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miR-145 expression level in tissue predicts prognosis of patients with esophageal squamous cell carcinoma

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ABSTRACT

Background: MicroRNA-145 (miR-145) is commonly down-regulated and has been identified as a tumor-suppressive miRNA in multiple types of cancers, as well as in esophageal squamous cell carcinoma (ESCC). In the present study, the clinical significance and prognostic value were investigated in ESCC.

Methods: A total of 126 patients with ESCC who underwent surgery were included in the present study. miR-145 expression was determined using quantitative real-time polymerase chain reaction assay (qRT-PCR) and was further correlated with patients' clinicopathological parameters. Overall survival was estimated by using Kaplan-Meier method, and univariate analysis was conducted by log-rank test. The Cox proportional hazards model was used in the multivariate analysis.

Results: miR-145 expression levels in ESCC tissues were significantly decreased compared with the adjacent normal zones ($P < 0.001$). We observed that the expression level of miR-145 was positively correlated with the tumor differentiation ($P = 0.015$), lymph node status ($P = 0.007$), distant metastasis ($P = 0.008$), and TNM stage ($P = 0.033$). ESCC patients with low miR-145 expression level had shorter overall survival than those with high miR-145 expression level (log-rank test, $P = 0.032$). Furthermore, multivariate Cox regression analysis showed that miR-145 expression level was independent factor in predicting the overall survival of ESCC patients (HR = 1.993, 95% CI: 1.277–8.283, $P = 0.023$).

Conclusions: Our findings indicated that miR-145 expression may be a useful prognostic marker that could be used for predicting overall survival of patients with ESCC.

1. Introduction

Esophageal cancer is the eighth most common malignant neoplasm and the sixth leading cause of cancer-related death worldwide [1]. There are two main subtypes of esophageal cancer, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), with ESCC being the most frequent type of esophageal malignancy. Despite advances in treatment of esophageal cancer approaches over the last decades, its prognosis has shown little improvement. Consequently, finding new molecular targets for its diagnosis, prognosis, and treatment has the potential to improve the clinical strategy and outcome of esophageal cancer [2,3].

MicroRNA (miRNA) are a class of small non-coding RNA molecules that are normally around 22 nucleotides in length. And they are evolutionarily conserved and functioned as negative regulators of gene expression at the post-transcriptional level, by binding to mRNA and suppressing translation [4,5]. MiRNAs were aberrantly expressed or mutated in human cancer, indicating that they might function as a novel class of oncogenes or tumor suppressor genes. A better knowledge of changes in miRNA expression during esophageal carcinogenesis might lead to possible improvements in the diagnosis and treatment for esophageal cancer [6].

miR-145 is commonly down-regulated and has been identified as a tumor-suppressive miRNA in multiple types of cancers, including non-small cell lung cancer cell lines (NSCLC) [7], colorectal cancer [8], bladder cancer [9], breast cancer [10], gastric cancer [11], and endometrial cancer [12]. Previously, miR-145 expression was found to be frequently downregulated in esophageal squamous cell carcinoma (ESCC) specimens and cell lines compared with adjacent normal tissues. Furthermore, overexpression of miR-145 inhibits tumor growth in part by targeting c-Myc, revealing that miR-145 may act as a tumor suppressor in ESCC [13]. Another experimental study suggested that miR-145-5p plays tumor-suppressive roles by inhibiting esophageal cancer cell migration, invasion and EMT through regulating the Sp1/NF-κB

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signaling pathway. However, its clinical significance and prognostic value have not been investigated.

2. Materials and methods

2.1. Patients and specimens

A total of 126 patients with ESCC had undergone routine surgery at Anhui provincial hospital from April 2013 to March 2018. Esophageal cancer samples and the corresponding adjacent esophageal tissues taken from the 126 patients were collected. None of the patients had received local or systemic therapy prior to surgery. All the patients included in the present study had not received preoperative chemotherapy and/or radiotherapy, had undergone R0 resection and esophagogastrostomy without perioperative death. Tumor samples were collected immediately following surgical resection, snap-frozen in liquid nitrogen and then stored at −80 °C until required for the extraction of total RNA. The histopathologic diagnosis of ESCC was verified based on sections stained with hematoxylin and eosin, according to the classification system of the World Health Organization. Tumor-node-metastasis (TNM) staging was determined based on the 7th classification guidelines of the American Joint Committee on Cancer. In this study, all experiments were performed in accordance with the principles outlined in the Declaration of Helsinki. All experimental protocols were approved by the Clinical Research & Ethic Committees at Anhui provincial hospital. All patients signed consent forms before enrollment in the study. Clinicopathological characteristics in our study are presented in Table 1.

