EXPRESSION OF MIRNA-483-5P IN ESOPHAGEAL CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FEATURES AND PROGNOSIS

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ABSTRACT

Objective*: To investigate the expression of miRNA-483-5p in esophageal cancer and its correlation with clinicopathological features and prognosis.*

Methods: *This study's 82 cases of esophageal cancer tissue were archived at the study hospital between March 2013 and May 2014. In addition, 28 cases of paracancerous tissue were archived. Real-time fluorescent quantitative polymerase chain reaction was used to detect the relative expression levels of microRNA-483-5p (miRNA-483-5p) in esophageal cancer and adjacent tissues. The relative expression level of miRNA-483-5p was analysed with clinicopathological parameters and prognosis of patients with esophageal cancer.*

Results: *The relative expression of miRNA-483-5p in esophageal cancer was (3.25±1.25), and the relative expression of miRNA-483-5p in adjacent tissue was (0.99±0.71). The difference was significant (P<0.05). The expression level of miRNA-483-5p was not related to the esophageal cancer patient's gender, age, or histological grade, nor to the depth of invasion of the tissue (P>0.05). It was related to lymph node metastasis and the TNM stage of the esophageal cancer patient (P<0.05). The five-year survival rate of patients with relatively high levels of expression of miRNA-483-5p was 43.90% (18/41), and the five-year survival rate of patients with relatively low levels of expression of miRNA-483-5p was 75.61% (31/41). There was a significant difference (P<0.05). Results from the COX proportional hazard regression model showed that miRNA-483-5p expression level, lymph node metastasis, and TNM stage were independent prognostic factors for patients with esophageal cancer.*

Conclusions: *The expression levels of miRNA-483-5p are elevated in esophageal cancer tissues, and its expression is related to lymph node metastasis and TNM staging in patients with esophageal cancer. MicroRNA-483-5p could be used as an independent risk factor in the prognoses of patients with esophageal cancer, being that it is of great significance in the onset and progression of esophageal cancer; it can be expected to become a new target for clinical treatment of esophageal cancer.*

Keywords: *miRNA-483-5p, esophageal cancer, clinicopathological features, prognosis.*

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Introduction

Esophageal cancer is a common malignant disease of the digestive tract; its morbidity and mortality vary significantly from country to country. Western countries regard adenocarcinoma as the only pathological type; Asian countries divide the disease into two subtypes, being esophageal squamous cell carcinoma and esophageal adenocarcinoma. Esophageal squamous cell carcinoma accounts for the majority[1]. Surveys have shown that compared with western Africa, the incidence of esophageal cancer in eastern Asia and south-eastern Africa is significantly higher[2]. China is in the 'esophageal cancer zone'. Studies have shown that esophageal cancer patients are more common in China, with Henan, Hebei and other regions being high-incidence areas. Populations in these areas demonstrate family-cluster tendencies[3]. Environmental factors are the main cause of esophageal cancer in Western countries. Drinking hot beverages is considered the main cause of esophageal cancer in Asian countries[4]. Most esophageal cancer patients develop advanced symptoms before they develop adverse symptoms, result-

ing in poor prognosis[5]. Therefore, early diagnosis along with timely and effective treatment are the key issues. Early diagnosis and treatment of esophageal cancer can significantly alleviate the patient's condition and improve the prognosis, which is particularly important for reducing the mortality rate.

However, the current lack of effective clinical biomarkers and minimally invasive early-diagnosis technologies has led to low early diagnosis rates and five-year survival rates for patients. Therefore, exploring molecular targets to prolong the survival time and treatment of esophageal cancer patients has become the focus of current clinical research. Although traditional serum-tumour markers have long been used for tumour diagnosis, these markers lack specificity and sensitivity, leading to a clinical lack of minimally invasive early-diagnosis markers for esophageal squamous cell carcinoma[6]. Clinical studies have confirmed that micro-RNA (mi-croRNA, miRNA) can interact with tumour-related genes and exert a series of biological reactions[7]. In this paper, the expression of miR-483-5p in esophageal cancer tissues was examined to explore its correlation with clinicopathological features and prognosis.

