Relationship Between Expression of MRP, LRP and Clinical Pathological Features in Esophageal Squamous Cell Carcinoma

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Abstract **Objective** To investigate the expression of MRP and LRP in esophageal squamous cell carcinoma (ESCC) tissues and the relationship between their expression and clinical pathological features together with prognosis of ESCC. **Methods** Elivision immunohistochemistry was used to detect the expression of MRP and LRP in 51 ESCC tissues and 18 surrounding noncancerous tissues. **Results** There were significant correlations between LRP expression and tumor size ($P<0.05$). The expression rate of MRP or LRP in tumor tissues was higher than that in normal tissues, the positive rate of MRP or LRP in ESCC with lymph node involvement was higher than that without lymph node metastasis. The three-year survival rate of negative patients was higher than that of positive patients, but there had no significant difference. No significant correlation was observed between MRP or LRP expression and patient age, cell differentiation, invasion depth, clinical staging. No significant correlation was observed between MRP and LRP expression. **Conclusions** The expression of MRP or LRP couldn´ t be regarded as an independent marker to predict malignant degree, lymph node metastasis and prognosis in ESCC. But it could remind us that there might have been the trends of easy metastasis and poor prognosis in positive patients.

Key words Esophageal carcinoma; MRP; LRP

Recently many studies have shown that the over-expression of resistance protein in tumor tissue is one of the major causes for ineffective anticancer drugs. These proteins lead to multidrug resistance by a variety of mechanisms. The tumor resistance transporter protein includes P-glycoprotein(Pgp), multidrug resistance-associated protein (MRP)and lung resistance protein (LRP), which had been studied more in these mechanisms. These resistant transporter proteins limited the chemotherapeutic agents to target cells by re-distributing cytotoxic antineoplastic agents between nucleolus and cytoplasm[1]. They are widely distributed in human tumor tissues and normal tissues. It is said that the expression of these transfer protein in tumor tissue was higher than that of normal tissue origin. The proteins over-expression in tumor tissue not only relates to drug resistance, but also to the malignant biological behavior [2]. Especially, in acute myelogenous leukemia and ovarian cancer, it may reflect the effects of chemotherapy and may serve as an independent prognostic indicator [3]. However, few studies in esophageal cancer, especially in the relationship between LRP expression and esophageal cancer have been reported. Using immunohistochemistry method, we investigate the expression of MRP and LRP in 51 cases of esophageal squamous cell carcinoma(ESCC) tissue. The relationship between the expression of MRP and LRP and clinical parameters, including the patient´ s survival time, was also investigated.

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MATERALS AND METHODS

Materials

Tumor specimen was obtained from 51 patients (44 men and 7 women)with ESCC who had received surgery in our hospital from March 1996 to October 1999. The normal esophageal tissure, obtained from 18 patients, 2cm beyond the cancer margin act as normal control. Specimens were fixed in 10% neutral formalin, embedded in paraffin, 4um serial sections. No patients

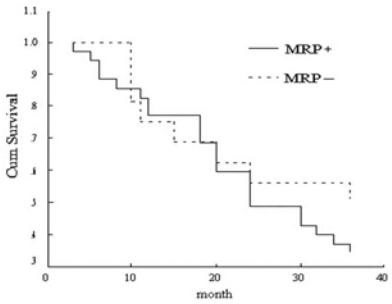

Fig.1 Kaplan−Meier Curve in MRP Positive and Negative Patients with ESCC

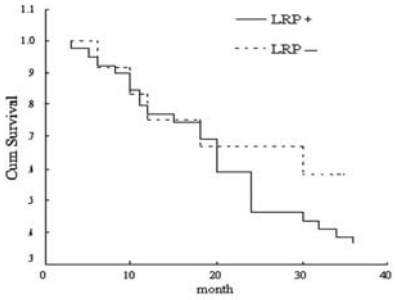

Fig.2 Kaplan−Meier Curve in LRP Positive and Negative Patients with ESCC

received preoperative radiotherapy or chemotherapy. The average age of patients was 58 years old (ranging from 36~70). TNM stage is classified under international law by this group stage, I phase 3 cases, IIA phase 20 cases, IIB phase 16 cases, Ⅲ phase 12 cases. They had been followed up for three years.

