

Serum levels of microRNA-21 and microRNA-10a can predict long-term prognosis in laryngeal cancer patients: a multicenter study

Shan Gao, Qin Xu, Yongbin Zhou, Qingchuan Yi

Department of Otolaryngology-Head and Neck Surgery, Zigong Fourth People's Hospital, Zigong 643000, China

Contributions: (I) Conception and design: S Gao; (II) Administrative support: Q Xu; (III) Provision of study materials: Y Zhou; (IV) Collection and assembly of data: S Gao, Q Yi; (V) Data analysis and interpretation: S Gao, Q Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Shan Gao. Department of Otolaryngology-Head and Neck Surgery, Zigong Fourth People's Hospital, Zigong 643000, China. Email: gs813124@126.com.

Background: Laryngeal squamous cell carcinoma (LSCC) ranks as the second most common subtype of squamous cell sarcoma (SCC) of the head and neck. In clinical practice, more tools are required to assist doctors in identifying patients who are at risk of poor prognosis at an early stage. Many studies have shown that microRNA-21 plays an important role in LSCC. MicroRNA-10a has also been found to be differentially expressed in squamous cell carcinomas of the head and neck. This study aimed to test whether circulating microRNA-21 and microRNA-10a could serve as prognostic predictors for patients with LSCC.

Methods: Between May 2009 and Jan 2015, laryngeal cancer patients who were treated at three medical centers were enrolled based on inclusion and exclusion criteria. Baseline blood samples were obtained before surgery, chemotherapy, or radiotherapy. MicroRNA array was used to detect the microRNAs that were differently expressed between the LSCC patients and healthy controls. The serum levels of selected microRNAs were tested in each patient using qRT-PCR. Clinical information was acquired from the patients' clinic or in-hospital records and all of the patients were followed-up.

Results: A total of 236 patients (average age, 59.4 ± 10.7 years; male, 223; 33.5% with supraglottic LSCC, and 66.5% with glottis LSCC) were enrolled into the final analysis. MicroRNA array showed that the serum level of microRNA-21 was higher and that of microRNA-10a was lower in the LSCC patients compared with the healthy controls. Patients at T I/II stage, N0 stage, M0 stage had lower serum level of microRNA-21 and higher serum level of microRNA-10a. Serum level of microRNA-21 and microRNA-10a both differed significantly between patients with LSCC of well, moderate and poor differentiation. Cox proportional hazards model revealed that T stage, N stage, M stage, age, and ratio of serum level of microRNA-21 and microRNA-10a were risk factors associated with 5-year survival rate in patients with LSCC. Kaplan Meier survival analysis revealed that lower baseline ratio of serum level of microRNA-21 and microRNA-10a predict better 5 years survival. The receiver-operating characteristic curve demonstrated the ratio had a good ability to discriminate patients survive at the end of five years follow-up, with an area under the curve (AUC) of 0.8965 (95% CI: 0.7849–0.9811).

Conclusions: The serum levels of microRNA-21 and microRNA-10a are associated with long-term prognosis in laryngeal cancer patients. Lower serum level of microRNA-21 and higher serum level of microRNA-10a predict better prognosis in LSCC patients.

Keywords: microRNA-21; microRNA-10a; laryngeal cancer

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Introduction

Laryngeal squamous cell carcinoma (LSCC) comprises 85–90% of laryngeal cancer cases (1). As the second most common form of squamous cell carcinoma (SCC) in the head and neck, around 12,800 new cases of LSCC are reported each year, and worldwide it accounts for 2.4% and 2.1% of all cancer cases and cancer-related deaths, respectively (2–4). In recent years, the incidence of LSCC has increased and has been more frequently diagnosed in middle-aged and elderly men. Chinese patients constitute about 12.8% of all laryngeal cancer cases diagnosed worldwide each year, and approximately 14.7% of related mortality.

For patients with early and localized LSCC, surgery, radiotherapy, and chemotherapy, are the main treatment option. However, for patients with advanced LSCC, radiochemotherapy is the only option and only serves to alleviate cancer-related symptoms. Despite the rapid improvements seen with therapeutic drugs, technology, and treatment strategy, the long-term prognosis of LSCC remains unsatisfactory (5). In clinical practice, more tools are required to enable doctors to identify patients who are at risk of poor prognosis at an early stage.