Materials and methods

Materials

The study's 82 samples of esophageal cancer tissue were archived at the study hospital between March 2013 and May 2014.

The inclusion criteria were as follows:

• Sample meets the diagnostic criteria for esophageal cancer in the 2nd edition of the 'Guidelines for the Standardized Diagnosis and Treatment of Esophageal Cancer';

• Sample was taken within four cm of the adjacent tissues;

• Diagnosis of esophageal cancer by senior experts;

• Patient agreed and signed the informed consent form before specimen was collected;

• Approval from hospital ethics committee.

The constitution of the sample follows. According to gender, there were 53 males and 29 females. According to age, 52 cases were 50 years old and over and 30 cases were under 50 years old. The average age was (47.69±9.78) years old.

According to histological classification, 35 cases were grade I, 25 cases were grade II, and 22 cases were grade III. According to depth of invasion, 28 cases were shallow and 54 were deep. There were 44 cases with lymph node metastasis and 38 cases without lymph node metastasis. According to TNM staging, 35 cases were grade I ~ II and 47 cases were grade III ~ IV. In addition, 28 cases of paracancerous tissue archived by the study hospital were collected; the distance between the cancerous tissue and the cancerous tissue was more than five cm.

Reagents and instruments

Reagents

Trizol Reagent was purchased from Anhui Jingke Biotechnology Co. Ltd. The reverse transcription kit was provided by Beijing Huada Protein R & D Center Co. Ltd. The cDNA amplification kit was obtained from Beijing Xiangsheng Xingye Technology Co. Ltd. The RNA extraction kit was purchased from Hangzhou Haoxin Biotechnology Co. Ltd. The fluorescent quantitative polymerase chain reaction (PCR) kit was obtained from Murray Biotechnology Co. Ltd. MicroRNA chips were provided by Agilent Technologies, Inc.

Instruments

Ultra-clean bench was purchased from Esko Trading Co. Ltd. The liquid nitrogen tank was provided by Guangzhou Bemart Instrument Equipment Co. Ltd. Pipettes were purchased from Eppendorf China Ltd. Haier vertical ultra-low temperature storage box was produced by Haier Company. The vertical pressure steam sterilizer was purchased from Dongguan Spectrum Standard Experimental Equipment Technology Co. Ltd.

Methods

Total RNA was extracted from esophageal cancer and adjacent tissues. The specimens were cut into small pieces and ground. A small amount of liquid nitrogen was added continuously during the grinding process. After grinding, RNAiso was added and mixed, then chloroform was added in a centrifuge. The supernatant was taken and mixed with isopropanol for 10 minutes. Absolute ethanol was added to the centrifuge to discard the supernatant and air dry. Then, diethylpyrocarbonate was added to dissolve the RNA pellet and measure the RNA concentration. Complementary DNA was obtained through a reverse transcription reaction using a reverse transcription kit.

Real-time fluorescent quantitative PCR was used to detect the relative expression level of miR-483-5p. The cDNA was diluted, and the RT-qPCR

reaction system was placed in a 96-well plate. At the same time, a reaction program was set. The denaturation was pre-denatured at 95 °C for five minutes, denatured at 95 °C for five seconds, extended at 72 °C for one minute, and extended at 72 °C for seven minutes. Three replicates were set for each reaction, and the mean value was recorded at the same time. Based on the median relative expression of the gene in esophageal cancer, the esophageal-cancer tissue samples were divided into two groups: relatively high levels of expression and relatively low levels of expression.

In this study, 82 patients with esophageal cancer who were included in the study were monitored for up for five years during the behavioural period; this mostly involved six-monthly telephone contact and outpatient review.

Statistical methods

The expression level of miR-483-5p for each tissue was expressed by ($\bar{x}\pm s$), and the t-test was used for comparison between the two groups. The relationship between the expression of miR-483-5p and the clinicopathological parameters of esophageal cancer patients was compared by χ^2. The Kaplan-Meier method was adopted to analyse the survival rate of patients with esophageal cancer, and the survival rate between the two groups was compared using a log-rank test. The COX proportional hazard regression model was used to analyse independent prognostic factors in patients with esophageal cancer. A P value of <0.05 was considered statistically significant.

