

Expression levels of MRP1, GST- π , and GSK3 β in ovarian cancer and the relationship with drug resistance and prognosis of patients

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Abstract. Expression levels of multidrug resistance-associated protein 1 (*MRP1*), glutathione S-transferase π (*GST- π*) and glycogen synthase kinase-3 β (*GSK3 β*) were investigated in ovarian epithelial cancer and the relationship with the primary drug resistance of patients with ovarian cancer to chemotherapy. One hundred and twenty-one ovarian cancer tissue samples from patients who underwent ovarian cancer resection from January 2013 to June 2015 in Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University were enrolled in the Experimental group, while 58 ovarian tissue samples from patients with fallopian tube obstruction but with no ovarian cancer who received surgical treatment for blocked fallopian tube were included in the Control group. After the detection of the expression levels of *MRP1*, *GST- π* , and *GSK3 β* mRNA by RT-PCR and the analysis of related clinical pathological factors, patients in the Experimental group were divided into the Chemotherapy-sensitive and Chemotherapy-resistant groups according to the chemotherapy efficacy. Additionally, with the mean expression levels of *MRP1*, *GST- π* , and *GSK3 β* in ovarian cancer tissues as the boundaries, the expression levels of the three genes in the Experimental group were classified into high expression and low expression. Ovarian cancer tissues had much higher expression levels of *MRP1*, *GST- π* , and *GSK3 β* mRNA than normal ovarian tissues ($P < 0.05$). The expression levels of *MRP1*, *GST- π* , and *GSK3 β* mRNA in the Chemotherapy-sensitive group were significantly lower than those in the Chemotherapy-resistant group ($P < 0.05$). Patients with high expression of *MRP1*, *GST- π* , and *GSK3 β* mRNA

had a much lower 3-year survival rate than patients with low expression of the genes ($P < 0.05$). Highly expressed in patients with ovarian cancer, *MRP1*, *GST- π* , and *GSK3 β* mRNA play an important role in the development and drug resistance of ovarian cancer, which ensures this study is of positive clinical guiding significance in developing proper treatment for ovarian cancer and evaluating the efficacy of chemotherapy.

Introduction

Ovarian cancer, a common gynecological malignant tumor, is one of the major malignant tumors causing female death (1). At present, the treatment of ovarian cancer mainly adopts methods of surgery and chemotherapy, among which intravenous chemotherapy can not only promote the atrophy of tumor lesions, but also significantly reduce the tumor recurrence rate and metastasis rate (2,3). However, some studies have found that intravenous chemotherapy for ovarian cancer can arouse a subsequent drug resistance of many patients, and that long-term chemotherapy and previous history of chemotherapy can also promote the development of drug resistance (4). At present, drugs used for the clinical chemotherapy for ovarian cancer are mainly paclitaxel and platinum drugs. However, patients receiving the same chemotherapy regimen can be affected with different efficacy and different risk due to the differences in the individual constitution and the sensitivity to chemotherapy, which is why the exploration of a molecular mechanism that can predict chemotherapy resistance is imperative (5).

Studies have shown that multidrug resistance-associated protein 1 (*MRP1*), glutathione S-transferase π (*GST- π*), and glycogen synthase kinase-3 β (*GSK3 β*) may be associated with chemotherapy resistance and prognosis of ovarian cancer (6-8). As a transmembrane protein-encoding gene, *MRP1* is able to cause drug resistance mainly by reducing the intracellular concentration of drugs (9). *GST- π* , is a detoxification enzyme, and its contribution to drug resistance is generally achieved by removing toxic substance from tumor cells to weaken the killing effect on cells (10). *GSK-3 β* is a key target gene downstream of the Akt signaling pathway, an important component of various signal transduction pathways in cells, and is involved in biological functions such as

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intracellular glucose metabolism and cell proliferation and differentiation (11). It has been previously shown that GSK-3 β , when its ser9 residue is inactivated by phosphorylation, has an inhibitory effect on apoptosis of ovarian cancer cells induced by cisplatin (12).

Owing to the complicated mechanism of drug resistance in tumor cells that probably involves multiple drug-resistance related genes, and the still few clinical studies on the prognosis and resistance to ovarian cancer concerning *MRP1*, *GST- π* , *P-GSK3 β* , this study investigated the expression of *MRP1*, *GST- π* , and *P-GSK3 β* in ovarian cancer patients with different chemotherapy outcomes and their relationship with the prognosis to providing more theoretical data for the prediction of chemotherapy resistance in ovarian cancer.