In recent years, many studies have demonstrated that microRNAs can serve as biomarkers for disease diagnosis and prognostic predictors. MicroRNAs are short non-coding RNAs 18 to 24 nucleotides in length, which are endogenously synthesized (6). MiRBase (release 22.1, Oct 2018) is a database of over 30,000 microRNAs, 2,600 of which belong to *homo sapiens*. The involvement of microRNAs in the regulation of mRNA expression has been revealed in experimental studies.

A number of studies have investigated the role of microRNA-21 in tumors and autoimmune diseases, among other conditions (7,8). MicroRNA-21 is overexpressed in many types of cancer, including lung, breast, and colorectal cancer (9). Many studies have shown that microRNA-21 plays an important role in LSCC (10), and it has also been associated with the proliferation and apoptosis of some cells (11). Clinical research has demonstrated that microRNA-21 could be used as a biomarker in colorectal and prostate cancer, and to predict the prognosis of some cancers, including LSCC (10,12). However, in these studies, microRNA-21 was mainly extracted from tissue samples rather than blood samples. The relationship of the serum (or circulating) level of microRNA-21 specifically in the prognosis of LSCC remains unclear. Meanwhile, based on isolated tumor

cells or histological samples, the differential expression of microRNA-10a has also been detected in cervical cancer, squamous cell carcinomas of the head and neck, acute myeloid leukemia, and pancreatic cancer (13–15). Based on these findings and the results in our microarray and qRT-PCR study, we hypothesized that the serum levels of microRNA-21 and microRNA-10a could be used to predict the long-term prognosis of LSCC.

Methods

Study population

Between May 2009 and January 2015, patients with LSCC who were treated at three medical centers were enrolled on this study.

The criteria for inclusion were as follows: (I) LSCC diagnosed by rapid intraoperative pathological examination, or endoscopic examination; (II) aged >18 years old; and (III) no history of surgery, radiotherapy, or chemotherapy. Patients who met any of the following criteria were excluded from the study: (I) metastatic laryngeal cancer; (II) a history of glucocorticoid or immunosuppressive agent therapy in the 1 month prior to blood sample collection; or (III) rheumatic diseases, renal dysfunction or dialysis, or liver or heart failure.

The original histopathological diagnoses of the patients had been made based on the 2005 WHO system (16). The included LSCC cases were classified into four grades (microinvasive, well-differentiated, moderately differentiated, and poorly differentiated SCC). The baseline information of patients was collected from in-hospital records and follow-up clinic records. All of the patients were followed-up before this paper was written.

All patients provided written informed consent. The study was approved by the ethics committees of Zigong Fourth People's Hospital (ID: 2009017).

Serum isolation and storage

Before surgery, radiotherapy or chemotherapy, blood samples were taken from each patient via direct venous puncture into tubes containing 10 mL sodium citrate. Serum was isolated within 4 h of collection of whole blood by centrifugation at 1,800 g for 10 min at room temperature and was aliquoted into RNase/DNase-free Eppendorf tubes and stored at –80 °C, before RNA isolation.

RNA extraction

We isolated RNA according to instruction described previously (17). In brief, serum was thawed on ice and RNA was isolated using the miRNeasy RNA isolation kit (Qiagen) according to the manufacturer's instruction. For microRNA array, six pools were created by mixing serum samples from 30 LSCC patients (a pool contained 10 serum samples) and 30 sex- and age-matched healthy controls for triple biological repeat (1 microRNA array chip for 1 pooled sample). After binding to the membrane of RNeasy Mini spin column and subsequent washing, we eluted RNA with 40 μ L of RNase-free water. For all qRT-PCR experiments, RNA extracted from 200 μ L of serum was eluted with 14 μ L of RNasefree water. To normalize the sample-to-sample variation in RNA isolation, synthetic *C. elegans* microRNAs, cel-miR-39 and cel-miR-54 (Qiagen) were added as control before acid phenol: chloroform extraction.

