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Original Article

MicroRNA-27a acts as a novel biomarker in the diagnosis of patients with laryngeal squamous cell carcinoma

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Abstract: Background: Increased evidences suggest that the microRNA-23a/24-2/27a (miR-23a/24-2/27a) cluster may play a crucial role in carcinogenesis and act as a novel oncogene. Among these members, miR-27a has been reported to promote proliferation and suppresses apoptosis in laryngeal carcinoma Hep2 cell lines. In this study, we examined the serum expression of miR-27a and its clinical significance in laryngeal squamous cell carcinoma (LSCC) patients. Methods: Serum miR-27a expression in 107 patients with LSCC and 104 healthy volunteers were detected using reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR). Then the relationship between its expression and clinical factors was analyzed. Finally, receiver operating characteristic (ROC) curve was established to estimate the diagnostic value of miR-27a in LSCC. Results: Serum miR-27a expression was higher in LSCC patients than in healthy volunteers ($P<0.05$). And the high miR-27a expression was significantly associated with clinical stage ($P=0.036$). However, there was no association between miR-27a expression and patients' age, gender, tumor site, cell differentiation, T classification and N classification ($P>0.05$). An AUC of 0.914 corresponding with a sensitivity of 86.0% and a specificity of 85.6% were obtained according to ROC curve. Besides, the optimal cutoff value was 3.155. These revealed miR-27a played an important role in the diagnosis of LSCC. Conclusion: Serum miR-27a expression was increased in LSCC patients compared to healthy controls. Moreover, it could be a potential diagnostic marker in LSCC.

Keywords: *MicroRNA-27a*, diagnosis, laryngeal squamous cell carcinoma

Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common malignancies in the head and neck region [1]. Most LSCC originate in glottis (>60%) and supraglottis, with the subglottis representing the minority of patients (<5%) [2]. The diagnostic methods for laryngeal cancer including physical examination, ultrasound, and computer tomography, but they are often insufficient for early detection [3]. Moreover, there are few typical symptoms in the early stages of LSCC and the lack of sensitive and specific bio-markers results in a delay of diagnosis [1]. Therefore, an accurate and reliable method for early diagnosis of LSCC was necessary in LSCC.

MicroRNAs (miRNAs) are an abundant class of endogenous non-coding small RNAs with a length of 18-25 nucleotides. They are highly conserved in the genomes of most species, including plants, animals and DNA viruses [4]. These miRNAs play important regulatory roles by sequence-specific base pairing on the 3' untranslated region (3'-UTR) of target messenger RNAs (mRNAs), in promoting mRNA degradation or inhibiting translation [5]. Growing evidences showed that miRNAs play an important role in a number of biological processes, including cell differentiation, proliferation, apoptosis and metabolism processes [6]. microRNAs have been functionally classified as proto-oncogenes or tumor suppressors and aberrantly expressed in different cancers [7]. More importantly, miRNAs have also been detected in human serum and plasma in remarkably stable forms, which makes the unique plasma/serum miRNA patterns possible to be noninvasive cancer biomarkers [8]. *MiR-27a*, located at chromosome 19p13.1, belongs to the *miR-23a/24-2/27a* cluster and widely exists in the verte-

Table 1. The relationship between *miR-27a* expression and clinicopathological features of LSCC patients

Characteristics	Cases (n=107)	*miR-27a* expression		*P* values
		Low (n=48)	High (n=59)	
Age (years)				
<60	45	23	22	0.212
≥60	62	25	37	
Gender				
Male	72	35	37	0.263
Female	35	13	22	
Tumor site				
Glottic	58	27	31	0.759
Supraglottic	35	14	21	
Subglottic	14	7	7	
Cell differentiation				
High	61	30	31	0.301
Middle and low	46	18	28	
T classification				
T1 + T2	59	29	30	0.322
T3 + T4	48	19	29	
N classification				
N0 + N1 + N2	52	28	24	0.794
N3 + N4	55	20	35	
Clinical stage				
I-II	64	34	30	0.036
III-IV	43	14	29	

brate genomes [9]. It has been demonstrated that members of this cluster play an important role in mammary tumorigenesis and act as a novel class of oncogenes [10, 11]. The aberrant expression of *miR-27a* has been observed in various human cancer types including laryngeal carcinoma [12]. However, the role of serum *miR-27a* and its clinical significance in LSCC patients remains unclear.

In this study, we examined the serum expression levels of *miR-27a* using qRT-PCR analysis and investigated the relationship between serum *miR-27a* expression and clinicopathological characteristics of LSCC patients. Moreover, we evaluated the potential role of *miR-27a* as a noninvasive bio-marker in the diagnosis of LSCC.

Materials and methods

Patients and samples collection

107 patients with pathologically diagnosed LSCC at the Fourth Hospital of Harbin Medical University during March 2006 until March 2007. None of the patients enrolled in this study had received any chemotherapy or radiotherapy before sampling. Besides, 104 healthy volunteers from with tumor-free and had no history of oncological diseases were recruited as healthy controls. The study was permitted by the Ethnic Committee of the hospital and all participant had signed written inform consent in advance.