Patients and methods

General information. One hundred and twenty-one ovarian cancer tissue samples from patients (with an average age of 51.23 \pm 11.31 years) who underwent ovarian cancer resection from January 2013 to June 2015 in the Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University (Shenyang, China) were retrospectively analyzed, including 31 cases of mucinous cystadenocarcinoma, 57 cases of serous cystadenocarcinoma, 23 cases of endometrial carcinoma, and 10 cases of clear cell carcinoma. There were 71 patients at stage I-II, 50 patients at stage III-IV on the basis of different clinical pathological stages and 52 cases of high differentiation, 41 cases of moderate differentiation, and 28 cases of poor differentiation according to the classification of histological grading. In total, 121 patients from whom ovarian cancer tissue samples were obtained (Experimental group) received chemotherapy after the ovarian cancer resection, while 58 subjects with fallopian tube obstruction but with no ovarian cancer all received surgical treatment for blocked fallopian tube before providing ovarian tissue samples for the Control group. No significant difference was proven between the two groups in terms of age and number of births ($P>0.05$). The general information of patients is shown in Table I.

Inclusion criteria were: patients confirmed to have ovarian cancer by pathological diagnosis. Exclusion criteria were: patients who received chemoradiotherapy before surgery; patients with severe liver and kidney dysfunction; ovarian cancer patients complicated with other tumors; patients with communication impairment or cognitive dysfunction; patients who did not cooperate with the experiment.

All the patients and their families signed the informed consent form of this experiment. The study was approved by the Ethics Committee of Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University.

Experimental reagents and drugs. Paclitaxel was purchased from Yunnan Hande Biotechnology Co., Ltd., Yunnan, China (license number of medical products permitted by SFDA: H109603221); cisplatin was purchased from Qilu Pharmaceutical Co., Ltd. Jinan, China (license number of medical products permitted by SFDA: H37021358). TRIzol reagent and cDNA reverse transcription kit used in this experiment were from Applied Biosystems-Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA. RT-PCR

kit was purchased from CWBio, Beijing, China. The primers were produced and designed by Sangon Biotech (Shanghai) Co. Ltd., Shanghai, China. Dexamethasone was purchased from Xi'an Reypnon Pharmaceutical Co., Ltd., Xi'an, China (license number of medical products permitted by SFDA: H20053754). Diphenhydramine was manufactured by Tonghua Mintai Pharmaceutical Co., Ltd., Tonghua, China (license number of medical products permitted by SFDA: H22025212).

Chemotherapy regimen. Dexamethasone (20 mg) was taken orally 12 h before the chemotherapy treatment, and diphenhydramine was taken 30 min before the chemotherapy treatment. The chemotherapy in this experiment used paclitaxel plus cisplatin: paclitaxel was infused at a concentration of 135 mg/m², and then an infusion of cisplatin at a concentration of 75 mg/m² was begun from the third day of chemotherapy. The chemotherapy was for 6 courses of treatment and each course equalled 3 weeks.

RT-PCR detection of *MRP1* mRNA, *GST- π* mRNA, and *GSK3 β* mRNA expression levels. The cut and ground, ovarian cancer tissues and normal ovarian tissues were mixed with TRIzol reagent for the extraction of RNA, the purity and concentration of which were then detected by UV spectrophotometer. Next, 1 μ g of total RNA from each sample was taken for reverse transcription of cDNA according to the kit instructions. The reaction parameters were: 37°C for 10 min, 42°C for 45 min, and 70°C for 5 min. Then TaqMan PCR (Beyotime, Shanghai, China) amplification of cDNA followed with *MRP1* and *GSK3* using *GAPDH* as internal reference and *GST- π* using β -actin as internal reference. Primer sequences are shown in Table II. PCR reaction conditions of *MRP1*: pre-denaturation at 94°C for 2 min, then 35 cycles of 94°C for 45 sec and 55°C for 60 sec, followed by a final extension at 65°C for 20 sec; PCR reaction conditions for *GST- π* mRNA: pre-denaturation at 94°C for 5 min, then 35 cycles of 94°C for 45 sec and annealing at 55°C for 35 sec, followed by a final extension at 72°C for 60 sec; PCR reaction conditions of *GSK3 β* : pre-denaturation at 94°C for 3 min, then 40 cycles of 94°C for 45 sec and annealing at 60°C for 40 sec, with a final extension at 72°C for 30 sec. The relative expression of the gene was expressed by 2^{- $\Delta\Delta C_q$} (13), and the RT-qPCR detection was repeated 3 times.