Results

Expression of miRNA-483-5p in esophageal cancer and adjacent tissues

The relative expression of miRNA-483-5p in esophageal cancer tissues was (3.25±1.25), and the relative expression of miRNA-483-5p in adjacent tissues was (0.99±0.71). The difference between the two was significant (P<0.05). The results are shown in Table 1.

Group	n	Relative expression of miRNA-483-5p
Esophageal cancer tissues	82	3.25±1.25
Adjacent tissues	28	0.99±0.71
t		9.063
P		<0.001

Table 1: Expression of miRNA-483-5p in esophageal cancer and adjacent tissues ($\bar{x}\pm s$).

Relationship between miRNA-483-5p expression and clinicopathological parameters in patients with esophageal cancer

The expression level of miRNA-483-5p was not related to the esophageal cancer patients' gender, age, or histological grade, nor the depth of invasion of the tissue (P>0.05).

It was related to lymph node metastasis and the TNM stage of esophageal cancer patients (P<0.05). The results are shown in Table 2.

Parameters	n	miR-483-5p		χ^2	*P*
		Low level of expression (n=41)	High level of expression (n=41)		
Gender				1.680	0.825
Male	43	22	21		
Female	39	19	20		
Age				0.351	0.647
≥50	52	27	25		
<50	30	14	16		
Histological grade				2.005	0.367
I	35	18	17		
II	25	13	12		
III	22	10	12		
Depth of invasion				1.856	0.641
Shallow	28	15	13		
Deep	54	26	28		
Lymph node metastasis				6.111	<0.001
Yes	58	21	37		
No	24	20	4		
TNM stage				37.216	<0.001
I~II	53	31	22		
III~IV	47	10	37		

Table 2: Relationship between miRNA-483-5p expression and clinicopathological parameters in patients with esophageal cancer.

Relationship between miRNA-483-5p expression and prognosis in patients with esophageal cancer

The five-year survival rate of patients with relatively high levels of expression of miRNA-483-5p was 43.90% (18/41), and the five-year survival rate of patients with relatively low levels of expression of miRNA-483-5p was 75.61% (31/41). There was a significant difference (P<0.05). The results are shown in Fig. 1.

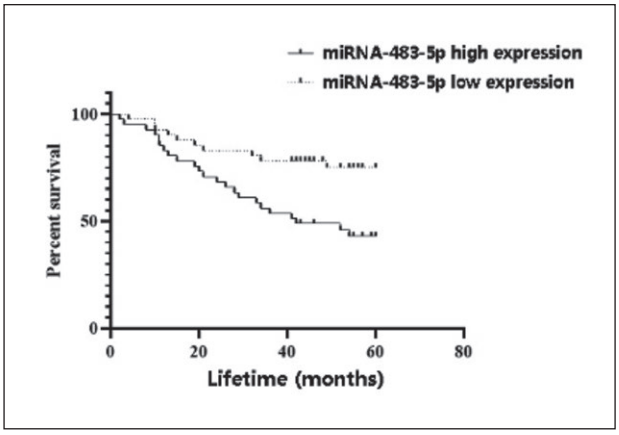

Figure 1: Relationship between miRNA-483-5p expression and prognosis in patients with esophageal cancer.

Analysis using COX model for patients with esophageal cancer

COX proportional hazard regression model results showed that miRNA-483-5p expression level, lymph node metastasis, and TNM stage were independent prognostic factors for patients with esophageal cancer. The results are shown in Table 3.

Parameters	Single factor analysis		Multi-factor analysis	
	95%CI	*P*	95%CI	*P*
Gender	1.031 (0.738~1.441)	0.92	-	-
Age	0.785 (0.471~1.315)	0.347	-	-
Histological grade	1.830 (0.941~3.568)	0.079	-	-
Depth of invasion	1.477 (1.056~2.068)	0.059	-	-
Lymph node metastasis	1.562 (1.121~2.177)	0.009	1.476 (1.051~2.076)	0.031
TNM stage	1.436 (1.015~2.031)	0.046	1.481 (1.056~2.080)	0.019
Expression of miRNA-483-5p	2.561 (1.331~5.106)	0.007	1.467 (1.028~2.095)	0.039

Table 3: Analysis using COX model for patients with esophageal cancer.

