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High Expression of MDR1 is Correlated with Poor Prognosis for Common Malignancies

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Authors' Contribution
BG and DL conceived and designed the study. FY and WC performed the experiments. XL and XL analyzed the data. BG wrote the manuscript.

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MDR1, Malignant tumor, Prognosis, ROC, Expression

ABSTRACT

The purpose of this study is to detect the relative expression level of Multi-drug resistance gene (MDR1) in common tumor malignancies, and evaluate the prognostic value of MDR1 expression in common malignancies. mRNA relative expression levels of MDR1 were detected byreal-time quantitative polymerase chain reaction (RT-qPCR) in tumor, adjacent, and non-cancerous tissues. The tumor markers were detected with COBAS 6000. The prognostic value of relative MDR1 expression level in malignant tumors was investigated by univariate survival and Cox regression model analyses, and survival times were compared using the log-rank test. At the same time, through receiver operating characteristic (ROC) curve analysis, their diagnostic threshold values were calculated. MDR1 expression levels were the highest in malignant tumor tissues, followed by adjacent tissues, and the lowest in non-cancerous tissues. Differences in expression level between varying degrees of differentiation and/or lymphatic metastasis, as well as variations in negative and positive expression between survival and recurrence times, were statistically significant. The level of tumor markers at 6 months after operation was significantly lower than that before operation in recurrent/non-recurrent and MDR1 positive/MDR1 negative group. There was a significant correlation between MDR1 expression and tumor markers (CA125 and CA153), regardless of whether recurrence was involved in PEOC and breast cancer. Multivariate logistic regression indicated that relative MDR1 expression levels in patients of the positive-group survival curves were lower than those in patients of the negative-group curves. High MDR1 expression is associated with clinicopathological features in malignant tumor patients. The detection of MDR1 expression combined with tumor markers can improve the sensitivity and specificity of predicting postoperative recurrence (especially breast cancer and PEOC).

INTRODUCTION

Tumors threaten human health and present a significant burden to families. In China, the incidence and mortality of common malignant tumors is high (Chen *et al*., 2016). At present, tumor drug therapy via chemotherapy and targeted therapy is the most widely used antitumor measure. Chemotherapy failure in most patients is caused by Multi-drug resistance (MDR) induced by chemotherapeutic drugs (Abraham *et al*., 2015). In MDR, tumor cells are not only resistant to antitumor drugs but also cross-resistant to other drugs with different structures and mechanisms. There are two types of MDR, the first is acquired resistance, in which some tumors have good early-treatment effects and poor efficacy in later stages, and the second is primary resistance, in which drug resistance is developed at the beginning of chemotherapy (Joshi *et al*., 2017). Overcoming the poor curative effects caused by MDR is one of the main directions of oncology research.

MDR1 encodes the drug transporter P-glycoprotein (P-gp), which is the earliest known drug resistance gene/protein. P-gp is considered a symbol of MDR because

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the mechanism it uses to regulate drug resistance is the classical mechanism of MDR. P-gp releases ATP to pump drugs and metabolic poisons out of the cell to reduce intracellular concentrations, thereby protecting cells from damage. This mechanism is primarily used by tumor cells to generate MDR (Yan *et al*., 2014). P-gP was mainly resistant to vinblastine and doxorubicin, but increased MDR expression can lead to accumulation of intracellular chemotherapeutic drugs, decreased drug sensitivity, and increased formation rates of MDR-derived malignant tumors (Yan *et al*., 2014), such as colorectal carcinoma (Liu *et al.,* 2014), lung cancer (Zhao *et al*., 2018), gastric cancer (Mieszala *et al*., 2018) and breast cancer (Ge *et al*., 2017), so it was of great clinical significance for tumor patients to choose chemotherapy regimen and prognosis. In this paper, the expression of MDR1/P-gp in common malignant tumors and the role of this expression in MDR tumors were studied. The relationship between expression and clinic pathological features and the value of MDR1/P-gp in predicting tumor prognosis were also analyzed.