Methods

Immunohistochemical staining was performed with two−step Elivision method. MRP monoclonal mouse anti−human antibody (OCRL1) and mouse anti−human monoclonal antibody LRP (LRP−56)were purchased from Zhongshan biotechnology company, China, which is working fluid of 3ml. Elivision immunohistochemical staining kit was purchased from Fujian Maixin company, China. Staining was performed according to the manufactures instruction of kit. The known positive tumor was served as positive control. With negative control, primary antibody was PBS instead of MRP or LRP. Sections were stained with DAB and restained with haematoxylin for visualization.

Analysis of immunohistochemical detection

Brown staining in cell cytoplasm was defined positive. Double blind method was used to count staining cells by two pathologists to observe 10 high visual fields. The proportion of positive cells was assessed. −: No staining cells or positive cells less than 10%. +: positive cells from 10% to 25%; ++: positive cells from 26% to 50%; +++: positive cells >50%.

Statistical Analysis

SPSS 11.0 software package was used to perform statistical analysis. Log−rank test was used to perform survival analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of MRP and LRP in ESCC tissues and surrounding noncancerous tissues

MRP and LRP positive particles are mainly located in cytoplasm of ESCC and normal epithelial cells of the esophagus mucosa. LRP expression in lymphocyte is also evident. It was coarse particles around nuclear cytoplasm and concentration. The positive rate of MRP or

Table 1 Expression of MRP and LRP in ESCC Tissues and Surrounding Noncancerous Tissues

Tissue type	n	MRP						LRP					
		−	+	++	+++	Positive rate	*P*	−	+	++	+++	Positive rate	*P*
ESCC	51	16	18	8	9	68.6%		12	10	13	16	76.5%	
Normal esophageal tissues	18	8	4	4	2	55.6%	>0.05	7	2	5	4	61.1%	>0.05

Table 2 Correlation between MRP or LRP Expression and Clinical Pathological Features of ESCC

Variable	n	MRP						LRP					
		−	+	++	+++	Positive rate(%)	*P*	−	+	++	+++	Positive rate(%)	*P*
Age													
≥60	27	9	8	4	6	66.67		5	7	5	10	81.48	
<60	24	7	10	4	3	70.83	0.775	7	3	8	6	70.83	0.519
Size													
≥5cm	16	4	4	4	4	75.00		1	2	5	8	93.75	
<5cm	35	12	14	4	5	65.71	0.189	11	8	8	8	68.57	0.013
Pathological grade													
Ⅰ	40	11	15	7	7	72.50		9	6	12	13	77.50	
Ⅱ	8	4	2	1	1	50.00		2	2	1	3	75.00	
Ⅲ	3	1	1	0	1	66.67	0.598	1	2	0	0	66.67	0.464
Invasion depth													
Muscular	33	7	15	7	14	78.79		9	6	9	9	72.72	
Full−thicknes	18	9	3	1	5	50.00	0.393	3	4	4	7	83.33	0.391
Clinical stage													
Ⅰ	3	1	2	0	0	66.67		2	0	0	1	33.33	
ⅡA	20	7	7	3	3	65.00		5	5	6	4	75.00	
ⅡB	16	3	6	4	3	81.25		3	2	4	7	81.25	
Ⅲ	12	5	3	1	3	58.33	0.611	2	3	3	4	83.33	0.467
Lymph node													
Metastasis	27	6	10	5	6	77.78		5	5	7	10	81.48	
Non−metastasis	24	10	8	3	3	58.33	0.122	7	5	6	6	70.83	0.274

LRP in ESCC was higher than that of normal esophageal squamous cell. Yet there was no significant difference (P>0.05) (Table 1).

Correlations between MRP or LRP expression and clinical pathological features of ESCC

LRP positive expression rate in tumor beyond 5cm in diameter was significantly higher than that in tumor less 5cm (P <0.05). The expression rate of MRP and LRP with lymph node metastasis was higher than those without, but there was no significant difference. There was no correlation between MRP, LRP expression and patient´s age, degree of tumor differentiation, invasion depth, clinical stages (Table 2).

The relationship between MRP,LRP expression and postoperative survival rate

The 3−year survival rates in MRP positive and negative were 37.14% (13/35) and 56.25% (9/16), in LRP they were 38.46% (15/39) and 58.33% (7/12) respectively. The 3−year survival rates in MRP and LRP negative expression was higher than that of the positive expression.No significant difference was found by Log−Rank test (P> 0.05).Kaplan−Meier survival curves of MRP, LRP expression were compared(Figure 1 and 2).

Correlations between MRP and LRP expression in ESCC

There was no correlation between the expression of MRP and LRP of ESCC (Table 3).