Observation indicators. i) A comparison was made of the *MRP1*, *GST- π* , and *GSK3 β* mRNA expression levels in ovarian cancer tissues and normal ovarian tissues. ii) The efficacy of chemotherapy in patients with ovarian cancer was evaluated, and patients were classified into four types according to the classification system for recurrent ovarian cancer of the American Gynecological Oncology group (14): chemotherapy-sensitive type (patients sensitive to platinum-based chemotherapy who reached a clinical remission period after chemotherapy, but ovarian cancer recurred 6 months after the end of chemotherapy), chemotherapy-resistant type (patients with a certain response to platinum-based chemotherapy and suffering tumor recurrence within 6 months after chemotherapy stopped), inveterate cancer type (patients with very limited response to platinum-based

Table I. General information [n (%)].

Factors	Experimental group (n=121)	Control group (n=58)	t/ χ^2 value	P-value
Age (years)			4.630	0.099
≤51	64 (52.89)	32 (55.17)		
>51	57 (47.11)	26 (44.83)		
BMI (kg/m ²)			0.225	0.894
≤21	58 (47.93)	27 (46.55)		
>21	63 (52.07)	31 (53.45)		
Marital status			0.915	0.633
Married	109 (90.08)	51 (87.93)		
Unmarried	12 (9.92)	7 (12.07)		
Childbearing history			0.368	0.832
Yes	97 (80.17)	47 (81.03)		
No	24 (19.83)	11 (18.97)		
Pathology type			-	-
Mucinous cystadenocarcinoma	31 (25.62)	-		
Serous cystadenocarcinoma	57 (47.11)	-		
Endometrial carcinoma	23 (19.01)	-		
Clear cell carcinoma	10 (8.26)	-		
Pathological stage			-	-
I-II	71 (58.68)	-		
III-IV	50 (41.32)	-		
Degree of differentiation			-	-
High	52 (42.98)	-		
Moderate	41 (33.88)	-		
Poor	28 (23.14)	-		

Table II. Related primer sequences.

Gene name	Forward primers	Reverse primers
<i>MRP1 mRNA</i>	5'-AACCTGGACCCATTCAGCC-3'	5'-GACTGGATGAGGTCGTCCGT-3'
<i>GSK3β mRNA</i>	5'-ATGCCACAGCAGCGTCAG-3'	5'-GGTCTGTCCACGGTCTCC-3'
<i>GAPDH</i>	5'-GCCACATCGCTCAGACACC-3'	5'-GATGGCAACAATATCCACTTTACC-3'
<i>GST-π mRNA</i>	5'-AATGGATCCTCCACCATGCCG CCCTACACCGTGGT-3'	5'-GACCTCGAGCTACTGTTTCCC GTTGCCAT-3'
<i>β-actin</i>	5'-TCAAGATCATTGCTCCTCCTGA-3'	5'-CTCGTCATACTCCTGCTTGCTG-3'

chemotherapy and thus with residual lesions), and refractory cancer type (patients unresponsive to platinum-based chemotherapy). Chemotherapy-sensitive patients were classified into the Chemotherapy-sensitive group, and patients of chemotherapy-resistant, inveterate cancer, and refractory cancer types were included in the Chemotherapy-resistant group. iii) An analysis was made of the comparison for the expression levels of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA between Chemotherapy-sensitive and Chemotherapy-resistant groups. iv) The relationship between the expression levels of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA and the clinical pathological features of ovarian cancer patients was explored. v) A follow-up for

patients was performed to record the 3-year survival rate and a comparison was made between the two groups.

Statistical analysis. In this study, statistical analysis of the data was prepared by SPSS20.0 software [Bizinsight (Beijing) Information Technology Co., Ltd., Beijing, China], and all the graphs in this experiment were drawn using GraphPad Prism 6 software. Comparison of countable data was conducted with the Chi-square test, measurement data were expressed as mean \pm standard deviation. The comparison between the two groups was made using the t-test, the comparison among multiple groups was analyzed by ANOVA and LSD post hoc test, and Kaplan-Meier and log rank test were used for the

Table III. Expression of all indexes in Chemotherapy-sensitive group and Chemotherapy-resistant group.

Gene name	Chemotherapy-sensitive group (n=71)	Chemotherapy-resistant group (n=50)	t value	P-value
<i>MRP1 mRNA</i>	1.51 \pm 0.21	2.71 \pm 0.17	33.41	<0.001
<i>GST-π mRNA</i>	1.62 \pm 0.19	2.66 \pm 0.18	30.29	<0.001
<i>GSK3β mRNA</i>	1.54 \pm 0.23	2.59 \pm 0.17	27.42	<0.001

Table IV. Relationship between MRP1 mRNA and different clinicopathological features in ovarian cancer.