MicroRNA array

We dephosphorylated and labeled pooled total RNA with pCp-Cy3 (Agilent Technologies) and T4 RNA ligase (GE Healthcare). Then the labeled sample was purified using Micro Bio-Spin 6 columns (Bio-Rad) and hybridized to Human miRNA Microarray V3 kit (Agilent Technologies) platform. After 20 h of hybridizations carried out at 55 °C, microarrays were washed and scanned using an Agilent scanner controlled by Agilent Scan Control software (version 7.0) and then analyzed with Agilent Feature Extraction software (version 9.5.3.1). The raw microRNA expression data were normalized using quantile normalization and analyzed with GeneSpring GX (Agilent Technologies, version 11.5) according to recommendations from Agilent Technologies.

qRT-PCR

Serum levels of microRNA-21 and microRNA-10a were tested by qRT-PCR using Taqman method [TaqMan miRNA Reverse Transcription Kit and miRNA-specific stem-loop primers (Applied Bio Systems)] in a small-scale RT reaction. The reverse transcriptase reaction consisted of a fixed volume of 1.67 μ L total RNA solution of each sample as input, 1.387 μ L of H₂O, 0.5 μ L of 10 $\times$ Reverse-Transcription Buffer, 0.063 μ L of RNase-Inhibitor (20 U/ μ L), 0.05 μ L of 100 mM dNTPs with dTTP, and

0.33 μ L of Multiscribe Reverse-Transcriptase (50 U/ μ L). The 5 μ L reactions were incubated in an Applied Biosystems 7500 Real Time PCR System in a 96-well plate at 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 5 min and held at 4 °C. We run all the reverse transcriptase reactions in duplicate. PCR reactions were incubated at 95 °C for five mins, followed by 40 cycles of 95 °C for 12 sec, 65 °C for 50 sec. MiR-21 primer: upstream: 5'-CGGCGGTAGCTTATCAGACTGA-3', downstream: 5'-GTGCAGGGTCCGAGGT-3'. MiR-10a primer: upstream: 5'-CAGGACGTGACGAGTGGAA-3', downstream: 5'- CCACTCACCGGCAGCCGTG-3'. Fold changes were calculated using the $2^{-\Delta\Delta C_t}$ method (18).

Statistical analysis

Continuous variables were expressed as the mean $\pm$ standard deviation (SD) and compared using an unpaired two-sided Student's *t*-test when normal distribution and equal dispersion were confirmed. The Mann-Whitney U test and the Wilcoxon's signed-rank test were used when the variance was unequal. Categorical variables were expressed as percentages (%) and compared using χ^2 analysis or Fisher's exact test if necessary. Kaplan-Meier survival analysis and the Cox proportional hazards model was used to analyze the predictive value of serum levels of microRNA-21 and microRNA-10a for the five-year survival rate in patients with LSCC. A P value <0.05 indicated statistical significance. All statistical analysis was performed with SPSS (version 17.0 for Windows, SPSS, Inc., Chicago, IL, USA).

Results

Baseline characteristics of patients with LSCC

According to the inclusion and exclusion criteria, 236 LSCC patients were enrolled into the final analysis. The patients had an average age of 59.4 $\pm$ 10.7 years, and 223 (94.5%) were male. Of the LSCC cases, 79 (33.5%) were supraglottic and 157 (66.5%) were glottic. No statistical difference existed in cancer stage between patients with supraglottic LSCC and those with glottic LSCC. In our study, most of the patients (225, 95.3%) had well or moderately differentiated tumors, and the majority had a history of smoking. The duration of follow-up ranged from 5.1 to 10.8 years. In the final analysis, we only calculated the 5-year survival rate. Details were listed in *Table 1*.

