

Original Article

MicroRNA-21-3p serves as a novel biomarker for diagnosis of laryngeal cancer

Enzhu Zhang, Xiao Feng, Ya Feng

Department of Otorhinolaryngology Head and Neck Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian 106011, Liaoning, China

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Abstract: MicroRNAs (miRNA) have been confirmed to play an important role in the diagnosis and prognosis of various cancers. There were many miRNAs had been considered act as a molecular marker in laryngeal cancer. However, the role of miR-21-3p in laryngeal cancer remains unknown. The purpose of this study was to investigate the function of miR-21-3p in the diagnosis of laryngeal cancer. Serum miR-21-3p expression was detected and compared in 112 patients with laryngeal cancer, 65 patients with sphagitis and 82 healthy people by qRT-PCR analysis. Meanwhile, mir-21-3p in preoperative and postoperative serum of patients with laryngeal cancer was measured, too. The result demonstrated that serum miR-21-3p was increased in patients with laryngeal cancer relative to that in sphagitis group and healthy people. Besides, postoperative serum expressed less miR-21-3p than preoperative. Moreover, serum miR-21-3p was found to be influenced by several clinicopathologic characteristics including tumor stage and differentiation. Then, we designed ROC curve using serum miR-21-3p which showed good diagnostic value with AUC of 0.840 combining sensitivity of 86.8% and a specificity of 78%. Taken together, miR-21-3p was over-expression and could be an independent diagnostic marker in laryngeal cancer.

Keywords: Laryngeal cancer, MiR-21-3p, diagnosis

Introduction

Laryngeal cancer is one of the most common type of head and neck squamous cell carcinomas and the second most frequently cancer occurred in respiratory tract [1]. The morbidity and mortality of laryngeal cancer were approximately 2.1/100,000 and 1.1/100,000 according to GLOBOCAN 2012. The occurrence of this disease was caused by many factors such as alcohol and tobacco abuse, exposure to hard-alloys dust, chlorinated solvents, and familiar genetic patterns and might affects the quality of life and became a burden to public health [2-4]. Moreover, the patients with laryngeal cancer usually at an advanced stage due to the lack of reliable and early diagnostic bio-markers [5]. Therefore, it is of great importance to look for appropriate and effective diagnostic markers in laryngeal cancer.

MiRNAs are a class of small endogenous non-coding RNA with a length of 18-25 nucleotides

and have been confirmed to be related to many progress of various cancers including cell growth, cell cycle, apoptosis, migration and invasion [6-8]. They can regulate the expression of its target gene via specific sites within the 3'-Untranslated Regions (3'-UTR) of a target-mRNA at post transcriptional level [9]. Now more than 1900 human miRNAs regulating about 60% of the genes in mammals have been identified [10]. The differently expressed of miRNAs in tumor tissues and normal controls can demonstrate their roles and confirm if they can be markers for the cancers [11, 12]. miR-21-3p, previously named miR-21*, had already detected in tumor samples and was believed to take effects in tumorigenesis [13, 14]. In the study of Lu et al, miR-21-3p was found to be up-regulated in laryngeal cancer [15]. However, its diagnostic value has never been reported.

In this study, we detected the expression of miR-21-3p via qRT-PCR analysis. And further investigated its relationship with clinicopatho-

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Table 1. Association between miR-21-3p and clinicopathological characteristics in 112 patients with laryngeal cancer

Clinicopathological characteristics	n	MiR-21-3p expression		P
		High	Low	
Age				0.177
<60	45	19	26	
≥60	67	37	30	
Gender				0.829
Female	29	15	14	
Male	83	41	42	
Tumor stage				0.000
I-II	54	13	41	
III-IV	58	43	15	
Smoking				0.344
Nonsmokers	59	27	22	
Current smokers	53	29	34	
Drinking				0.571
Drinker	57	27	30	
Nondrinker	55	29	26	
Differentiation				0.828
Well	28	13	15	
Moderate	41	20	21	
Poor	43	23	20	
Lymph node metastasis				0.014
Absent	53	23	20	
Present	59	33	36	
Tumor depth (pT)				0.107
T1	21	13	8	
T2	29	9	20	
T3	36	19	17	
T4	26	15	11	

logic characteristics. Meanwhile, the role of miR-21-3p in the diagnosis of laryngeal cancer was estimated through establishing ROC curve.

Materials and methods

Patients and samples

112 patients who were diagnosed as laryngeal cancer in The First Affiliated Hospital of Dalian Medical University were collected in our study (Table 1). All of them had never received any physical and chemical therapy before sampling. Besides, 65 patients with sphagitis and 82 healthy people were taken as benign lesions controls and healthy controls, respectively. The study was permitted by the Ethnic Committee

of the hospital and written informed consents were signed by each participator in advance.