2.2. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from tissues using TRIZOL reagent according to the manufacturer's protocol (Invitrogen). RNA was reverse transcribed using SuperScript First Strand cDNA System (Invitrogen) according to the manufacturer's instructions. Primers for miR-145 and endogenous control U6 snRNA were obtained from Applied Biosystems (Foster City, California, USA). Quantitative real-time PCR assay was performed to evaluate miR-145 expression using SYBR Premix Ex Taq (Takara Co. Ltd) and measured in a LightCycler 480 System (Roche, Basel, Switzerland). The amplification profile was denatured at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. All quantitations were normalized to the level of human U6 snRNA in the reaction. The comparative threshold cycle (CT) (DDCT) method, which compares differences in CT values between common reference RNA and target gene RNA, was used to obtain the relative fold changes in gene expression.

Table 1
Summary of clinicopathologic features of the ESCC patients.

Variables	Cases (n)	miR-145 expression		P value
		Low (n = 62)	High (n = 64)	
Gender				
Male	71	34	37	0.858
Female	55	28	27	
Age (years)				
< 60	69	35	34	0.724
≥60	57	27	30	
Tumor location				
Up/middle	74	35	39	0.718
Lower	52	27	25	
Differentiation				
Well/Moderate	81	33	48	0.015
Poor	45	29	16	
Lymph node metastasis				
Yes	31	22	9	0.007
No	95	40	55	
Distant metastasis				
Yes	13	11	2	0.008
No	113	51	62	
TNM stage				
I/II	86	33	53	0.033
III/IV	40	29	11	

2.3. Follow-up

At this study, patients are routinely followed once every 2–4 months in the first three years, regardless of the stage of their disease. Time to death is defined from the date of surgery. At each visit, patients undergo a medical history and physical examination. Imaging tests and required examinations are performed when clinically indicated.

2.4. Statistical analysis

Statistical analyses were performed with SPSS 18.0 software (SPSS, Inc., Chicago, IL). Data were analyzed using independent two-tailed *t*-test. Overall survival was estimated by using Kaplan-Meier method, and univariate analysis was conducted by log-rank test. The Cox proportional hazards model was used in the multivariate analysis. Two-tailed P-value of < 0.05 was considered statistically signifcant.

3. Results

3.1. The expression level of miR-145 in human ESCC tissues

miR-145 expression was determined using quantitative real-time polymerase chain reaction assay. The analysis of miR-145 relative expression levels (comparing with U6) in ESCC tissue specimens indicated significantly decreased expression in the malignant specimens compared with the adjacent normal zones (shown in Fig. 1, $P < 0.001$). The relative miR-145 expression level was classified as high or low in relation to the median value.

3.2. Relationship between miR-145 expression and clinicopathological characteristics in ESCC patients

The relationships between miR-145 expression levels and clinicopathological characteristics in individuals with ESCC are summarized in Table 1. We did not find a significant association of miR-145 expression levels with patient's gender ($P = 0.858$), age ($P = 0.724$), and tumor location ($P = 0.718$) in 126 ESCC cases. However, we observed that the expression level of miR-145 was positively correlated with the tumor differentiation ($P = 0.015$), lymph node status ($P = 0.007$), distant metastasis ($P = 0.008$), and TNM stage ($P = 0.033$) in ESCC patients (shown in Table 1).

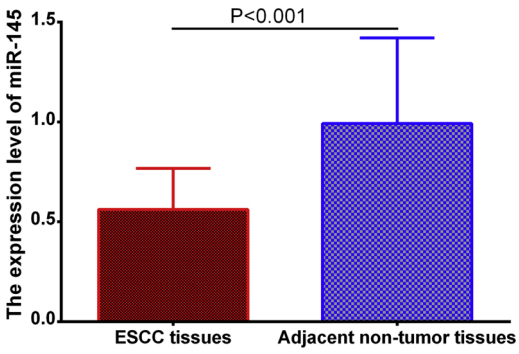

Fig. 1. The expression level of miR-145 was significantly downregulated in ESCC tissues when compared with matched adjacent normal tissues ($P < 0.001$).