Table 1 Baseline characteristics of 236 LSCC patients

Items	General (n=236)	Supraglottic (n=79)	Glottic (n=157)	t/ χ^2 value	P value
Male (n, %)	223 (94.5)	73 (92.4)	150 (95.5)	0.993	0.319
Age (years)	59.4±10.7	61.0±11.7	58.6±11.2	1.530	0.127
Smoke (n, %)	181 (76.7)	64 (81.0)	117 (74.5)	1.239	0.266
Alcohol (n, %)	47 (19.9)	17 (21.5)	30 (19.1)	0.192	0.662
Hypertension (n, %)	21 (8.9)	6 (7.6)	15 (9.6)	0.249	0.618
Diabetes (n, %)	17 (7.2)	7 (8.9)	10 (6.4)	0.488	0.485
COPD (n, %)	13 (5.5)	5 (6.3)	8 (5.1)	0.154	0.695
SBP (n, %)	131.8±22.5	132.7±23.6	131.3±22.9	0.439	0.661
Fast glucose (mmol/L)	5.5±1.4	5.7±1.6	5.4±1.5	1.418	0.158
TC (mmol/L)	5.12±1.67	5.22±1.74	5.07±1.59	0.662	0.508
TG (mmol/L)	1.92±0.88	1.99±0.94	1.88±0.96	0.836	0.404
ALT (U/L)	28.5±7.8	29.8±8.4	27.8±7.3	1.887	0.060
Cr (umol/L)	77.6±18.5	79.1±19.8	76.8±20.1	0.834	0.405
T-stage (n, %)				0.983	0.805
I	28 (11.9)	11 (13.9)	17 (10.8)		
II	71 (30.1)	23 (29.1)	48 (30.6)		
III	95 (40.3)	33 (41.8)	62 (39.5)		
IV	42 (17.8)	12 (15.2)	30 (19.1)		
N-stage (n, %)				1.900	0.387
N0	76 (32.2)	21 (26.6)	55 (35.0)		
N1	89 (37.7)	31 (39.2)	58 (36.9)		
N2	71 (30.1)	27 (34.2)	44 (28.0)		
M-stage (n, %)				2.280	0.131
M0	211 (89.4)	74 (93.7)	137 (87.3)		
M1	25 (10.6)	5 (6.3)	20 (12.7)		
Differentiation (n, %)				1.458	0.483
Well	67 (28.4)	19 (24.1)	48 (30.6)		
Moderate	158 (66.9)	57 (72.1)	101 (64.3)		
Poor	11 (4.7)	3 (3.8)	8 (5.1)		
Follow-up (years)	7.4±2.8	7.1±2.9	7.6±3.1	-1.194	0.234

LSCC, laryngeal squamous cell carcinoma; COPD, chronic obstructive pulmonary disease; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; ALT, alanine aminotransferase.

Table 2 Fold change (LSCC vs. healthy Control) of selected microRNAs in microRNA array

microRNA	microRNA array		qRT-PCR	
	Fold change	Adjusted P value	Fold change	P value
miR-21	1.67	0.004	1.48	0.007
miR-10a	0.79	0.004	0.72	0.005

LSCC, laryngeal squamous cell carcinoma

Table 3 Serum levels of microRNA-21 and microRNA-10a in the different subgroups ($2^{-\Delta\Delta C_t}$)

Items	n	miR-21	t/ χ^2 value	P value	miR-10a	t/ χ^2 value	P value
General	236	1.48±0.42			0.72±0.22		
Localization		350.09			170.69		
Supraglottic	79	1.53±0.47	1.127	0.261	0.69±0.23	-1.371	0.172
Glottic	157	1.46±0.44			0.74±0.28		
T-stage (n, %)							
I/II	99	1.27±0.48	-5.987	<0.001	0.85±0.27	6.886	<0.001
III/IV	137	1.64±0.46			0.63±0.22		
N-stage (n, %)							
N0	76	1.21±0.41	-6.562	<0.001	0.91±0.31	7.600	<0.001
N1/N2	160	1.61±0.45			0.63±0.24		
M-stage (n, %)							
M0	211	1.42±0.42	-6.440	<0.001	0.75±0.21	5.316	<0.001
M1	25	2.02±0.59			0.50±0.31		
Differentiation (n, %)			15.691	<0.001		8.157	<0.001
Well	67	1.35±0.48			0.81±0.26		
Moderate	158	1.49±0.45			0.70±0.24		
Poor	11	12.2±0.49			0.53±0.28		

MicroRNA array result

MicroRNA array revealed that microRNA-21 and microRNA-10a were significantly differentially expressed between the LSCC group and healthy control group (see *Table 2*). This result was further validated by qRT-PCR. The fold change of these 2 microRNAs in qRT-PCR was also showed in *Table 2*.

