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Down-regulation of lncRNA MIR31HG correlated with aggressive clinicopathological features and unfavorable prognosis in esophageal squamous cell carcinoma

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Abstract. – OBJECTIVE: Long non-coding RNA MIR31HG (MIR31HG) has been shown to affect numerous tumorigenesis. However, the function of MIR31HG in esophageal squamous cell carcinoma (ESCC) remains unclear. The aim of this study was to investigate whether the levels of MIR31HG could be served as a prognostic factor in patients with ESCC.

PATIENTS AND METHODS: MIR31HG expression was detected in 185 samples of surgically resected ESCC and matched normal tumor-adjacent tissues by qRT-PCR. The association between MIR31HG expression levels in tissue and characteristics was examined. Overall survival (OS) curves were conducted to compare MIR31HG level and clinical characteristics. Cox regression analysis was conducted to determine the prognostic value of MIR31HG.

RESULTS: The levels of MIR31HG were decreased in the ESCC tissues from patients with ESCC compared with the control ($p < 0.01$). In malignant cases, lower expression MIR31HG levels were significantly associated with poor differentiation ($p < 0.001$), advanced lymph node metastasis ($p = 0.006$), positive distant metastasis ($p = 0.005$) and TNM stage ($p = 0.004$). Kaplan-Meier analysis indicated that patients presenting with reduced MIR31HG expression exhibited poorer OS ($p = 0.0002$). Univariate and multivariate analysis suggested that MIR31HG expression was an independent prognostic marker for survival in patients with ESCC.

CONCLUSIONS: We observed that down-regulated MIR31HG in ESCC patients was associated with malignant clinical characteristics. MIR31HG might be considered as a potential prognostic indicator and a potential target for therapeutic targets in ESCC.

Key Words:
Long non-coding RNA, MIR31HG, ESCC, Prognosis.

Introduction

Esophageal cancer is one of the most aggressive carcinomas and has been ranked as the sixth most common cause of cancer-related death worldwide[1]. There are two major histological types of esophageal cancer, viz. esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC)[2]. Importantly, ESCC is the most common pathological type (90-95%) in China[3]. Despite the rapid advancement in diagnosis and therapy, the 5-year overall survival rate is only about 25-30% among patients who have undergone curative surgical resections[4,5]. Therefore, it is required to identify specific and reliable biomarkers involved in development and progression of ESCC to predict the clinical outcome of the tumor. Increasing studies have focused on long non-coding RNAs (lncRNAs), which are greater than 200 nt and are unable to be translated into proteins[6]. The functions of a number of lncRNAs have been characterized, and it is evident that they play crucial roles in development and progression of tumors[7,8]. Growing evidence suggests that cancer lncRNAs may serve as a tumor suppressor or promoter in various tumors and may be a new class of cancer biomarkers and therapeutic targets[9]. For instance, lncRNA PANDAR was reported to be downregulated in non-small cell lung cancer tissues and its overexpression significantly inhibit tumors cell proliferation by regulating Bcl-2[10]. It has been reported that lncRNA UCA1 may promote breast cancer cell invasion and migration through enhancing Wnt/beta-catenin signaling pathway[11]. Another lncRNA BANCR was reported to serve as a tumor promoter and its up-regulation was associated with poor pro-

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gnosis in patients with ESCC[12]. Their findings highlighted the role of lncRNA in progression of tumors. However, the function of most lncRNAs has not been confirmed.

MIR31HG (also known as LOC554202) is an lncRNA located in 9p21.3 and 2166 bp in length. Recently, MIR31HG has been shown to be aberrantly expressed in various types of tumor tissues, as well as playing significantly roles in a number of biological processes including proliferation, migration or apoptosis[13-15]. However, the potential role of MIR31HG as a prognostic biomarker in ESCC has not been investigated. Therefore, the primary aim of this study was to investigate MIR31HG clinical prognosis significance in patients with ESCC.

Patients and Methods

Tissue Samples

Between October 2008 and April 2011, 185 ESCC samples and paired adjacent non-cancerous esophageal tissues were collected from ESCC patients, who underwent surgical resection at our hospital. The surgical tissues were carefully examined by a pathologist, and the final surgical pathology reports were recorded. Eligibility was granted if primary diagnosis occurred ≤ 6 months prior to study enrollment and. None of the ESCC patients had received any anticancer treatment prior to sampling. All patients were clinically staged according to the seventh edition of the American Joint Committee on Cancer (AJCC) system for esophageal cancer[16]. Overall survival was defined as the time between the date of surgery and the date of death. Study approval was obtained from independent Ethics Committees at Chinese PLA General Hospital. All participants provided written consent.