Clinico-pathological factor	n	MRP1		GST- π		GSK3 β	
		mRNA	t/F value P-value	mRNA	t/F value P-value	mRNA	t/F value P-value
Age (years)			1.278 0.204		0.815 0.417		1.498 0.137
\leq 51	64	2.24 \pm 0.21		2.35 \pm 0.13		2.10 \pm 0.16	
>51	57	2.19 \pm 0.22		2.33 \pm 0.14		2.06 \pm 0.13	
Pathological type			1.209 0.310		327.5 <0.050		1.522 0.198
Mucinous cystadenocarcinoma	31	2.41 \pm 0.22		2.36 \pm 0.27		2.17 \pm 0.18	
Serous cystadenocarcinoma	57	2.32 \pm 0.23		3.27 \pm 0.28		2.09 \pm 0.17	
Endometrial carcinoma	23	2.33 \pm 0.18		1.69 \pm 0.15		2.10 \pm 0.19	
Clear cell carcinoma	10	2.35 \pm 0.21		1.42 \pm 0.11		2.08 \pm 0.15	
Pathological stage			23.72 <0.050		45.70 <0.050		39.01 <0.050
I-II	71	1.68 \pm 0.25		2.01 \pm 0.11		1.92 \pm 0.14	
III-IV	50	2.89 \pm 0.31		3.09 \pm 0.15		3.09 \pm 0.19	
Degree of differentiation			790.8 <0.050		1070 <0.050		1084 <0.050
High	52	1.25 \pm 0.21		1.27 \pm 0.22		1.24 \pm 0.19	
Moderate	41	2.34 \pm 0.25		2.21 \pm 0.17		2.59 \pm 0.21	
Poor	28	3.26 \pm 0.19		3.46 \pm 0.21		3.27 \pm 0.20	

MRP1, multidrug resistance-associated protein 1; GST- π , glutathione S-transferase π ; GSK3 β , glycogen synthase kinase-3 β .

survival analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in ovarian cancer and normal ovarian tissues. The expression levels of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in ovarian cancer tissues of the Experimental group were 2.23 \pm 0.23, 2.34 \pm 0.12 and 2.07 \pm 0.17, respectively, significantly higher than those in normal ovarian tissues of the Control group which had an expression of MRP1 mRNA at 1.01 \pm 0.12, GST- π mRNA at 0.94 \pm 0.14, and GSK3 β mRNA at 0.89 \pm 0.11, with a statistical difference between the two groups ($P < 0.05$) (Fig. 1). According to the average expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in ovarian cancer tissues, the expression levels of the three mRNAs were divided into high expression and low expression.

Expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in Chemotherapy-sensitive group and Chemotherapy-resistant group. According to the evaluation of chemotherapy efficacy for the Experimental group, 71 patients were

chemotherapy-sensitive and 50 were chemotherapy-resistant. The expression levels of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in the Chemotherapy-sensitive group were statistically lower than those in the Chemotherapy-resistant group ($P < 0.05$) (Table III).

Relationship between MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA in ovarian cancer tissues and different clinical pathological features. The expression of MRP1 mRNA was associated with pathological stage and pathological differentiation ($P < 0.05$), having no significant relationship with age and pathological type ($P > 0.05$). GST- π mRNA expression was linked to pathological type, pathological stage, and degree of differentiation ($P < 0.05$), but not significantly related to age ($P > 0.05$). GSK3 β mRNA expression was associated with pathological stage and pathological differentiation ($P < 0.05$), with no obvious connection with age and pathological type ($P > 0.05$) (Table IV).

Relationship between MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA expression and 3-year survival rate in patients after operation. In the Experimental group, MRP1 mRNA was highly expressed in 68 patients and expression was