Serum levels of selected microRNAs

The fold changes of microRNA-21 and microRNA-10a by qRT-PCR in different subgroups are shown in *Table 3*. In general, the serum level of microRNA-21 was 1.13

to 2.27 (1.48±0.42) and the serum level of microRNA-10a ranged from 0.34 to 0.96 (0.72±0.22). No statistical difference in serum level of microRNA-21 or microRNA-10a existed between the patients with supraglottic LSCC and those with glottic LSCC. Meanwhile, patients with T I/II stage, N0 stage, and M0 stage had lower serum levels of microRNA-21 and higher serum levels of microRNA-10a. The serum levels of both microRNA-21 and microRNA-10a differed significantly between LSCC patients with well-, moderately and poorly differentiated tumors. We subsequently calculated the ratio of serum level of microRNA and microRNA-10a in every patient, and the result was 1.22 to 7.31 (2.06±1.25).

Outcome at follow-up

The follow-up time ranged between 5.2–10.8 (7.4±2.8) years, during which time 71 (30.1%) patients died. At 5 years, the survival rate was 77.1% (182 patients survived and 54 patients died). We divided the patients into subgroups according to their serum level of microRNA-21 and microRNA-10a, using the general average level as a cut-off point. The results showed that patients with higher serum level of microRNA-21 or lower serum level of microRNA-10a had a lower five-year survival rate. For further analysis, we divided the patients into two groups based on the average ratio of serum level of microRNA-21 and microRNA-10a and found that patients with a higher ratio had a lower five-year survival rate. Multivariate analysis by Cox proportional hazards model revealed that T stage, N stage, M stage, age, and the ratio of serum levels of microRNA-21 and microRNA-10a were risk factors associated with survival rate in patients with LSCC. The details are listed in *Tables 4* and *5*. Kaplan Meier survival analysis revealed that a lower baseline ratio of serum levels of microRNA-21 and microRNA-10a predicted better survival after five years (*Figure 1*). The receiver-operating characteristic curve demonstrated that the ratio had good discrimination ability in predicting patient survival at the end of five years of follow-up, with an area under the curve (AUC) of 0.8965 (95% CI: 0.7849–0.9811; *Figure 2*).

Discussion

In present study, we found that the serum levels of microRNA-21 and microRNA-10a both differ significantly in LSCC patients of different subgroups. Higher serum level of microRNA-21 and lower serum level of microRNA-10a both predict lower five-year survival. Further analysis demonstrated that serum levels of microRNA-21 and microRNA-10a could also be used in combination to predict five-year survival in LSCC patients.

There has been a significant amount of in-depth investigation of microRNA-21 in recent years. Experimental studies have shown that microRNA-21 is involved in many tumors and other diseases, including lung, liver, colorectal, and pancreatic cancer, head and neck squamous cell carcinoma (HNSCC), hypertension (19), and coronary artery disease (20), especially acute myocardial infarction (21). A correlation has also been identified between the level of microRNA-21 expression in tumor tissue and the diagnosis and prognosis of human tumors.