Discussion

The onset of esophageal cancer involves a series of evolutions, and there is also abnormal genetic and epigenetic accumulation. Current studies have shown that there are genetic mutations in esophageal cancer, but its relationship with tumour progression has not been elucidated. In addition, clinical researchers have not found genes that can specifically induce esophageal cancer[8]. Studies have shown that miRNAs play a role in multiple signalling pathways by regulating many target genes; detecting abnormally expressed miRNAs in tumours could further understanding of their genesis and their action mechanisms. Clinical reports have shown that miRNAs could regulate many transcriptional genes and that they are important for screening and verifying cancer[9]. Each tumour has a specific miRNA-expression profile. If the expression of miRNAs is abnormal during tumorigenesis, development, invasion, and metastasis, it could further reveal the role and evolution of tumours[10].

Serum miRNA is obtained relatively simply, is stable and reproducible, and is resistant to adverse environments. Clinical studies in 2008 confirmed the presence of miRNAs in the blood with potential for tumour evaluation[11]. Although esophageal cancer generally results in a poor prognosis, related reports have shown that miRNAs play an important role in the early diagnosis of esophageal cancer and are also closely related to the prognoses of patients[12]. The gene miRNA-483-5p was taken as the research object to further clarify its correlation with the pathogenesis of esophageal cancer.

The gene miRNA-483-5p was first discovered in human embryonic livers. Previous reports have confirmed that it is abnormally expressed in colon cancer, esophageal cancer and other cancers[13]. Through study of adrenocortical carcinoma, it was found that the expression of miRNA-483-5p is up-regulated in cancer tissues, and its expression level relates to the patient's prognosis, indicating that high levels of miRNA-483-5p expression indicates a poor prognosis[14]. Esophageal-cancer-related studies have shown that the expression level of miRNA-483-5p in esophageal cancer tissues is up-regulated compared with adjacent tissues, and that the miRNA-483-5p expression is related to TNM stage and lymph node metastasis in patients with esophageal cancer[15]. This study's experimental results show that miRNA-483-5p is expressed at high levels in cancer. In this study, the expression levels of miRNA-483-5p in esophageal cancer and normal-adjacent tissue were measured and then analysed in the context of the clinical-pathological parameters of patients. The results were consistent with the studies referenced. This suggests that miRNA-483-5p expression is elevated in esophageal cancer and may be involved in the onset and progression of esophageal cancer. However, several experiments have shown that miRNA-483-5p expression is reduced in some cancers[16]. In a study of human neurotic cell carcinoma, it was found that miRNA-483-5p expression was significantly lower than that of adjacent tissues. It has been reported that the disordered expression of miRNA-483-5p is related to the low survival rate of some cancer patients[17].

In order to further explore the clinical value of miRNA-483-5p in the prognosis evaluation of patients with esophageal cancer, monitoring of all patients in the group continued. The results showed that the five-year survival rate of patients with relatively high levels of expression of miRNA-483-5p was significantly lower than

that of patients with relatively low levels of expression of miRNA-483-5p. The results using the COX proportional hazard regression model showed that miRNA-483-5p expression level, lymph node metastasis, and TNM stage were independent prognostic factors for patients with esophageal cancer. These results confirmed that miRNA-483-5p demonstrates abnormally high levels of expression in esophageal cancer and esophageal cancer, and it participates in the development of esophageal cancer and plays an important role.

The expression of miRNA-483-5p is elevated in esophageal cancer tissues, and its expression is related to lymph node metastasis and TNM staging in patients with esophageal cancer. Expression of miRNA-483-5p could be used as an independent risk factor for the prognosis of patients with esophageal cancer, being that it has been shown to be linked to the onset and progression of esophageal cancer; it can, therefore, be expected to become a new target for the clinical treatment of esophageal cancer.

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