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for MIR31HG

Total RNA was isolated from human tissues using TRIzol reagent (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. First-strand cDNA was synthesized using the SuperScript III first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). The expression levels of MIR31HG were detected with the use of RT-qPCR, which were conducted using an SYBR Green mix in a 10-μl reaction volume on an ABI Prism 7900 Sequence Detector System (TaKaRa, Dalian, China). GAPDH was used as an endogenous control. Fold changes were calculated through relative quantification ($2^{-\Delta\Delta Ct}$). The sequences were as follows: MIR31HG primers: 5'-TCTCTGGTGCTTCCCTCCTT-3' (forward) and 5'-GATCTAAGCTTGAGCCCCCA-3' (reverse). GAPDH primers: 5'-GTCAACGGATTTGGTCTGTATT-3' (forward) and5'-AGTCTTCTGGGTGGCAGTGAT-3' (reverse). Each assay was performed in triplicate, and the average was calculated.

Statistical Analysis

The data for statistical analyses were performed by using the SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL, USA). The X^2-test was performed to determine the significance of MIR31HG expression as being correlated with clinicopathologic factors in ESCC. The relevance between the MIR31HG expression and the overall survival of patients with ESCC was assessed using log-rank test and Kaplan-Meier method. Univariate and multivariate survival analysis were performed using the Cox proportional hazards model. $p \leq 0.05$ was considered to indicate a statistically significant difference.

Results

MIR31HG was Frequently Downregulated in Human ESCC Tissues

Expression of MIR31HG was measured in all 185 GC samples and adjacent non-tumor tissues using Real-time PCR. As shown in Figure 1, we found that MIR31HG was significantly downregulated in ESCC tissues compared with matched non-tumor tissues ($p < 0.01$).

Downregulation of MIR31HG Associates with Advanced Clinicopathological Features of ESCC

To further investigate the clinicopathological significance of MIR31HG levels in patients with ESCC, we divided the 185 patients with ESCC into a high-expression group (n = 95) and a low-expression group (n = 90) based on the median expression level of MIR31HG. The correlation of MIR31HG expression with different clinicopathological features in ESCC was illustrated in Table I. Low MIR31HG expression was observed to be closely correlated with poor differentiation ($p < 0.001$), advanced lymph node metastasis ($p = 0.006$), positive

Table I. Association between MIR31HG expression and clinicopathological features of ESCC patients.

Variables	Cases (n)	MIR31HG expression		p-value
		Low (n = 90)	High (n = 95)	
Gender				NS
Male	118	57	61	
Female	77	33	34	
Age (years)				NS
< 55	100	51	49	
≥ 55	85	39	46	
Tumor location				NS
Middle	75	34	41	
Lower	110	56	54	
Differentiation				< 0.001
Well+ moderate	118	45	73	
Poor	67	45	22	
Lymph node metastasis				0.006
Yes	47	31	16	
No	138	59	79	
Distant metastasis				0.005
Yes	58	37	21	
No	127	53	74	
TNM stage				0.004
I + II	123	56	77	
III + IV	62	34	18	

distant metastasis (p = 0.005) and TNM stage (p = 0.004). However, there was no association between MIR31HG expression and age or gender (p > 0.05).

Correlation Between MIR31HG Expression and Prognosis of ESCC Patients

In order to explore the clinical significance of MIR31HG in progression of ESCC, the prognostic value of MIR31HG expression in human ESCC was further investigated by Kaplan-Meier analysis and the log-rank test. As shown in Figure 2, we observed that patients with high MIR31HG levels had better prognosis than those with low MIR31HG levels (p = 0.0020). The differentiation, lymph node metastasis, distant metastasis, TNM stage and MIR31HG expression, were distinctly associated with poor overall survival of ESCC patients based on the univariate analysis (p=0.001, 0.003, 0.001, 0.002, and 0.005, respectively) (Figure 2). Furthermore, multivariate analysis revealed that differentiation (p = 0.001), lymph node metastasis (p = 0.005), distant metastasis (p = 0.002), TNM stage (p = 0.004) and MIR31HG expression (HR=2.231, 95% CI, 1.118-3.899; p=0.007) were the influencing factors to lifetime of ESCC (Table II).

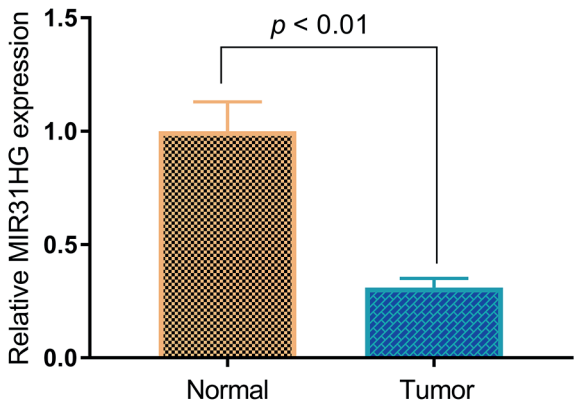

Figure 1. MIR31HG expression in ESCC tissues and adjacent normal tissues was detected by RT-qPCR.