Serum samples from patients with laryngeal cancer and with sphagitis as well as healthy controls were extracted and put into blood collection tube of EDTA severally. All serum samples were stored at -80°C for RNA extraction. The expression of miR-221-3p was detected by quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from all serum samples with TRIzol (Invitrogen, Carlsbad, CA, USA), respectively. Then reverse transcriptase was conducted to synthesize the first chain of cDNA with TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR reaction was performed in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). The GAPDH was taken as the endogenous control for miR-21-3p. The comparative cycle threshold (CT) method was used to evaluate the relative quantification of miR-21-3p. Each sample was in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 software (SPSS Inc, IL, USA). All quantified data were presented as mean ± SD. The differences between two groups were analyzed via students' t while one way ANOVA was used to compare the variance among three or more groups. ROC curve was established to estimate the diagnostic value of miR-21-3p. The difference was considered to be significant when $P < 0.05$.

Results

miR-21-3p was up-regulated in the serum of patients with laryngeal cancer

The expression of miR-21-3p in the serum of patients with laryngeal cancer, benign lesions controls and healthy controls was detected by qRT-PCR analysis, respectively. As shown in Figure 1, the serum miR-21-3p expression was significantly higher in patients with laryngeal cancer than that in benign lesions controls and healthy controls ($P < 0.05$). This revealed miR-

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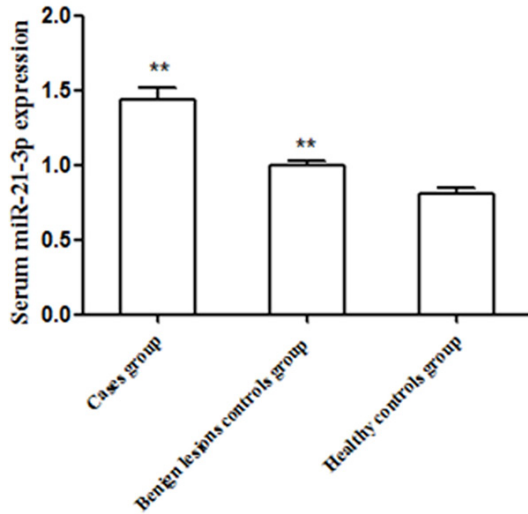


Figure 1. The expression of serum miR-21-3p in patients with laryngeal cancer, benign lesions controls and healthy controls. Serum miR-21-3p expression was significantly higher in patients with laryngeal cancer than that in benign lesions controls and healthy controls ($P<0.05$).

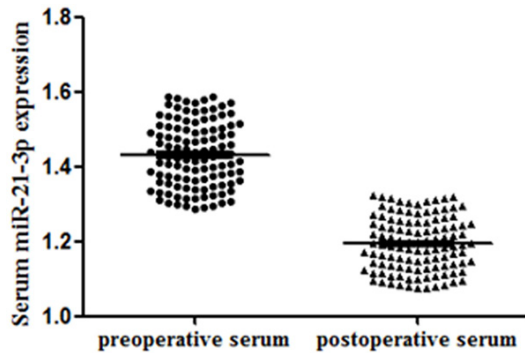


Figure 2. The expression of serum miR-21-3p in the preoperative and postoperative serum of patients with laryngeal cancer. Serum miR-21-3p expression was obviously decreased after operation compared with preoperative ($P<0.05$).

21-3p was related to laryngeal cancer and it might be an oncogene in this cancer.

Expression of miR-21-3p in the preoperative and postoperative serum of patients with laryngeal cancer

The preoperative and postoperative serum was extracted from patients with laryngeal cancer. And the expression of miR-21-3p in then was measured by qRT-PCR. The result demonstrated that the miR-21-3p expression in postoperative serum was obviously decreased compared

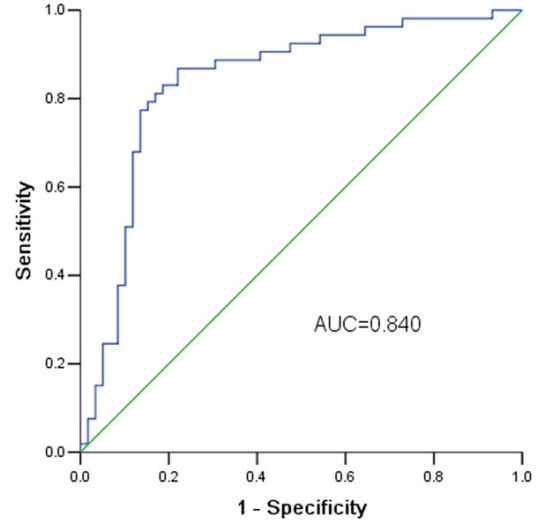


Figure 3. ROC curve analysis in the laryngeal cancer for detecting miR-21-3p expression showed a high AUC of 0.840 (95% CI=0.762 to 0.919).

with that in preoperative serum ($P<0.05$) which indicated that miR-21-3p could help distinguish the patients with laryngeal cancer (**Figure 2**).