MATERIALS AND METHODS

Specimens and patients

Different types of fresh specimens were collected from May 2008 to August 2011 in Taihe Hospital. All specimens were collected including cancer tissues, adjacent tissues (>2 cm distance to the resection margin) and non-cancerous (>5 cm distance to the resection margin), some patients did not collect adjacent tissues or non-cancerous. All patients were collected with relevant case data, including gastric cancer (gender, size, pathogenetic location, lymph node status, histologic type), colorectal cancer (gender, age, size, lymph node status, differentiation, Duke stage), primary epithelial ovarian cancer (age, histologic type, stage, differentiation), non-small cell lung cancer (gender, age, histologic type, stage, differentiation, lymph node status), breast cancer (age, histologic type, TNM stage, menopausal status). There were not statistically significant with the demographic and clinical information in all cases. According to the imaging and laboratory examination, the possibility of postoperative recurrence of malignant tumor can be determined. None of the patients underwent preoperative radiotherapy or chemotherapy. All patients had complete clinical date, and were followed up from 6 to 60 months. In accordance with the Declaration of Helsinki, all protocols were approved by the Ethics Committee of Taihe Hospital.

Detection of tumor markers in common tumor malignancies

Peripheral blood 5ml was collected pre-operation into a heparin anticoagulant tube for the isolation of plasma. The tumor markers were detected with COBAS 6000 (Roche) at two time points, in one week of post-operation and 6 months after the surgery, with CEA>10μg/L, CA724>6 KU/L, CA153>25KU/L, CA125>35KU/L, and CYFRA 21-1>3.3ng/mL being regarded as elevated status.

Real-time PCR analysis

Total RNA of Tissues and cells were extracted using TRIzol® Reagent according to the instruction. The absorbance of RNA was determined at 260nm and 280nm with Nano Drop-2000. cDNA was synthesized according to the instruction by the Reverse Transcription System kit and was stored at −80˚C until use. The primers were used for Realtime quantitative PCR (RT-qPCR) as follows: Forward, 5'-CCC ATC ATT GCA ATA GCA GG-3' and reverse, 5'-TGT TCA AAC TTC TGC TCC TGA-3' for human MDR1; and forward, 5'-GAA GGT GAA GGT CGG AGT C-3' and reverse, 5'- GAA GAT GGT GAT GGG ATT TC-3' for human GAPDH. MDR1, GAPDH primers yielded products of 158, 226 bp, respectively. PCR amplification system: Mg^{2+} 2.4μl, 5' and 3' primer 2 μl, 2mmol/L dNTP 1.5μl, 10×SYBR-Green 1μl, Taq 0.3μl,10×Buffer 3μl, cDNA5μl, with sterile water total volume filled 30μl. Reaction conditions: 95˚C denaturation for 5 minutes, 94˚C 30 seconds, 60˚C 30 seconds, 72˚C 1 min with 35 cycles, and dissociation curve analysis was performed after all amplification. MDR1 gene relative expression level was normalized by GAPDH in each sample and determined by the $2\text{-}\Delta^{\Delta Ct}$ method.

Statistical analysis

Statistical analysis was performed with SPSS software version 16.0 and $P<0.05$ was used to indicate statistically significant difference. The measured data were expressed as the mean ± standard deviation. Survival time analysis was compared using the log rank test. A Student's t-test was used to determine the expression differences between the different groups, Univariate survival analysis and Cox regression model analysis were used to compare different clinical feature groups. Receiver operating characteristic (ROC) curve was established to evaluate the recurrent value of MDR1 and tumor markers in common malignant tumor. Partial correlations were used to analyze the correlation between MDR1 expression and tumor markers' levels.

RESULTS

MDR1 gene relative expression levels in all samples

MDR1 was highly expressed in all malignant tumor tissues, partially expressed in adjacent tissues, and almost not expressed in non-cancerous tissues, the difference of expression between different groups were statistically

significant (Fig. 1). Statistical analysis found that the differences in MDR1 expression levels of differentiation degree/lymph node status were statistically significant (Table I).

