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Reduced miR-300 expression predicts poor prognosis in patients with laryngeal squamous cell carcinoma

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Abstract. – OBJECTIVE: miR-300 has been demonstrated to play an important role in the progression of several tumors, but its role in tumorigenesis of laryngeal squamous cell carcinoma (LSCC) is still unclear. The purpose of this study was to explore miR-300 expression in LSCC patients and analyze its association with clinicopathological factors and prognosis.

PATIENTS AND METHODS: In the present study, we measured the expression level of miR-300 in LSCC tissues by RT-PCR. Associations between miRNA-300 expressions and various clinicopathological characteristics were analyzed. Patient survival and their differences were determined by Kaplan-Meier method and log-rank test. The univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: miR-300 expression was significantly increased in LSCC tissues compared with that in adjacent non-cancerous tissues ($p < 0.01$). In addition, lymph node metastasis ($p = 0.004$) and TNM stage ($p = 0.001$) were obvious influence factors for the expression of miR-300. More importantly, Kaplan-Meier analysis showed that LSCC patients with low miR-300 expression tended to have shorter overall survival ($p < 0.001$). Finally, multivariate analysis revealed that miR-300 expression was an independent prognostic factor for LSCC patients.

CONCLUSIONS: Our results pointed to miR-300 as a powerful prognostic marker in LSCC and as a novel target for tumor-suppressive therapy.

Key Words:
miR-300, LSCC, Prognosis, Overall survival.

Introduction

As a common head and neck malignancy, laryngeal carcinoma has a high incidence of approximately 2.4% of new cases around the world every year[1]. In 2008, laryngeal carcinoma was responsible for 82,000 deaths worldwide[2]. Laryngeal squamous cell carcinoma (LSCC) is the most common type of laryngeal carcinoma[3]. Despite the great progress that has been made in the treatment of the disease, including surgery or radiotherapy, the overall 5-year survival rates for laryngeal carcinoma were less than 50%, which are mainly due to the metastasis and recurrence. Therefore, it is necessary to explore effective biomarkers for early-stage diagnosis and potential targets for therapy.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs with 20-22 nucleotides[4]. Mature miRNAs can be generated from sequential processing of primary miRNA transcripts by Drosha and Dicer, and they can silence their cognate target genes by specifically binding and cleaving mRNAs or inhibiting their translation[5,6]. Previous evidence revealed that the expression patterns of miRNAs played an important role in cancer progression[7,8]. Emerging studies have reported that some of miRNAs are commonly dysregulated in LSCC. For example, miR-206[9], microRNA-34a/c[10] and miR-144[11] were reported to be down-regulated always, while miR-155[12], miR-744-3p[13], and miR-145[14] were overexpressed in LSCC. It has been shown that miRNAs play an important role in all type of cancers. However, the expression of miRNAs in cervical cancer has not been studied in-depth.

In the present study, we focused on miR-300. We firstly determine the expression levels of miR-300 in LSCC tissues and match normal non-tumor tissues. Next, we evaluate the relationship between its expression and the clinical parameters of LSCC. Moreover, we investigated whether miR-300 expression was associated with the outcome of LSCC patients. Overall, This study will provide valuable evidence for the identification of novel prognostic biomarkers for LSCC.

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Patients and Methods

Patients and Clinical Specimens

Seventy-one cases of human LSCC tissue specimens were obtained at the time of surgical resection from Yidu Central Hospital of Weifang. Tissue samples were snap-frozen in liquid nitrogen at the time of total thyroidectomy and subsequently stored at -80°C. These patients were pathologically diagnosed with LSCC. Of the 133 patients, 87 were males and 46 were females, with a mean age of 61.33 ± 7.86 years. The characteristics of the patients are described in Table I. All patients provided written informed consent for the use of their tissues. The study approved by the Human Research Ethics Committee of Yidu Central Hospital of Weifang

Quantitative Real-time Polymerase Chain Reaction (qPCR)

Total RNA was isolated from fresh tissues and cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse transcribed with a miRNA-specific primer, followed by real-time PCR using TaqMan probes. The expression level of miR-300 was measured by quantitative real-time PCR (qRT-PCR), which was performed using the Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA). The PCR results were analyzed using the Mastercycler ep Realplex Program and reported as relative quantities with respect on a calibrator sample using the 2-ΔΔCt method. The expression levels of miR-300 were normalized to U6. The specific primers were shown in Table I.