All blood specimens from patients and tumor-free volunteers were collected. The n the samples were separated by centrifuging the blood at 1200× g for 15 min at 4°C. Finally, the obtained serum samples were stored in 1.5 mL RNase free tubes at -80°C for RNA extraction. The clinicopathological characteristics of the LSCC patients are summarized in **Table 1**.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from serum of patients with LSCC and healthy controls with TRIzol reagent (Invitrogen). RNA was reverse transcribed into cDNA with TransScript First-Strand cDNA synthesis supermix (TransScript, Invitrogen) following the manufacturer's instructions. Real-time PCR was performed on an ABI 7500 Real-Time PCR instrument (Applied Biosystems, Inc). Primers of *miR-27a* and *U6* were obtained from Ribobio (Guangzhou, People's Republic of China). Each sample was examined in triplicate and the expression of *miR-27a* was normalized with *U6* using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

All statistical analysis was carried out using Origin pro 9.0. All experiments were repeated at least three times, and the data were presented as means ± SD. The difference between two groups was analyzed using students' t tests. The relationship between serum *miR-27a* expression and clinicopathological factors were assessed using x^2 tests or Fisher's exact tests. Receiver operating characteristic (ROC) analysis was established to determine the diagnostic performance of serum *miR-27a* in patients with LSCC. $P<0.05$ were considered to be statistically significant.

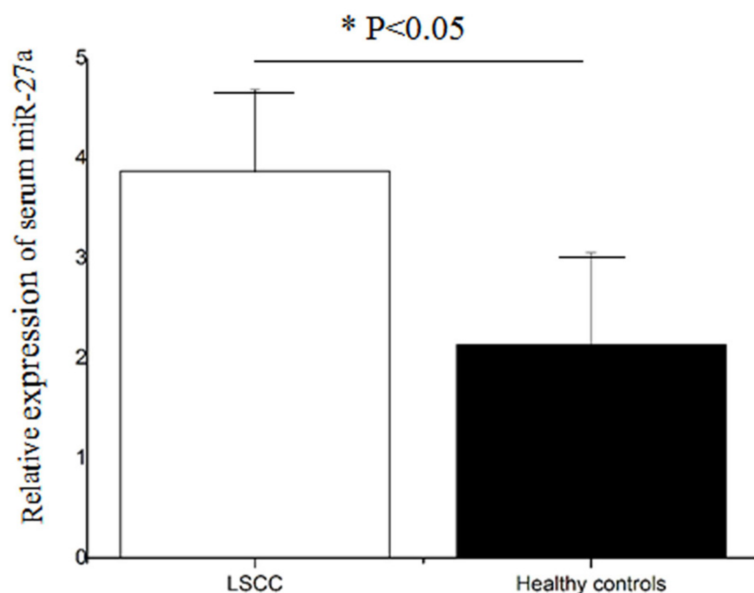

Figure 1. Serum *miR-27a* expression level in patients with LSCC and healthy controls. It was increased in patients with LSCC compared to healthy controls (*P*<0.05).

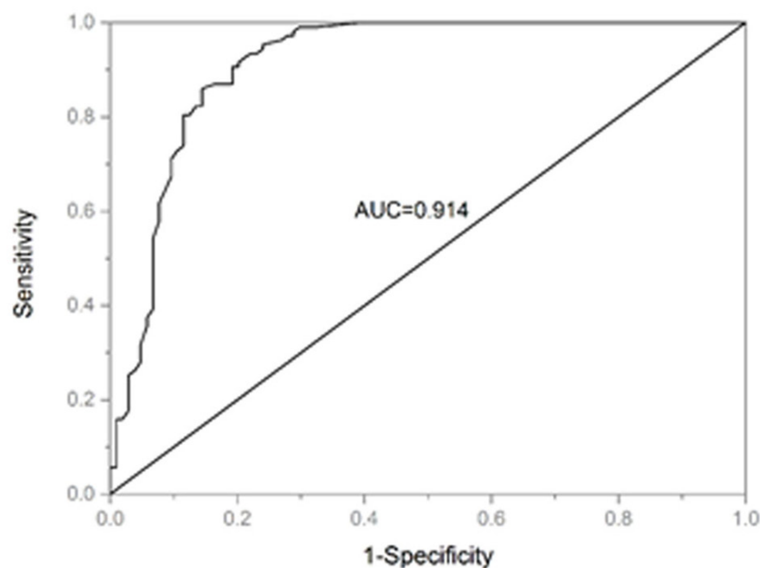

Figure 2. ROC curve for evaluating the diagnostic value of serum *miR-27a*. The AUC was 0.914 combing with a sensitivity of 86.0% and a specificity of 85.6%. The ideal cutoff value of *miR-27a* expression level was 3.155.