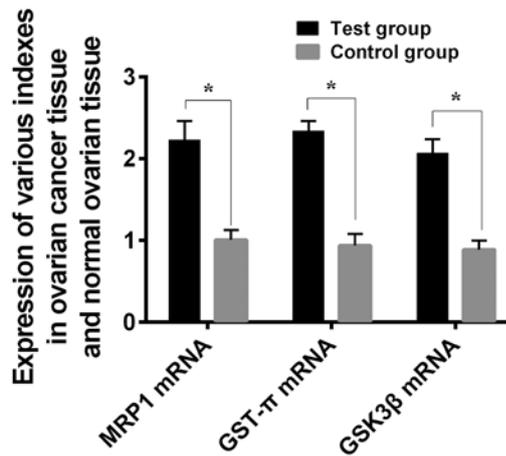


Figure 1. Comparison of MRP1, GST- π , and GSK3 β mRNA expression levels in ovarian tissues between the Experimental group and the Control group. The results of RT-PCR revealed that the expression levels of MRP1, GST- π and GSK3 β mRNA in the ovarian cancer tissues of the Experimental group were statistically much higher than those in the ovarian tissues of the Control group ($P < 0.05$). MRP1, multidrug resistance-associated protein 1; GST- π , glutathione S-transferase π ; GSK3 β , glycogen synthase kinase-3 β .

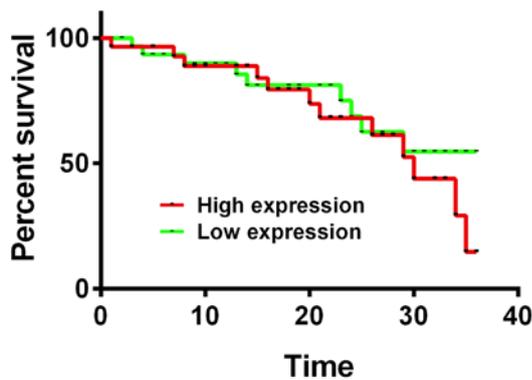


Figure 2. Association between MRP1 mRNA expression and 3-year survival rate after surgery. Kaplan-Meier survival analysis and log rank test revealed that the 3-year survival rate of patients with high MRP1 mRNA expression was significantly lower than that of patients with low expression, and the difference was statistically significant ($P < 0.05$). MRP1, multidrug resistance-associated protein 1.

low in 53 patients; GST- π mRNA was highly expressed in 65 patients and expression was low in 56 patients; GSK3 β mRNA was highly expressed in 69 patients and expression was low in 52 patients. The 3-year survival rates of patients with high expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA were 33.82, 36.92 and 34.78%, respectively, much lower than the 3-year survival rates of patients with low expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA (52.83, 53.57, and 51.92%, respectively), and the difference was statistically significant ($P < 0.05$) (Figs. 2-4).

Discussion

Ovarian cancer, a common gynecological malignant tumor, with few obvious symptoms in its early stage, is often not correctly diagnosed until reaching the middle and advanced stages when surgical resection is not practicable (15). Therefore, chemotherapy has become the main treatment for

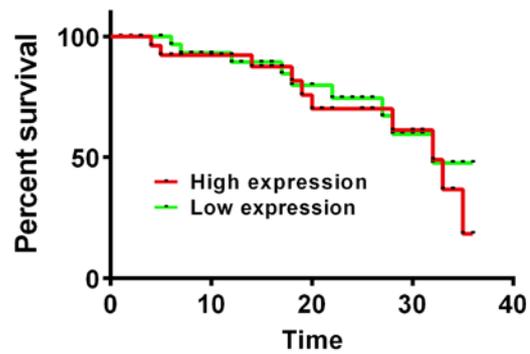


Figure 3. The relationship between GST- π mRNA expression and 3-year survival rate after surgery. Kaplan-Meier survival analysis and log rank test presented a statistically lower 3-year survival rate in patients with high GST- π mRNA expression than patients with low expression ($P < 0.05$). GST- π , glutathione S-transferase π .

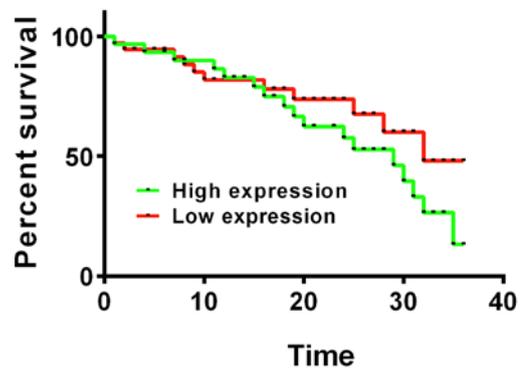


Figure 4. The relationship between GSK3 β mRNA expression and 3-year survival rate after surgery. Kaplan-Meier survival analysis and log rank test showed that patients with high expression of GSK3 β mRNA had a lower 3-year survival rate than patients with low expression, and the difference was statistically significant ($P < 0.05$). GSK3 β , glycogen synthase kinase-3 β .