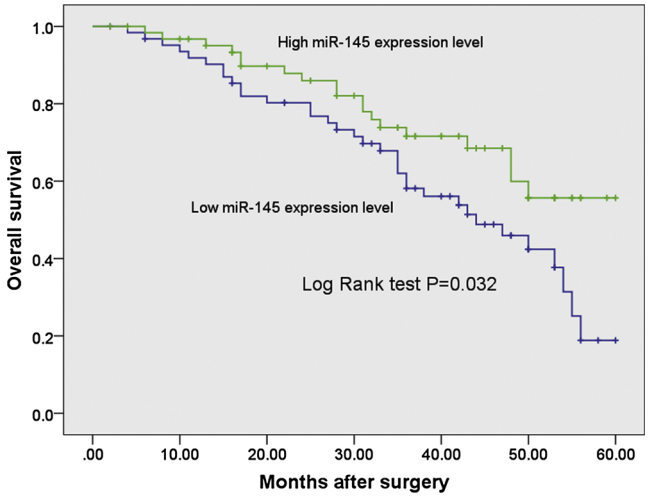

Fig. 2. ESCC patients with low miR-145 expression level had shorter overall survival than those with high miR-145 expression level (P = 0.032).

Table 2
Multivariate Cox regression analysis of overall survival in 126 patients with ESCC.

Variable	Hazard ratio	95% CI	P-value
Gender	0.827	0.591–2.182	0.572
Age	1.226	0.893–3.018	0.113
Tumor location	0.629	0.782–2.446	0.711
Differentiation	3.016	1.937–9.023	0.028
Lymph node metastasis	2.782	1.628–10.283	0.017
Distant metastasis	3.927	2.017–11.337	0.002
TNM stage	3.012	1.883–10.365	0.006
miR-145 expression	1.993	1.277–8.283	0.023

3.3. The expression level of miR-145 was associated with ESCC prognosis

To evaluate the prognostic value of miR-145 expression in ESCC patients, survival curves were constructed by Kaplan–Meier method and compared by the log-rank test. As shown in Fig. 2, ESCC patients with low miR-145 expression level had shorter overall survival than those with high miR-145 expression level (log-rank test, P = 0.032). To determine the possibility of miR-145 as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-145 expression were evaluated by multivariate Cox regression analysis. Results showed that miR-145 expression level (HR = 1.993, 95% CI: 1.277–8.283, P = 0.023) was independent factor in predicting the overall survival of ESCC patients (shown in Table 2). In advanced subanalyses, it was found that reduced miR-145 expression level was associated shorter overall survival in both stage I–II ESCC (log-rank test, P = 0.001, Fig. 3A) and stage III–IV cases (og-rank test, P < 0.001, Fig. 3B).

4. Discussion

Esophageal cancer is one of the most common gastrointestinal cancers. Up to 300 thousand people worldwide die from this disease each year. More than 50% of the global incidence of esophageal cancer is in China. Despite advances in its diagnosis and multimodal therapies, the prognosis for patients with esophageal cancer remains poor. Therefore, the goal to attain a more thorough understanding of the molecular biology, genetic causes, and cellular origin of esophageal cancer is of great significance in the development of improved therapeutic strategies and in the identification of prognostic markers.