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The unpaired t-test was applied to test the differential expression of miR-300 in cancer tissues compared to adjacent non-malignant tissues. The chi-square and t-tests were performed to assess the relationship between miR-300 expression levels and clinicopathological features. The Kaplan-Meier survival curves were plotted, and the log-rank test was done. The univariate analysis was used in multivariate analysis on the basis of Cox proportional hazards model. In all cases, a $p < 0.05$ was considered statistically significant.

Table I. RT-PCR primers.

The specific primers	Sequence
MiR-300	F:5'-TATACAAGGGCAGACTCTCT-CT-3'
	R:5'-GTGCAGGTTCCGAGGT-3'
U6	F:5'-CTCGCTTCGGCAGCACATATACT-3'
	R:5'-ACGCTTCACGAATTTGCGTGTC-3'

Results

Up-regulation of miR-300 in LSCC

qRT-PCR was performed to detect the expression levels of miR-300 in 133 pairs of LSCC and adjacent non-tumor tissues. Our results showed that miR-300 expression in LSCC tissues was significantly higher than in paired nontumor tissues ($p < 0.05$, Figure 1), suggesting that miR-300 may play an anti-oncogenic role in LSCC.

Correlation between miR-300 Expression and Clinical Features

To determine the correlation of miR-300 expression levels with the clinical features of LSCC, we divided patients into two groups based on the levels of miR-300 expression. As shown in Table II, no significant correlations were identified between miR-300 expression and clinicopathological parameters such as age, gender, thyroid cartilage invasion, and T classification. However, lymph node metastasis ($p = 0.004$) and TNM stage ($p = 0.001$) were obvious influence factors for the expression of miR-300.

miR-300 Expression and Patients' Survival

Since miR-300 was associated with lymph node metastasis and TNM stage of LSCC pa-

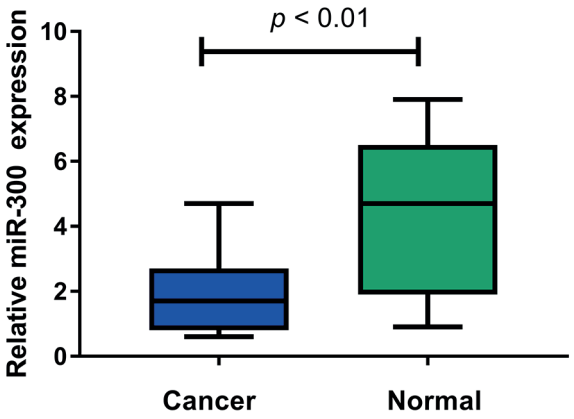

Figure 1. miR-300 was detected in LSCC tissues and matched non-cancerous tissues using qRT-PCR.

Table II. Association between miR-300 expression and different clinicopathological features of human LSCC.

Characteristics	All cases	miR-300 Low expression	miR-300 High expression	*p*-value
Age				0.703
<50	47	30	17	
≥50	86	52	34	
Gender				0.376
Male	87	56	31	
Female	46	26	20	
Thyroid cartilage invasion				0.301
Yes	40	22	18	
No	93	60	33	
T classification				0.127
T1-2	67	37	30	
T3-4	66	45	21	
Lymph node metastasis				0.001
Yes	73	54	19	
No	60	28	32	
TNM stage				0.004
I/II	65	32	33	
III/IV	68	50	18	

tients, we wonder whether miR-300 was correlated with survival of the patients. As expected, Kaplan-Meier survival analysis showed that high miR-300 expression predicted significantly better OS ($p < 0.001$, Figure 2).

Univariate analysis showed that lymph node metastasis, TNM stage, and miR-300 expression levels were significantly related to overall survival ($p = 0.011$, $p = 0.008$, and $p = 0.003$, resp., Table III). Multivariate analysis showed lymph node metastasis, TNM stage, and miR-300 expression levels were independent prognostic factors ($p < 0.014$, $p = 0.011$, and $p = 0.007$, resp., Table III).