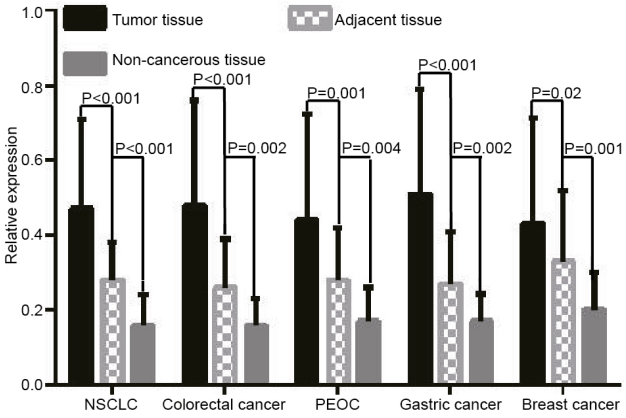

Fig. 1. The relative expression levels of MDR1 in all tissues.

Recurrence time and survival time in different groups

Most patients had relapses during the follow-up period, and the recurrence rate of non-small cell lung cancer was 53.2% (25/47), gastric cancer 30.2% (16/53), primary epithelial ovarian cancer was 73.7% (42/57), colorectal carcinoma was 56.9% (29/51), breast cancer was 29.2% (14/48). According to the expression of resistance gene, it was divided into positive and negative group. The survival time and recurrence time of the follow-up patients were shown in Figure 2.

The levels of different tumor markers in different groups

In the malignant tumors, the tumor markers were reduced in both the recurrence group/the non-recurrence group and MDR1 positive group/MDR1 negative group, the difference was statistically significant (P<0.001). Before the operation, there was no statistically significant difference in the level of tumor markers between the recurrence group/the non-recurrence group and MDR1 positive group/MDR1 negative group. But after surgery, there was statistically significant difference in the level of tumor markers between the recurrence group/the non-recurrence group and MDR1 positive group/MDR1 negative group in PEOC, breast cancer and colorectal cancer (P<0.05). The levels of different tumor marker in different groups were shown in Table II.

MDR1 expression and tumor marker level in common malignancies

Receiver operating characteristic (ROC) curve of the predicted probability of logistic regression were constructed, and relative sensitivity and specificity of MDR1 expression and tumor markers were calculated. Area under ROC curves of MDR1 expression and tumor markers were shown in Table III and Figure 3. And partial correlations were used to analyze the correlation between MDR1 expression and tumor markers, we found a significant correlation between MDR1 expression and tumor markers, regardless of whether recurrence was involved in PEOC and breast cancer. The correlation between the level of MDR1 expression and tumor markers were shown in Table IV.

Table I. Relationship between the expression of MDR1 and clinicopathologic paramenters in common malignancies.

Tumors	Characteristics	n	Expression	P
NSCLC				
	Differentiation			
	Well	26	0.56±0.20	
	Moderately +poorly	21	0.36±0.24	0.004
	Lymphatic metastasis			
	positive	30	0.59±0.21	
	negative	17	0.31±0.20	0.001
Colorectal cancer				
	Duck stage			
	A+B	26	0.40±0.22	
	C+D	25	0.56±0.31	0.047
	Lymphatic metastasis			
	positive	34	0.55±0.27	
	negative	17	0.34±0.24	0.01
PEOC				
	Differentiation			
	Well+moderately	32	0.51±0.26	0.03
	Poorly	25	0.35±0.28	
Gastric cancer				
	Differentiation			
	Well+moderately	28	0.66±0.24	
	Poorly	25	0.46±0.26	0.007
Breast cancer				
	Lymphatic metastasis			
	positive	18	0.67±0.21	
	negative	30	0.29±0.21	0.001
	Tumor metastasis			
	Yes	23	0.63±0.23	
	No	25	0.24±0.17	0.001

Note: PEOC, primary epithelial ovarian cancer; NSCLC, non-small cell lung cancer. MDR1, multidrug resistance 1.