Relationship between clinicopathologic characteristics and the expression of miR-21-3p in patients with laryngeal cancer

To explore whether miR-21-3p was involved in the development of laryngeal cancer, we analyzed its relationship between its expression and clinicopathologic characteristics. The outcome manifested that tumor stage ($P=0.000$) and lymph node metastasis ($P=0.014$) were important influence factors for the expression of miR-21-3p.

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As the abnormal expression of miR-21-3p in serum of patients with laryngeal cancer, benign lesions controls and healthy controls, we inferred miR-21-3p might be linked with the diagnosis of laryngeal cancer. Therefore, we established ROC curve. The curve showed miR-21-3p had a high diagnostic value with AUC of 0.840, corresponding with a sensitivity of 86.8% and a specificity of 78%. The cut-off value was 1.422 (**Figure 3**).

Discussion

miRNAs have been verified to be closely related to occurrence and development of multiplicate

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cancers with the widely use of various detecting techniques and further research of miRNAs. The potential effects of oncogene or anti-tumor make miRNAs to be vital molecular markers in the diagnosis and prognosis of cancers so that provide new therapy strategies.

In previous studies, there were many miRNAs had been found play a role in laryngeal cancer no matter in its diagnosis or prognosis or other processes. miR-299-3p was proved to be down-regulated and increased its expression could suppress the cell growth of laryngeal cancer cells [16]. Tian et al., found the up-regulation of miR-27a promoted cell proliferation and inhibited apoptosis which made it a potential diagnostic marker in laryngeal cancer [17]. In the study of Saito et al., miR-196a was specifically expressed and it might be a promising biomarker in the diagnosis and therapy of laryngeal cancer [18]. miR-206 was decreased and significantly inhibited the tumorigenesis via inhibiting cell proliferation, migration, invasion and promoting cell apoptosis by regulating VEGF expression according to Zhang et al. [19]. Wu et al., studied the expression trend and function of miR-16 and confirmed its up-regulation targeted by zyxin might be a promoter for the cell motility and be useful in the diagnosis and therapy of laryngeal cancer [20]. The high expression of miR-23a was reported to be an independent prognostic marker in laryngeal cancer [21]. The up-regulation of miR-21 and down-regulation of miR-375 were shown that they may be associated with the development and prognosis of laryngeal cancer, what's more, they were suggested to be potential diagnostic markers [22]. However, the role of miR-21-3p in laryngeal cancer remains unknown.

In current study, we detected the expression of miR-21-3p in the serum of patients with laryngeal cancer, with sphenitis and healthy people, respectively. The outcome manifested the expression of miR-21-3p was increased in patients with laryngeal cancer which was consistent with the previous study [15]. Then we further explored the role of miR-21-3p in the progress of laryngeal cancer. Tumor stage and differentiation were significantly influence the expression of serum miR-21-3p. In advanced stage of laryngeal cancer, the serum miR-21-3p expression was much higher. The lower differentiation level, the higher expression level of miR-21-3p and the more malignancy of laryn-

geal cancer. These might revealed that the up-regulation of miR-21-3p participated in the development of laryngeal cancer.

The early detection is meaningful for the effective and timely treatment of cancers. As the pathogenesis of laryngeal cancer is still incompletely known and its poor prognosis, this cancer might be a big threat to people's healthy. Hence, the timely diagnosis might help decrease the harm of laryngeal cancer. During the past time, many researchers had found several bio-markers for the diagnosis of laryngeal cancer. For instance, Qiu et al., found the increase of serum HMGB1 had a high diagnostic value with a high specificity in laryngeal squamous cell carcinoma [23]. Serum miR-21 combined with HOTAIR aberrantly expressed in laryngeal squamous cell carcinoma and this expression manner was considered to be valuable biomarker in the early detection of laryngeal squamous cell carcinoma [24]. Zhang et al., measured the expression of kallikrein 6 and verified it was significantly up-regulated in the plasma of patients with laryngeal cancer so that inferred kallikrein 6 can serve as a diagnostic marker [25]. In present study, we evaluated the diagnostic value of the up-regulation of miR-21-3p using ROC curve. Our analysis demonstrated that miR-21-3p had a high diagnostic value with AUC of 0.840 in laryngeal cancer. Besides, the sensitivity and specificity of miR-21-3p were relatively high. We speculated miR-21-3p might be a useful and effective diagnostic indicator in the early detection of laryngeal cancer.

In summary, miR-21-3p increases in serum of patients with laryngeal cancer and its expression is impacted by tumor stage and differentiation of the cancer. In addition, miR-21-3p has a good diagnostic value in laryngeal cancer. However, as the limits of samples scale and other unfavorable factors in the study, the conclusion still needs to be validated.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ya Feng, Department of Otorhinolaryngology Head and Neck Surgery, The First Affiliated Hospital of Dalian Medical University, 222 Zhongshan Road, Dalian 106011, Liaoning, China. Tel: +86-18098876539; Fax: +86-

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411-83635963-3141; E-mail: fengya1102@126.com

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