarcinoma	48	29 (64.4)	29 (60.4)	38 (79.2)	35 (72.9)
Mucinous cystadenocarcinoma	19	9 (47.4)	8 (42.1)	13 (68.4)	15 (78.9)
Endometrioid carcinoma	13	8 (61.5)	10 (76.9)	10 (76.9)	9 (69.2)
Histological grade					
I	19	11 (57.9)	7 (36.8)*	10 (52.6)*	16 (84.2)
II	29	18 (62.1)	16 (55.2)	23 (79.3)	20 (69.0)
III	32	17 (53.1)	24 (75.0)	28 (87.5)	23 (71.9)
FIGO stage					
I/II	27	11 (40.7)*	11 (40.7)*	18 (66.7)	15 (55.6)*
III/IV	53	35 (66.0)	36 (67.9)	43 (81.1)	44 (83.0)
Size of tumour (cm)					
≤10	26	13 (56.6)	12 (48.0)	18 (69.2)	20 (76.9)
>10	54	33 (61.1)	35 (64.8)	43 (79.6)	39 (72.2)
Size of residual focus (cm)					
≤2	38	20 (52.6)	21 (55.2)	30 (78.9)	24 (63.2)
>2	42	26 (61.9)	26 (61.9)	31 (73.8)	35 (83.3)
Total	80				

*$P<0.05$

Table 3. COX multivariate analysis on the factors influencing survival time in ovarian carcinoma.

	B (regression coefficient)	Standard error	χ^2	Degree of freedom	*P* value	Relative risk
Age	–0.013	0.015	0.749	1	0.387	0.987
Pathological type	–0.134	0.233	0.333	1	0.564	0.874
Histological grade	–0.164	0.236	0.486	1	0.486	0.848
FIGO stage	2.247	0.919	5.973	1	0.015	9.460
PGP	0.718	0.355	4.078	1	0.043	2.049
GST-π	0.897	0.398	5.069	1	0.024	2.452
Topo-II	0.687	0.436	2.486	1	0.115	1.989
LRP	0.959	0.484	3.926	1	0.048	2.609
Size of primary tumour	0.182	0.420	0.187	1	0.665	1.200
Size of residual focus	0.455	0.490	0.860	1	0.354	1.576
Seroperitoneum	–0.494	0.578	0.731	1	0.392	0.610
CA125	0.000	0.000	0.389	1	0.533	1.000
Curative effect of chemotherapy	0.349	0.395	0.779	1	0.377	1.417

efflux through PGP. In the present study, the positive expression rate of GST-π increased with age in normal ovarian tissues, while expression became stronger in the later clinical stages in patients with tumours. Concurrent de-differentiation of ovarian tissue is possibly related to the fact that GST-π expression increases during malignant tumour development. However, increased expression of GST-π showed no correlation with histological type, size of original tumour or size of residual focus.

Kaplan-Meier survival analysis demonstrated that the median survival time of GST-π-positive patients was 36 months, while that of GST-π-negative patients was 41 months. These data were significantly different between the two groups. At the same time, GST-π negative expression resulted in a constant survival time of 40 months. Therefore, the follow-up visit and treatment regimen should be enforced after 40 months in order to maximise survival rate.

COX regression analysis demonstrated that the relative death risk for GST-π-positive patients was 2.452-fold greater than GST-π-negative patients. GST-π expression has a predictive value for drug resistance to chemotherapy in the treatment of ovarian carcinoma, and can be considered an important prognostic factor of post-operative survival time.

Topo-II activity is related to the cytotoxicity of many anticancer drugs, including adriamycin, etoposide, Pt and 5-fluorouracil.[9,10] In the present study, Topo-II expression in poorly differentiated tumour cells was higher than that in the medium to well-differentiated cells. This suggests that Topo-II levels tend to increase in poorly differentiated tumours due to their rapid proliferation rate.

Topo-II is an important target for tumour chemotherapeutics, and Topo-II expression reflects the sensitivity of anticancer drugs. When Topo-II is highly expressed, Topo-II inhibitors are suitable for use in tumour chemotherapy and subsequently enhance the curative effect. When Topo-II expression decreases, such inhibitors are ineffective because the tumour is not sensitive to them. The DNA topoisomerase inhibitor topotecan is an effective anticancer drug for second-line chemotherapy that lacks cross-drug resistance with Pt, etoposide and paclitaxel.[11]

Lung resistance-related protein is superior to PGP for the prediction of multidrug resistance *in vitro*, and also for non-multidrug-resistant cisplatinum.[12] Art *et al*.[13] analysed the expression of LRP in specimens from 115 cases of ovarian carcinoma and found that grade I and II tumour lesions, as well as residual lesion <2 cm at stage I and II, was easier to observe than grade III tumours, as well as residual lesion <2 cm at stage III and IV. They suggest that LRP expression is related to tumour stage, size of residual tumour, poor differentiation, remission stage and survival time. Moreover, LRP could be considered an independent prognostic factor.

In the present study, LRP expression was considerably higher in carcinoma than that in benign tissues and normal tissues, and the expression level increased with clinical stage. These data demonstrate that LRP exhibited a constant expression level in ovarian carcinoma tissues. The expression was parallel to ovarian epithelial cells during metastasis and was associated with disease course.

Data from the present study were studied using Kaplan-Meier survival analysis and COX regression analysis. The results demonstrate that LRP-positive patients had a different median survival time compared to LRP-negative patients (37 months *vs*. 55 months). The relative death risk of LRP-positive patients was 2.609-fold higher than that of LRP-negative patients. These data suggest that LRP expression has a considerable influence on survival time. Therefore, LRP can be considered a marker for predicting drug resistance of chemotherapeutics and assessing prognosis.

In summary, PGP, GST-π, Topo-II and LRP are all expressed in primary ovarian carcinoma. This reflects the complexity of ovarian carcinoma multidrug resistance mechanisms, and also provides a new route to investigate the reversal of drug resistance. Combined identification of drug resistance gene products in ovarian carcinoma tissues may provide a comprehensive screen for effective low-toxicity drugs with new drug resistance mechanisms. This will be of benefit in defining individual chemotherapy regimes and may facilitate the optimisation of chemotherapeutics and the use of reversal agents and prognostic indices following surgery. □

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