Results

Expression level of miR-27a in LSCC patients

QRT-PCR was used to evaluate serum *miR-27a* expression level in 107 LSCC patients, and 104 healthy volunteers. As shown in **Figure 1**, the serum *miR-27a* expression was significantly lower in patients with LSCC than that in healthy controls (*P*<0.05).

Relationship between miR-27a expression and clinicopathological characteristics of patients with LSCC

To further investigate whether the expression level of *miR-27a* was correlated with the development of LSCC, we further associated the relationship between *miR-27a* expression and the clinicopathological factors. It was found that *miR-27a* expression was tightly associated with clinical stage (*P*=0.036). However, there was no association between *miR-27a* expression and patients' age, gender, tumor site, cell differentiation, T classification and N classification (*P*>0.05; **Table 1**).

Diagnostic value of miR-27a for LSCC

ROC curves showed that the *miR-27a* expression had a high diagnostic value with an AUC of 0.914. Moreover, it had a high sensitivity (86.0%) and specificity (85.6%) (**Figure 2**). The ideal cutoff value of *miR-27a* expression level was 3.155. Therefore, *miR-27a* might be an independent diagnostic marker in LSCC.

Discussion

LSCC is one of the most fatal human carcinomas that often occurs in larynx which is a specialized area that is meaningful for breathing, sound production, and protecting the trachea against food aspiration in human [13]. A conventional biopsy from the primary tumor act as an accurate technique for the diagnosis of LSCC and an invasive examination, but it is often performed under local even general

anesthesia with physical or psychological discomfort. Therefore, it is particularly necessary to identify novel and efficient bio-markers for improving the strategies for the diagnosis of patients with LSCC.

In previous studies, many molecules had been confirmed to be related to the diagnosis of LSCC. For instance, serum HMGB1 was over-expression in LSCC and could serve as an independent diagnostic and prognostic marker [14]. Cyclin D1 was confirmed to be a highly sensitive marker in differentiating LSCC from LD or HLM via the study of Jovanovic et al. [15]. FGF3 and p21 protein expression were detected in LSCC and Wang et al., found the combination expression of serum exosomal *miR-21* and HOTAIR had a high diagnostic value with a AUC of 0.876 as well as a good prognostic value in LSCC [16]. Besides, the expression of *miR-21, miR-375* and *miR-106b* were also proved to be important diagnostic marker in LSCC according to stimulated studies [17, 18].

miR-27a has been reported to have both oncogenic and tumor suppressive functions in different cell lines and human cancer tissues. Tang *et al.* reported that *miR-27a* expression might be elevated and have clinical potentials as a non-invasive diagnostic bio-marker in osteosarcoma patients [19]. It was revealed that oncogenic *miR-27a* could play an important role in the cell growth and metastasis of ovarian cancer [20]. Pan *et al.* found that *miR-27a* had the function as an oncogene by targeting *MAP2K4* in the osteosarcoma MG63 cell lines [21]. *miR-27a* was considered to play an oncogenic role by targeting Spry2 and modulating the malignant, biological behavior of pancreatic cancer cells [22]. Sun *et al.* demonstrated that *miR-27a* was more highly up-regulated in cancer, plasma, and adipose samples from obese liver cancer cases [23]. In contrast, Miao *et al.* reported that *miR-27a* was down-regulated by regulating the expression of MET and EGFR in small cell lung cancer [24]. In colorectal carcinogenesis and progression, *miR-27a* was identified as a tumor suppressor and it took effects by targeting SGPP1 and Smad2 [25]. Tian *et al.* indicated that *miR-27a* might act as an oncogene through suppressing the expression of *PLK2* and serve as a useful bio-marker in the diagnosis of LSCC [12]. In our study, we found that the serum *miR-27a* expression was higher in LSCC patients than that in healthy controls. These results were in agreement with the previous studies [12]. However, the other functions of *miR-27a* were never reported.

Then we explored the relationship between *miR-27a* and clinicopathological characteristics to estimate whether it was related to the development of LSCC. It is worth noting that high expression of *miR-27a* appeared to be significantly correlated with clinical stage which indicated it participated in the progression of LSCC. Finally, we investigated the clinical significance of *miR-27a* via establishing its ROC curve. The results indicated that *miR-27a* exhibited a high diagnostic value in LSCC with a high value of AUC and sensitivity as well as specificity. To the best of our knowledge, the current study represented the first demonstration that serum *miR-27a* expression may function as a diagnostic bio-marker in LSCC patients.

In conclusion, these findings provides the convincing evidences for the first time that the down-regulation of *miR-27a* may serve as a novel molecular marker in the diagnosis of LSCC, and its expression level was influenced by clinical stage. However, there are some limitations. Firstly, the sample size is small. Secondly, the current study has not elucidated the exact molecular mechanisms of *miR-27a* acting on LSCC. Therefore, some further studies are still need to be done.

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Disclosure of conflict of interest

None.

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