In a systematic review and meta-analysis, Guraya *et al.* analyzed data from 31 studies (including 3,273 patients with different kind of cancers) and concluded that a high level of microRNA-21 was related to worse overall survival (22). In another study, Liu *et al.* found that when compared with non-tumor controls, microRNA-21 was significantly upregulated in the tumor tissue of LSCC patients with (10). Similarly, Hu *et al.* tested microRNA-21 and microRNA-375 in freshly frozen primary tumors and non-cancerous laryngeal squamous epithelial tissues and found that patients who exhibited high miR-21 or low miR-375 expression in tumor tissues had poorer prognoses compared to patients with lower miR-21 or higher miR-375 expression. They also found that the miR-21/miR-375 expression ratio was highly sensitive (0.94) and specific (0.94) for predicting disease (12). Cao *et al.* used microRNA array to screen microRNA expression in 6 pairs of laryngeal SCC and adjacent normal tissues; they detected 29 differentially expressed microRNAs and confirmed 6 microRNAs by qRT-PCR. MiR-21, miR-93, miR-205, and miR-708 were upregulated, while miR-125b and miR-145 were downregulated (23). Zhang *et al.* also used microRNA array to screen the expression of 1,145 human microRNAs in LSCC tissue based on 10 patients and normal tissue from controls. MicroRNA-21, microRNA-19a, and microRNA-33a were found to be upregulated (24). In a recent study, Erkul *et al.* found that microRNA-21 could serve as a diagnostic biomarker and was expressed at lower levels in patients with early-stage disease compared to patients with late-stage disease; however, no statistical significance was observed (25). In an early study, Child *et al.* found that microRNA-21 was frequently overexpressed in human HNSCC tumors but univariate and multivariate statistical models detected no correlation between level of microRNA-21 and tumor recurrence or overall survival (26). The findings of these studies suggest that microRNA-21 could be used as biomarker for LSCC.

Other studies have indicated that a relationship exists between microRNA-21 and patients' prognosis. Hedbäck *et al.* found that level of microRNA-21 in tumor tissue was correlated with disease-free survival (27). Through multivariate analysis carried out by Hu *et al.* a significant correlation was found between a high level of microRNA-21 in tumor tissue and overall survival (12).

As described earlier in this paper, the aforementioned studies mainly focused on microRNA from tissue, which did not facilitate convenient monitoring during follow-up. In their report, Erkul *et al.* pointed out that circulating

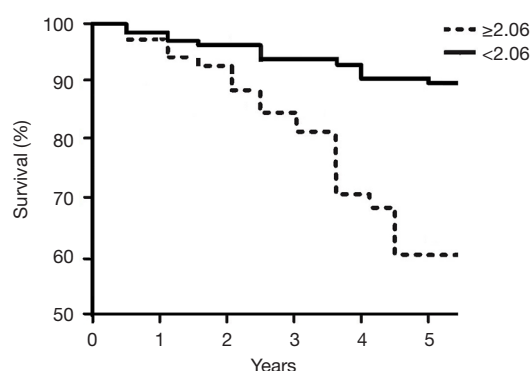
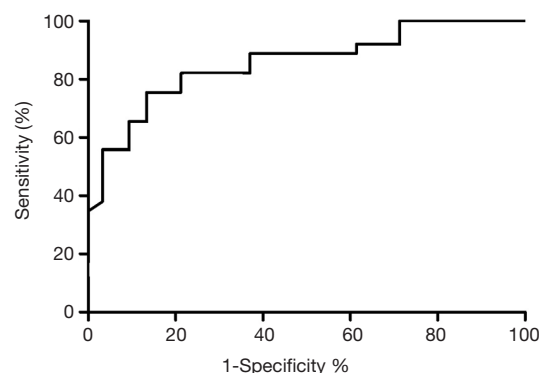
Table 4 The 5-year survival rate of different subgroups

Items	n	5-year survival	t/ χ^2 value	P value
General	236	182 (77.1)		
Gender			0.438	0.508
Male	223	171 (76.7)		
Female	13	11 (84.6)		
Age, years			4.703	0.030
≥ 65	94	63 (67.0)		
< 65	142	113 (79.6)		
Localization			0.399	0.528
Supraglottic	79	59 (74.7)		
Glottic	157	123 (78.3)		
T-stage (n, %)			11.190	0.001
I/II	99	87 (87.9)		
III/IV	137	95 (69.3)		
N-stage (n, %)			4.491	0.034
N0	76	65 (85.5)		
N1/N2	160	117 (73.1)		
M-stage (n, %)			26.792	< 0.001
M0	211	173 (82.0)		
M1	25	9 (36.0)		
Differentiation (n, %)			8.776	0.012
Well	67	57 (85.1)		
Moderate	158	120 (75.9)		
Poor	11	5 (45.5)		
miR-21			33.223	< 0.001
≥ 1.48	107	64 (59.8)		
< 1.48	129	118 (91.5)		
miR-10a			15.469	< 0.001
≥ 0.72	121	106 (87.6)		
< 0.72	115	76 (66.1)		
Ratio			54.967	< 0.001
≥ 2.06	98	52 (53.1)		
< 2.06	138	130 (94.2)		