DISCUSSION

No consistency conclusions have been reached yet

Table 3 Correlation between MRP and LRP Expression in ESCC

LRP	MRP			
	−	+	++	+++
−	2	6	0	4
+	6	3	1	0
++	4	5	3	1
+++	4	4	4	4

on the relationship among MRP, LRP expression and clinical pathological features and prognosis of tumor. Some believe there are relationships between the expression of MRP and lymph node metastasis, and the MRP expression in esophageal cancer tissue is significantly higher than that in surrounding normal tissue. MRP expression in tumor with deep invasion into muscular layer is significantly higher than in the tumor restricted at superficial muscular layer [4]. However, studies have shown there is no relationship between MRP and the pathological type or grade of renal cell carcinoma[5]. LRP expression in ovarian cancer or acute myeloid leukemia is a strong predictor of the chemotherapy effect and an independent prognostic indicator [3]. In our group, MRP and LRP positive particles are mainly distributed in the cytoplasm of ESCC, not in the membrane. In some interstitial cells,especially in lymphocytes, LRP expression is visible. It is coarse particles concentrated around the nuclear, with a clear yellow or pale brown. Presumably, it has played the role at transport cytotoxic antineoplastic agents away from the target through re−distributing in nucleocytoplasmic transport. Expression of MRP and LRP in tumor tissue are higher than in the surrounding normal tissue, but no significant difference. It is indicated that there exists resistance in ESCC and primary esophageal epithelium tissue, and it would be further increased in ESCC. The reason is thought to be esophageal mucosa exposed to the external environment and harmful substances, hence MRP or LRP expression increase to withstand external noxious substance stimulus. There are significant correlations between tumor size and LRP expression. No significantly correlated connections between the expression of MRP or LRP and the age, differentiation, invasion depth, and clinical stages of 51 ESCC cases. The MRP and LRP expression in cancer with lymph node metastasis is higher than that without lymph node metastasis, but no significant difference. We hold that the tumor cell proliferates and survives more easily with higher LRP expression because of the selective action in a variety of esophageal cancer suppressor factors. The tumor cells with LRP positive could transfer exogenous or endogenous harmful substances away from nuclei, so the tumors proliferate more easily. It coincides with the physiological functions of LRP. There has been the trend that cells with LRP or MRP positive would survive and metastasize more easily because they could transfer harmful substances.

No relationship between MRP expression and cell differentiation and Dukes stage of rectal cancer is proved, according to Pengs research [6]. But there is shorter survival time in MRP positive cells than in negative through 5−year follow−up. They hold that MRP could be used as an independent prognostic indicator in rectal cancer. Studies show that there is no obvious correlations between LRP and histological type, sex, age, invasion depth, metastasis in non−small cell lung cancer. However, the chemotherapy effect is worse in LRP positive patient than in LRP negative. So correlations exist between LRP expression and multidrug resistance in non−small cell lung cancer. Furthermore, it could affect chemotherapy and prognosis [7]. To analyze the follow−up data of 51 cases of ESCC, we find that the 3−year survival rate in patients with MRP or LRP negative and positive are 56.25%, 58.33% and 37.14%, 38.46%, respectively. However, there is no statistical difference. Therefore, we conclude that ESCC, MRP or LRP expression may be a hint that tend to transfer easier and poor prognosis, yet not as an independent indicator. And no significant correlations between MRP and LRP expression emerge in 51 ESCC tissues by Spearman rank correlation analysis, which is similar to the research of Peng in rectal cancer [6]. We consider that there are more than two kinds of resistance protein expression in ESCC. They pump or re−distribute the drugs far away from the targets, causing multidrug resis-

tance through different mechanisms. The distribution of these proteins and the different mechanisms in the cell determine that their existences are not dependent on each other. They play a different role in the process of tumor growth. Therefore, the sensitive chemotherapy drugs and different reversed resistance protein can be a choice to improving the efficacy of chemotherapy in esophageal cancer. In spite of that, the results of this study may be less concerned in the number of cases, we should accumulate more cases and follow−up data, so as to examine the different expression of protein even further. The significance of resistance protein in esophageal cancer should be evaluated more accurately. The relationship and control mechanism between resistance protein and tumor suppressor gene/ promoting gene requires further study. Once the mechanism is identified and effective multi−drug resistance reversal of esophageal cancer was adopted, it would be expected to improve the survival rate of patients with esophageal cancer significantly.

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