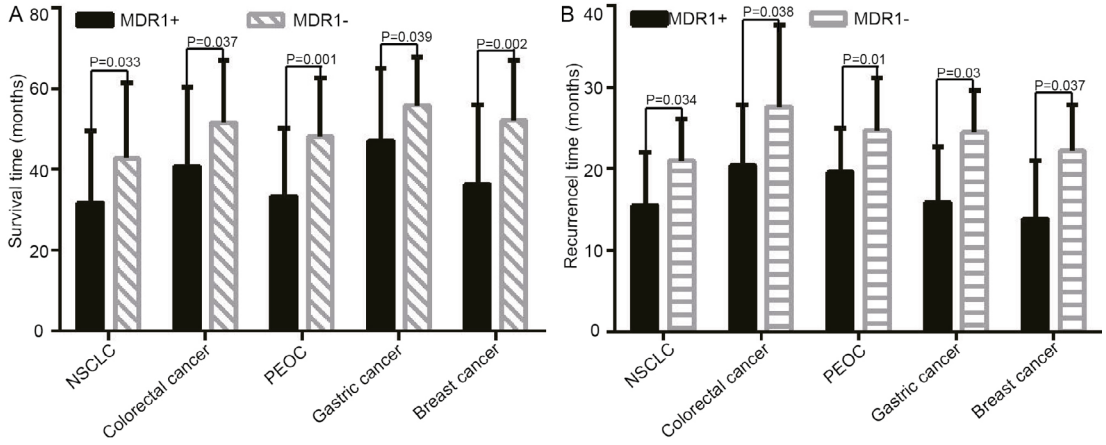

Fig. 2. Recurrence time and survival time in different groups. PEOC: primary epithelial ovarian cancer; NSCLC: non-small cell lung cancer; MDR1: multidrug resistance 1; MDR1+: MDR1 positive group; MDR1-: MDR1 negative group.

Table II. The levels of different tumor markers in different groups.

		Recurrent	Non-recurrent	P	MDR1 +	MDR1 -	P
NSCLS	CYFRA21-1						
	pre-operation	4.89±1.39	4.73±1.01	0.66	5.06±1.22	4.52±1.19	0.14
	post-operation	3.82±1.17	3.17±1.01	0.05	3.64±1.17	3.35±1.10	0.389
Colorectal cancer	CEA						
	pre-operation	13.08±4.79	12.35±4.69	0.59	13.06±5.39	12.43±3.89	0.63
	post-operation	8.36±2.10	6.17±2.24	0.001	8.05±2.41	6.71±2.23	0.046
PEOC	CA125						
	pre-operation	31.09±9.46	27.42±5.76	0.164	31.58±8.09	28.27±9.35	0.17
	post-operation	15.04±4.25	11.07±3.76	0.002	15.84±4.48	11.63±3.16	<0.001
Gastric cancer	CA724						
	pre-operation	8.79±3.01	9.68±2.84	0.31	9.18±2.76	9.70±3.09	0.523
	post-operation	3.61±1.79	4.71±1.83	0.052	4.80±2.02	3.87±1.57	0.072
Breast cancer	CA153						
	pre-operation	32.76±5.96	29.18±5.66	0.055	31.37±4.77	28.62±7.04	0.114
	post-operation	17.72±5.68	14.02±4.12	0.015	17.57±3.96	13.34±4.75	0.002

Note: PEOC, primary epithelial ovarian cancer; NSCLC, non-small cell lung cancer. MDR1, multidrug resistance 1.