Currently, investigators have focused on the potential of miRNAs to serve as biomarkers for cancer. Aberrant expression of miRNAs in cancer tissue has been reported in various types of cancers, and increasing evidence suggested the potential of miRNA as prognostic markers in cancer Chen, 2018 #18; Feng, 2018 #19}. miR-145 is commonly down-regulated and has been identified as a tumor-suppressive miRNA in multiple types of cancers. For example, Liu Q et al. found that miR-145 was downregulated in NSCLC cancer tissue compared with that in adjacent normal tissue. NSCLC cell lines, namely H1299, PC7, and SPCA-1, also demonstrated miR-145 downregulation, which is correlated well with their invasive ability, assessed by the Matrigel invasion assay. Furthermore, miR-145 is an important molecule in NSCLC that regulates cancer epithelial-mesenchymal transition (EMT) through targeting ZEB2 [7]. In the study by Tang H et al., miR-145 expression was found to be downregulated in CRC tissues and cell lines, and miR-145 regulated tumor suppressor candidate 3 and mitogen-activated protein kinase pathway to inhibit the progression of colorectal cancer [8]. Zhang H et al. showed that the miR-145 was downregulated in bladder cancer. miR-145 overexpression inhibited cell proliferation and migration. Moreover, miR-145-5p directly targeted TAGLN2, and TAGLN2 expression was increased in bladder cancer. In addition, the high expression of TAGLN2 promoted cell proliferation and migration in bladder cancer. miR-145 appeared to regulate TAGLN2 in bladder cancer and it also inhibited the cell proliferation and migration [9].

Previously, miR-145 expression was found to be frequently downregulated in esophageal squamous cell carcinoma (ESCC) specimens and cell lines compared with adjacent normal tissues. Furthermore, overexpression of miR-145 inhibits tumor growth in part by targeting c-Myc, revealing that miR-145 may act as a tumor suppressor in ESCC [13]. However, its clinical significance and prognostic value have not been investigated. In the present study, the analysis of miR-145 expression levels in ESCC tissue specimens indicated significantly decreased expression in the malignant specimens compared with the adjacent normal zones. The relationships between miR-145 expression levels and clinicopathological characteristics in individuals with ESCC are analysed, and we observed that the expression level of miR-145 was positively correlated with the tumor differentiation, lymph node status, distant metastasis, and TNM stage in ESCC patients. To evaluate the prognostic value of miR-145 expression in ESCC patients, survival curves were constructed by Kaplan–Meier method and compared by the

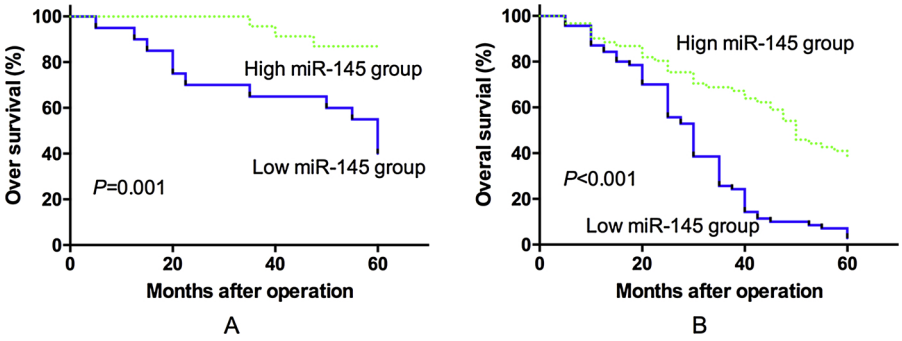

Fig. 3. ESCC patients with low miR-145 expression level had shorter overall survival than those with high miR-145 expression level in both TNM I–II and TNM III–IV group. A) Low miR-145 expression level had shorter overall survival in TNM I–II group (P = 0.001). B) Low miR-145 expression level had shorter overall survival in TNM III–IV group (P < 0.001).

log-rank test. We found that ESCC patients with low miR-145 expression level had shorter overall survival than those with high miR-145 expression level. To determine the possibility of miR-145 as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-145 expression were evaluated by multivariate Cox regression analysis. Results showed that miR-145 expression level was independent factor in predicting the overall survival of ESCC patients.

It is also important to detect the circulating biomarkers of different disease because is convenient to get blood samples of the patients. There were a lot previous clinical studies focusing on circling miRNAs expression in different diseases. The present study aimed to investigate the feasibility of using serum microRNAs as biomarkers for ESCC. In this study, we focus on the expression and prognostic effect of miR-145 in ESCC because we can use it as a prognosis biomarker in the following study. Also, we will conduct some other experimental study focusing on the effect of miR-145 on ESCC and this experiment needs the miRNA expression profile of ESCC.

Taken together, our findings indicated that miR-145 might be a tumor-suppressive miRNA in ESCC, and its expression may be a useful prognostic marker that could be used for predicting overall survival of patients with ESCC.

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