Table 5 Cox proportional hazards model analysis

Factors	β	SE	Wald	P	RR	95% CI	
						Low	Up
T stage	0.667	0.331	5.479	0.025	1.853	1.241	2.305
N stage	0.735	0.382	4.816	0.033	2.108	1.618	2.846
M stage	0.891	0.293	4.472	0.017	1.612	1.092	1.985
Age	0.598	0.268	5.015	0.041	1.449	1.086	1.792
Ratio*	1.634	0.194	6.624	0.006	1.969	1.374	2.901

*, ratio of serum levels of microRNA-21 and microRNA-10a.

Figure 1 Kaplan-Meier survival curve, $P < 0.001$.Figure 2 ROC curve of ratio ($P = 0.006$).

markers may prove more useful than tissue markers, because they can be assayed before surgery and can be monitored throughout life (25). In contrast, in the present study, we tested the serum levels of microRNA-21 and microRNA-10a. As baseline parameter, high serum level of microRNA-21 and low serum level of microRNA-10a predicted a poor five-year survival rate. In fact, many studies have demonstrated the level of circulating microRNA-21 to be a powerful prognostic tool and the expression of microRNA-21 to be associated with poor overall survival and poorer disease-free survival in esophageal squamous cell carcinoma (ESCC), pancreatic ductal adenocarcinoma, and colorectal carcinoma (22).

MicroRNA-10a is also involved with many diseases, including glioma (28), lung cancer (29), gastric cancer (30), hepatocellular carcinoma (31), cervical cancer (32), pancreatic cancer (33), breast cancer (34), and renal cell carcinoma (35). Lu *et al.* observed that in human non-small cell lung cancer, downregulation of microRNA-10a mediated the anti-tumor effect of icaritin (36). Furthermore, Chen *et al.* found that

microRNA-10a could promote cancer cell proliferation in oral squamous cell carcinoma by upregulating GLUT1 and promoting glucose metabolism (37). In contrast with our findings, Bi *et al.* similarly found that abnormal high expression of microRNA-10a/b may result in unlimited proliferation of immature blood progenitors and repression of mature blood cell differentiation and maturation, leading to the occurrence of acute myeloid leukemia (38). On the contrary, in ESCC tissue and cell lines, Liu *et al.* concluded that microRNA-10a cell proliferation and metastasis could be inhibited through the targeting of Tiam 1 (39). Notably, Inoue *et al.* found that microRNA-10a expression was comparably downregulated in the tumors of high-grade intraepithelial neoplasm and non-invasive ESCC, while the expression levels of microRNA-10a were elevated in invasive ESCC tumors (40). In short, the findings of these studies are not consistent, suggesting multi-center, large sample size, standardized research studies are needed in the future.

Some studies also focused on a combination of two or more microRNAs as biomarkers or risk factors for a certain

disease (41-43). In our study, we combined microRNA-21 with microRNA-10a based on the ratio of their serum levels. We found this ratio to be well correlated with the five-year survival rate of the LSCC patients in our study. ROC curve analysis also suggested that this ratio had good discrimination ability in identifying patients who would survive after five years of follow-up. Future study will continue to evaluate the prognostic value of microRNA-21 and microRNA-10a and their use in combination for patients with LSCC.

Conclusions

The serum levels of microRNA-21 and microRNA-10a are associated with long-term prognosis in laryngeal cancer patients. Lower serum level of microRNA-21 and higher serum level of microRNA-10a, especially the ratio of these two microRNAs predict better prognosis in LSCC patients. The limitation of this study is the relatively small sample of LSCC patients. Further study should enroll more patients to validate present findings.

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Footnote

Data Sharing Statement: Available at <http://dx.doi.org/10.21037/tcr-20-1758>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr-20-1758>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the ethics committees of Zigong Fourth People's Hospital (ID: 2009017). All patients provided written informed consent.

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