Discussion

Approximately 200,000 people worldwide die from ESCC each year, and China is a particularly high incidence area[17]. Accumulating evidence has suggested that lncRNAs play a crucial role in the pathogenesis of ESCC[18,19]. Moreover, accumulating scholars have suggested that lncRNAs could serve as ideal prognostic biomarkers in various diseases including cancer because they are easy

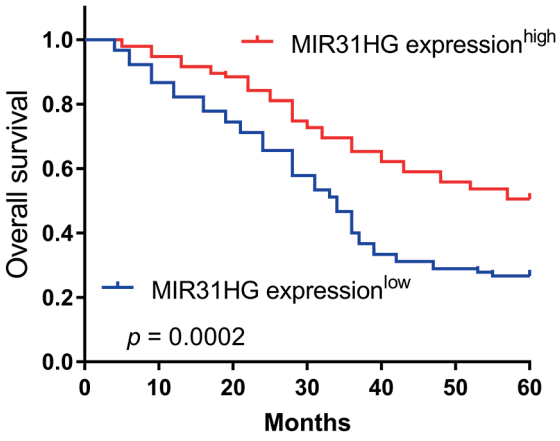

Figure 2. Kaplan-Meier survival analysis was utilized to determine the association of MIR31HG expression with the prognosis of the patients with ESCC.

to detect and strongly associated with clinical prognosis[20-22].

MIR31HG has been reported to function as a tumor suppressor or oncogene in a variety of human cancers. For instance, Yang et al[23] reported that knockdown of MIR31HG significantly suppressed pancreatic ductal adenocarcinoma cell growth and invasion whereas enhanced expression of MIR31HG had the opposite effects. They further confirmed that its expression was negatively regulated by miR-193b. In contrast, Nie et al[24] found that MIR31HG was correlated with gastric cancer proliferation and served as a marker of poor prognosis in gastric cancer patients. Ding et al[25] reported that the overexpression of Loc554202 (MIR31HG) decreased the cell proliferation and induced apoptosis *in vitro* and hindered tumorigenesis *in vivo* through the activation of specific caspase cleavage cascades. Also, Yang et al[26] further indicated that the patients with the lower expression of Loc554202 had a worse outcome compared to patients with higher Loc554202 expression. However, there were no reports about the prognostic significance of MIR31HG expression in human ESCC.

In the present study, we firstly determined the expression pattern of MIR31HG in ESCC tissues and matched normal tissues by RT-PCR. The results showed that the mean level of MIR31HG expression in ESCC tissues was significantly lower than that in the adjacent non-tumor tissues. Moreover, the clinical clinicopathological significance of MIR31HG expression in ESCC was explored, and differentiation, lymph node metastasis, distant metastasis and TNM stage were found to be correlated with MIR31HG expression. Next, Kaplan-Meier analysis showed that patients with low MIR31HG expression level had a significantly shorter overall survival than those with high MIR31HG expression level, which was consistent with reports of tumor-suppressive role of MIR31HG in colorectal cancer. Moreover, multivariate Cox analysis proved that MIR31HG was an independent prognostic indicator for ESCC patients. Thus, MIR31HG may be a prognostic predictor for ESCC.

Conclusions

We provided the first evidence that MIR31HG was low expressed in ESCC tissues, and that decreased expression of MIR31HG was associated with poor clinical outcome. Thus, MIR31HG may function as a potential diagnostic biomarker and therapeutic target for ESCC. Importantly, the mechanism of translocation and regulation of MIR31HG in HCC need to be studied.

Founding

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Table II. Univariate and multivariate analyses for overall survival in ESCC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	0.892	0.677-1.342	0.362	-	-	-
Age	1.213	0.833-1.679	0.315	-	-	-
Tumor location	1.455	0.791-1.893	0.266	-	-	-
Differentiation	3.542	1.774-5.933	0.001	2.893	1.556-5.145	0.001
Lymph node metastasis	3.156	1.556-4.972	0.003	2.456	1.269-4.288	0.005
Distant metastasis	4.145	1.681-6.326	0.001	3.785	1.388-5.766	0.002
TNM stage	3.885	1.559-5.894	0.002	3.235	1.217-5.211	0.004
MIR31HG expression	2.893	1.441-4.346	0.005	2.231	1.118-3.899	0.007

Conflict of interest

The authors declare no conflicts of interest.

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