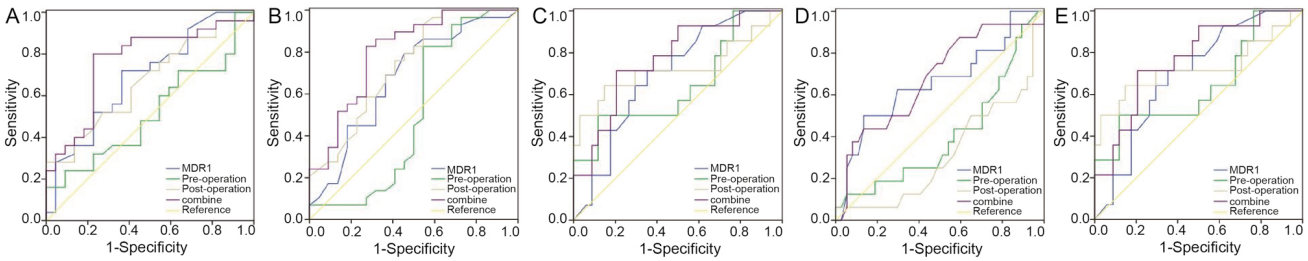

Fig. 3. The ROC analysis of MDR1 expression and tumor markers in the prognosis of common malignant tumor.

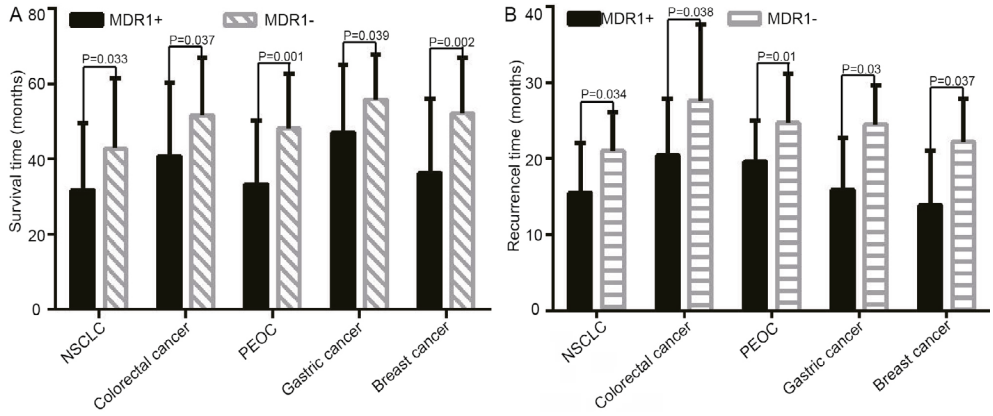

Fig. 4. Survival curves of MDR1 mRNA-positive and negative patients. A: non-small cell lung cancer; B: colorectal cancer; C: primary epithelial ovarian cancer; D: gastric cancer; E:breast cancer.

Table III. Area under ROC curves of MDR1 and tumor markers.

Variable	Area	Standard errora	Approximation Sig.b	95%CI
NSCLS				
MDR1	0.69	0.078	0.027	0.54-0.84
CYFRA 21-1 (pre-operation)	0.52	0.086	0.84	0.35-0.69
CYFRA 21-1 (post-operation)	0.67	0.079	0.051	0.51-0.82
MDR1+CYFRA 21-1	0.77	0.072	0.002	0.62-0.91
Colorectal cancer				
MDR1	0.68	0.078	0.032	0.52-0.83
CEA (pre-operation)	0.51	0.092	0.93	0.33-0.69
CEA (post-operation)	0.74	0.071	0.004	0.60-0.88
MDR1+CEA	0.80	0.064	<0.001	0.68-0.93
PEOC				
MDR1	0.69	0.077	0.033	0.54-0.84
CA125 (pre-operation)	0.67	0.07	0.059	0.53-0.80
CA125 (post-operation)	0.76	0.074	0.003	0.61-0.90
MDR1+CA125	0.81	0.059	<0.001	0.69-0.92
Gastric cancer				
MDR1	0.66	0.087	0.07	0.49-0.82
CA724 (pre-operation)	0.41	0.088	0.29	0.24-0.58
CA724 (post-operation)	0.32	0.082	0.161	0.16-0.48
MDR1+CA724	0.68	0.082	0.04	0.52-0.84
Breast cancer				
MDR1	0.70	0.078	0.035	0.54-0.85
CA153 (pre-operation)	0.64	0.095	0.123	0.46-0.83
CA153 (post-operation)	0.73	0.097	0.015	0.53-0.92
MDR1+CA153	0.77	0.074	0.004	0.62-0.91

Note: PEOC, primary epithelial ovarian cancer; NSCLC, non-small cell lung cancer.