Discussion

miRNAs do not encode any proteins. However, over the past decades, it has known to us that they participate in the regulation of cellular differentiation, proliferation, apoptosis, and development[15,16]. Novel prognostic markers are very important in the diagnosis and treatment of LSCC, and miRNAs are currently one of the promising candidates. Previous studies have shown the role of miR-300 in different types of tumors. For instance, Xue et al[17] showed that the expression of miR-300 was upregulated in osteosarcoma tissues, and overexpression of miR-300 promoted cell proliferation and invasion and induced EMT. Furthermore, They also identified BRD7 as a target of miR-300. Another study by Liu et al[18] revealed that serum miR-300 was an independent prognostic marker for osteosarcoma. Shen et al[19] found that over-expression of miR-300 could promote cell proliferation and cell cycle progression by targeting p53. Zhou et al[20] reported that miR-300 expression was downregulated in glioblastoma tissues, and

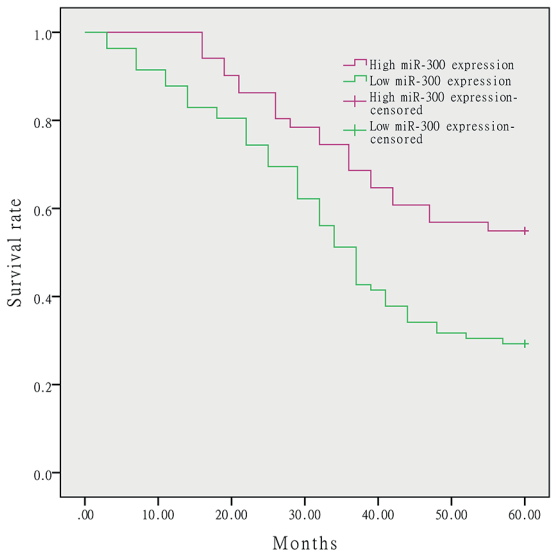

Figure 2. Kaplan-Meier curves of the overall survival of 133 LSCC patients.

Table III. Univariate and multivariate analysis of overall survival in LSCC patients.

Variables	HR	Univariate 95% CI	*p*-value	HR	Multivariate 95% CI	*p*-value
Age	1.41	0.55-2.63	0.461			
Gender	1.24	0.83-2.11	0.335			
Thyroid cartilage invasion	1.17	0.58-1.77	0.148			
T classification	1.63	0.79-2.26	0.231			
Lymph node metastasis	3.23	1.13-3.89	0.011	3.12	0.98-3.16	0.014
TNM stage	2.66	1.21-3.15	0.008	2.16	0.77-2.47	0.011
miR-300 expression	2.32	0.78-2.67	0.003	1.89	0.66-2.33	0.007

overexpression of miR-300 inhibited cell proliferation, cell cycle, and invasion in glioblastoma cell. The above findings revealed that miR-300 served as a tumor suppressor or a tumor promoter in different tumors. Recently, Ge et al[21] reported that miR-300 expression was downregulated in LSCC tissues, and over-expression of miR-300 suppresses LSCC proliferation and metastasis by targeting ROS1. This result suggested that miR-300 play an anti-oncogene in LSCC. Thus, we wonder whether miR-300 has associated whit the prognosis of LSCC patients.

In the present study, we explored the expression of miR-300 in LSCC tissues and matched normal tissues. Our qRT-PCR data showed that miR-300 expression was downregulated in tumor tissues compared with the adjacent non-tumor tissues. Meanwhile, we found that low expression of miR-300 was significantly correlated with lymph node metastasis and TNM stage of the disease. Furthermore, depending on the data of Kaplan-Meier method, miR-300 overexpression was observed to be associated with favorable prognosis in LSCC. Finally, according to multivariate analysis, miR-300 was an independent poor prognostic factor for LSCC patients.

Conclusions

The expression of miR-300 was decreased in LSCC tissues, suggesting that miR-300 may be a negative prognostic factor for LSCC patients. We provided the evidence that miR-300 may have a diagnostic and therapeutic potential in LSCC.

Conflict of interest

The authors declare no conflicts of interest.

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