patients with advanced ovarian cancer, but the accompanied drug resistance can have a serious impact on the treatment efficacy and the survival of patients (16). Moreover, drug resistance in intravenous chemotherapy can increase the recurrence rate of ovarian cancer and lead to an exacerbation of the prognosis of patients (17). *MRP1*, a drug resistance gene, has a strengthened ability to transport a negatively charged drug when highly expressed so as to reduce the drug concentration (18). *GST- π* can activate ATP-dependent transport enzymes on cancer cell membranes to promote excretion of chemotherapy drugs from cancer cells to reduce drug concentration (19). Many studies have indicated that the subsequent drug resistance of residue ser-9 after its activation makes a great contribution to GSK-3 β 's function of cancer suppression in the occurrence and development of various tumors (20). The aforementioned three factors are currently the most studied drug-related factors in clinical research. Considering the serious impact of chemotherapy resistance of ovarian cancer on patients, an effective prediction of the chemotherapy sensitivity of patients is essential.

In this study, the expression of MRP1 mRNA, GST- π mRNA, and GSK3 β mRNA by RT-PCR was compared between ovarian cancer tissues and normal ovarian tissues and their relationship in ovarian cancer with clinical pathological

features and drug resistance of patients was analyzed. According to the experiment results, the expression of MRP1 mRNA was much higher in ovarian cancer tissues and in patients in the Chemotherapy-sensitive group, compared with its expression in normal ovarian tissues and in patients in the Chemotherapy-resistant group. Associated with pathological stage and pathological differentiation instead of age and pathological type, high MRP1 mRNA expression indicated a much shorter 3-year survival time than low MRP1 mRNA expression, suggesting a possible involvement of MRP1 in the chemotherapy resistance of ovarian cancer. Previous findings have shown that the expression of MRP1 in patients with advanced ovarian cancer is significantly higher than that in patients with early ovarian cancer, which is consistent with the conclusion of this study (21). Despite the lack of detailed study on the resistance mechanism of MRP1, some studies believed that MRP1's function of deconcentrating intracellular drugs is from its combination with glutathione (22). Similar to MRP1 mRNA, GST- π mRNA and GSK3 β mRNA were also more highly expressed in ovarian cancer tissues and in Chemotherapy-resistant group than in normal ovarian tissues and in Chemotherapy-sensitive group, and patients with high expression of GST- π mRNA and GSK3 β mRNA had a much shorter 3-year survival time than patients with low GST- π mRNA and GSK3 β mRNA expression. Such results suggest that both GST- π and GSK3 β may be involved in the chemotherapy resistance and prognosis of ovarian cancer. Previous findings identified that GST- π , with high expression in ovarian cancer tissues, together with MRP1 that has a strong positive expression in ovarian cancer, can be used as predictors of tumor recurrence, drug resistance, and poor prognosis (23,24). When exploring the relationship between GST- π expression and chemotherapy in patients with ovarian cancer it was found that patients with GST- π -positive expression were less sensitive to chemotherapy than patients with GST- π -negative expression, which is similar to the findings of this study (25). A study concluded that the sustained inhibition on GSK3 β activity, a potential hallmark of tumorigenesis, may reduce apoptosis induced by chemotherapeutic drugs, suggesting that suppression on GSK3 β activity may be one of the mechanisms of chemical resistance in tumor cells (26). In a previous study, the conclusion on the mechanism of the tumorigenesis of ovarian cancer after inhibiting the activity of GSK-3 β in breast cancer cell lines drawn was that the inhibition of GSK-3 β activity leads to abnormal activation of Wnt pathway, which causes the accumulation of β -catenin in the nucleus of tumor cells and the accelerated proliferation of tumor cells, thereby promoting the occurrence of breast tumors (27).

In summary, this study explored several important factors involved in drug resistance: MRP1, GST- π , and GSK3 β which are highly expressed in ovarian cancer and thus involved with the drug resistance and prognosis and capable of performing as a target factor for predicting chemotherapy resistance and prognosis of ovarian cancer and supporting the prediction of clinical chemotherapy efficacy. However, the specific mechanisms of MRP1, GST- π , and GSK3 β in chemotherapy resistance in patients with ovarian cancer have not been explored in detail. Thus continued research efforts should be made to identify the underlying mechanism.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XT wrote the manuscript. XT and JZ analyzed and interpreted the patient general data. YZ and PM performed PCR. XW was responsible for the analysis of the observation indicators. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Liaoning Cancer Hospital and Institute, Cancer Hospital of China Medical University (Shenyang, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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