Prognostic value of MDR1 expression in common malignancies

Overall survival analysis in the all patients with different types common malignancies based on the relative expression level of MDR1 was carried out by log-rank test with Kaplan-Meier method. And the statistical results showed that there were significant differences in the survival curve between the positive group and the negative group based on MDR1 relative expression level in common malignant tumor cases (Fig. 4). Multivariate logistic regression analysis and Univariate logistic regression analysis indicated that high MDR1expression was significantly associated with common malignancies patients prognosis and was an independent risk factor for malignancies prognosis (Table V).

Table IV. The correlation between the level of MDR1 expression and tumor markers.

		CYFRA21-1		CEA		CA125		CA724		CA153	
		r	P	r	P	r	P	r	P	r	P
1	MDR1	0.052	0.73	0.22	0.19	0.42	0.001	0.018	0.9	0.38	0.007
2	MDR1	0.039	0.80	0.089	0.54	0.33	0.013	0.037	0.80	0.32	0.03

Note: r: Partial correlation coefficient; 1: including recurrent factor; 2: no-including recurrent factor; MDR1, multidrug resistance 1.

Table V. Cox regression analyses for MDR1 in common malignancies.

Tumors	Characteristics	Univariate analysis			Multivariate analysis		
		B	Exp (B)	P	B	Exp (B)	P
NSCLC							
	Lymph node status						
	metastasis vs No-metastasis	4.19	0.015	0.024			
	MDR1						
	positive vs negative	3.75	42.65	0.005	1.49	4.46	0.003
Colorectal cancer							
	Duck Stage						
	A+B VS C+D	1.77	5.84	0.047			
	MDR1						
	positive vs negative	2.00	7.36	0.028	4.02	5.47	0.043
PEOC							
	Differentiation						
	Well+moderately VS Poorly	1.70	0.18	0.022			
	MDR1						
	positive vs negative	2.38	0.092	0.002	1.27	3.58	0.001
Gastric cancer							
	MDR1						
	positive vs negative	2.20	9.00	0.013	4.41	82.40	0.039
Breast cancer							
	MDR1						
	positive vs negative	4.09	60.00	0.002	1.67	5.30	0.005
	Lymph node status						
	metastasis vs No-metastasis	1.08	2.96	0.04			

Note: PEOC, primary epithelial ovarian cancer; NSCLC, non-small cell lung cancer; B, regression coeffcient; Exp (B), odds ratio.

DISCUSSION

MDR is the manifestation of the declining sensitivity of tumor cells to drugs with different structures and mechanisms (Hu *et al*., 2017). Among the numerous mechanisms used to produce MDR, the most important one is the protein transport mechanism in cell and nuclear membranes, which are the means through which chemotherapeutic drugs and harmful toxins are transported out of cells. Different types of antitumor drugs can be expelled out of the cell (Levatić *et al*., 2013; Loo *et al*., 2013). P-gp is encoded by MDR1 and plays a particularly important role as a drug carrier in different MDR mechanisms. High expression of resistance genes is the main cause of tumor cell resistance to chemotherapy (Chufan *et al*., 2015; Hou *et al*., 2008). The drug resistance gene may be an important biomarker in predicting and improving tumor prognosis.

In this study, we found that relative MDR1 gene expression levels in common malignancies were significantly higher than those in adjacent and non-cancerous tissues. None of the patients underwent preoperative radiotherapy or chemotherapy, which suggests that primary drug resistance is associated with common malignancies. MDR1 expression was also associated with clinic pathological features that may represent tumor prognosis, such as differentiation degree or lymphatic metastasis. Thus, high MDR1 expression may be involved in the prognosis of common malignant tumors. The prognosis and recurrence of tumors may be closely related to the high expression of MDR1 (Sun *et al*., 2016).

We divided all of the cases into a positive-MDR1 expression group and a negative-MDR1 expression group according to expression level and studied the recurrence and survival times of patients in both groups. Statistical analysis revealed that, in several common malignancies, the recurrence and survival times of positive-MDR1 expression patients were shorter than those of negative-expression patients. Survival curve analysis demonstrated that MDR1 expression in common malignant tumor patients of the negative-group survival curves was higher than that in the positive-group curves. This phenomenon has also been observed in cervical cancer (Yang *et al*., 2017) and liver cancer (Gao *et al*., 2015), thereby suggesting that high MDR1 expression may be closely related to the prognosis of common malignancies and that high expression level could indicate poor prognosis for common malignant tumor cases.

A large body of evidence shows that drug resistance genes could be used as prognostic indicators of cancer (Janikova *et al*., 2016; Pan *et al*., 2016). In the present study, we investigated the prognostic value of the relative expression of the MDR1 gene in common malignant tumor cases. Multivariate and univariate logistic regression analyses indicated that high relative expression level of the MDR1 gene could be an independent prognostic risk factor for malignant tumor cases. The expression level of various resistance genes can be used as prognostic markers for cervical and liver cancer (Kim *et al*., 2016; Dufour *et al*., 2015). However, the importance of these relative expression level has rarely been reported in clinical applications. Therefore, in future studies (including *in vitro* and *in vivo*), increasing the sample size is necessary to confirm the prognostic value of the relative expression the MDR1 gene in common malignant tumor cases.

As the primary treatment for most malignant tumors, surgery can achieve a certain therapeutic effect, the level of tumor markers after surgery was significantly reduced, and tumor markers can be maintained at a normal level with postoperative chemotherapy. However, multidrug resistance can affect the sensitivity of tumor cells to chemotherapeutic agents and lead to tumor recurrence. Studies have shown that CA125, CA153 and CEA may be useful in predicting the recurrence of ovarian cancer (Guo *et al*., 2017), breast cancer (Li *et al*., 2017) and colorectal cancer (Gao *et al*., 2018). When the value of CA125 is higher than 10 U/ml and continuously increased, indicates a relative risk of recurrence and need to be vigilant (Guo *et al*., 2017). Nicholson BD (Nicholson *et al*., 2015) recommend monitoring for colorectal cancer recurrence with more than one diagnostic modality but applying the highest CEA cut-off assessed (10 μg/L). In this study, we also studied the value of MDR1 expression and tumor markers in predicting recurrence of malignant tumors. Relative sensitivity and specificity of MDR1 expression and tumor markers were calculated by ROC in this study. The detection of MDR1 expression combined with tumor markers can improve the sensitivity and specificity of predicting postoperative recurrence. After surgery, there was statistically significant difference in the level of tumor markers between the recurrence group/the non-recurrence group and MDR1 positive group/MDR1 negative group in PEOC, breast cancer and colorectal cancer. Besides, we found a significant correlation between MDR1 expression and tumor markers, regardless of whether recurrence was involved in PEOC and breast cancer by partial correlations analysis, which suggested that the expression of MDR1 can indirectly predict the postoperative level of CA125 and CA153. Although CA724 and CYFBA21-1 can be used for non-specific diagnosis of gastric cancer (Li *et al*.,2013) and non-small cell lung cancer (NSCLC) (Yu *et al*., 2017), they need to be further studied as tumor markers for predicting postoperative recurrence.

CONCLUSION

In conclusion, high MDR1 expression is related to aggressive clinical characteristics in common malignant tumor cases. Thus, MDR1 may be a high-risk factor of prognosis for common malignancies.

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Statement of conflict of interest

There is no conflict of interest.

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