Relationship of Common Variants in miR-27a-3p Gene with Susceptibility and Prognosis of Oral Cancer

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Research

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Abstract

Background: *miR-27a-3p* has been found dysexpressed in various cancers. The aim of the present study was to clarify the prognostic value of *miR-27a-3p* in patients with oral cancer.

Methods: We used quantitative real-time polymerase chain reaction (qRT-PCR) assay to detect the expression of *miR-27a-3p* in the tissue of oral cancer and adjacent normal specimens. The association of *miR-27a-3p* with clinicopathological characteristics was analyzed via the Chi-square test. Kaplan-Meier survival and Cox regression analysis were performed to evaluate the prognostic value of *miR-27a-3p* in oral cancer patients.

Results: The down-regulated expression of *miR-27a-3p* was found in oral cancer tissues compared with the matched noncancerous samples ($P<0.05$). And its expression was influenced by TNM stage ($P=0.032$), T stage ($P=0.014$) and lymph node metastasis ($P=0.025$). Kaplan–Meier analysis result showed that the decreased level of *miR-27a-3p* expression was associated with a poor overall survival of oral cancer patients. Additionally, multivariate cox regression analysis revealed that the low expression of *miR-27a-3p* was an independent prognostic maker in oral cancer patients (HR=0.462, 95% CI=0.223–0.957, $P=0.038$).

Conclusions: Taken together, the expression pattern of *miR-27a-3p* was decreased in oral cancer tissues. The decreased expression of *miR-27a-3p* was a potential prognostic biomarker in patients with oral cancer.

Background

Oral cancer is one of the most common malignancies in head and neck that defined as any cancerous tissue growth located in the oral cavity [1]. According to the American Cancer Society, approximately 45,780 new cases and 8,650 cancer deaths attributed to this condition occur in 2015 in United States [2]. Approximately 90% of oral neoplasms are oral squamous cell carcinoma (OSCC), most of which are invasive growth, invasion of surrounding tissue and easy to occur lymph node metastasis [3, 4]. Surgical resection with chemotherapy and radiotherapy are the the most effective therapeutic methods for treatment oral cancer, but the 5-year survival rate of patients with in advanced clinical stages remained around 50–55% over the past several decades [5, 6]. Therefore, it is of great importance to identify reliable molecular markers that may be beneficial to improve the poor prognosis of oral cancer.

Recent studies suggest that aberrant microRNAs (miRNAs) expressions have been found in various human cancers [7–9]. miRNAs are a group of small, noncoding, single-stranded RNAs of 19–25 nucleotides in length that play important roles in modulating cell differentiation, growth, apoptosis and proliferation [10–12]. miRNAs are found highly stable and abundant in serum, urine and tissues, which play crucial roles in tumorigenesis and cancer diagnosis or prognosis in various cancers [13–15]. *miR-27a-3p* found by Zeng et al. could inhibit the YAP1 directly by post-transcriptionally silencing and potentially suppress EMT process, suggesting it plays pivotal role in effectively manipulating the invasion

and metastasis in oral squamous cell carcinoma cells [16, 17]. However, the clinical significance of *miR-27a-3p* in the prognosis of oral cancer was still unclear.

In this study, we mainly focused on the serum expression levels of *miR-27a-3p* and investigated the relationship between its expression and clinicopathological characteristics in patients with oral cancer. Thereby further evaluating its value as a prognostic marker in oral cancer.

Methods And Materials

The subjects under study included 136 patients with oral cancer who underwent surgery in Chinese PLA General Hospital. Patients with oral cancer who had received prior radiotherapy to the head and neck area, were excluded from the study. All the fresh oral cancer tissues and adjacent normal tissues specimens were surgically removed and put immediately into liquid nitrogen, then stored at -80°C until use, respectively. Clinicopathological characteristics of the patients are summarized in Table 1, including age, gender, TNM stage, T stage, lymph node metastasis, differentiation and smoking status. A 5-years' follow-up was conducted.

Table 1

The association between serum *miR-27a-3p* expression and clinicopathological characteristics of oral cancer

Characteristics	No. (n = 136)	<i>miR-27a-3p</i> expression		χ^2	P values
		Low (n = 75)	High (n = 61)		
Age(year)				0.019	0.891
< 60	70	39	31		
≥ 60	66	36	30		
Gender				1.592	0.207
Male	75	45	30		
Female	61	30	31		
TNM stage				4.594	0.032
I-II	62	28	34		
III-IV	74	47	27		
T stage				6.098	0.014
T1-T2	71	32	39		
T3-T4	65	43	22		
Lymph node metastasis				5.024	0.025
negative	68	31	37		
positive	68	44	24		
Differentiation				0.806	0.369
Moderate + high	70	36	34		
poor	66	39	27		
Smoking				0.159	0.690
non-smoker	71	38	33		
smoker	65	37	28		

This study was approved by the Medical Ethics Committee of Chinese PLA General Hospital, and written informed consents were obtained from each patients prior to surgery.

RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted from matched oral cancer tissues and adjacent tissues, using the Trizol reagent (Invitrogen, Burlington, ON, USA) according to the manufacturer's instruction. Isolated RNA was used as a template for reverse-transcribing into cDNA with the Reverse Transcriptase MMLV (TaKaRa Bio, Shiga, Japan) according to the manufacturer's protocol. The quantitative real-time PCR (qRT-PCR) reaction was performed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Thermal cycling conditions included an initial step at 98 °C for 30 s, and 40 cycles at 95 °C for 2 s and at 63–66 °C for 5 s for each miRNA-specific primer. The relative mRNA expression of target gene was calculated by $2^{-\Delta\Delta CT}$ method and *U6* was used as endogenous control. Experiments were performed in triplicate.

Statistical analysis

All statistical analyses were performed using the SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Data are presented as mean normalized gene expression $\pm$ standard deviation (SD) from independent experiments. Student's test was used to evaluate the difference between tumor and normal groups. The associations between mRNA expression and clinicopathological factors were assessed using the χ^2 test. The survival curves were constructed by the Ksplan-Meier method and compared using the log-rank test. A multivariate analysis with cox regression analysis was conducted to evaluate the prognostic value of *miR-27a-3p* in oral cancer. The levels of significance was set at $P < 0.05$.

Results

The expression of miR-27a-3p was decreased in oral cancer

We used qRT-PCR analysis to measure *miR-27a-3p* expression levels in oral cancer tissues and adjacent normal tissues. As shown in Fig. 1, the *miR-27a-3p* expression levels were significantly lower in oral cancer tissues compared with adjacent healthy tissues ($P < 0.01$).

Relationship between miR-27a-3p and clinicopathological characteristics of oral cancer

To explore whether *miR-27a-3p* was involved in the development of oral cancer, we further explored the association between the expression of *miR-27a-3p* and clinicopathological characteristics of patients. We divided the patients into two classed (high- and low-expression group) using the average level of patients as the cutoff value. As indicated in Table 1, *miR-27a-3p* expression was positively associated with TNM stage ($P = 0.032$), T stage ($P = 0.014$) and lymph node metastasis ($P = 0.025$). However, there was no significant relationship between *miR-27a-3p* expression and other parameters, including age, gender, differentiation and smoking status (all $P > 0.05$).

Association of miR-27a-3p expression with prognosis in oral cancer patients

Kaplan-Meier survival analysis was done to evaluate the association of *miR-27a-3p* expression with overall survival. Patients with negative expression of *miR-27a-3p* had significantly poorer overall survival

as compared to patients with high expression of *miR-27a-3p* (Fig. 2, log rank test, $P < 0.05$). We further evaluate the correlation between clinicopathological parameters and patients outcomes using multivariate Cox regression analysis. The cox analysis results suggested that the expression of *miR-27a-3p* was an independent prognostic factor in patients with oral cancer (Table 2, HR = 0.462, 95% CI = 0.223–0.957, $P = 0.038$).

Table 2
Multivariate analysis adjusted for clinical variables for the prognostic value of *miR-27a-3p* in oral cancer patients

Variable	HR	95%CI	Pvalue
Low <i>miR-27a-3p</i> expression	0.462	0.223–0.957	0.038
High <i>miR-27a-3p</i> expression	-	-	-
HR: hazard radio, 95% CI: 95% confidence interval. $P < 0.05$ was considered to be statistically significant.			

Discussion

As oral cancer is generally asymptomatic at an early stage, most of patients are diagnosed at advanced stage, resulting the poor prognosis. Currently, the advances in surgery operation, chemotherapy and radiotherapy have been performed, and the prognosis of patients with oral cancer has slowly but steadily improved, but the overall 5-year survival rate is still showing poor [18]. Thus, it is important to identify new biomarkers that, using surgical samples, predict cancer recurrence in oral cancer.

Recent studies have demonstrated that miRNAs can play important roles in cancer development and progression for several types of cancer including oral squamous cell carcinoma (OSCC) [19–21], and as such they may be useful as biomarkers and treatment targets..

It has been reported that *miR-27a-3p* dysexpressed in many cancers [22–27]. Wataru Nakata et al. identified miRNAs that are up-regulated in clear cell renal cell carcinoma (ccRCC) and revealed that high levels of *miR-27a-3p* correlated with a worse progression-free survival rate [23]. XU et al. showed that overexpression of miR-24-3p and *miR-27a-3p* could promote cell proliferation and observed that *miR-27a-3p* is upregulated in glioma tissues [24]. So far, dysregulation of *miR-27a-3p* has not been reported in oral cancer. Therefore, we were interested to validate its roles in in oral cancer after the aberrant overexpression of *miR-27a-3p* was further confirmed in paired oral carcinomas and normal tissues.

In the present study, we investigated the expression pattern of *miR-27a-3p* in oral cancer. The results indicated that the level of *miR-27a-3p* was decreased in oral cancer tissues than in adjacent normal tissues, which suggests that *miR-27a-3p* may be a tumor suppressor gene in oral cancer. The down-regulated expression of *miR-27a-3p* was positively associated with TNM stage ($P = 0.032$), T stage ($P = 0.014$) and lymph node metastasis ($P = 0.025$), whereas no significantly associations was found between

miR-27a-3p and other characteristics. These data above suggested that *miR-27a-3p* plays crucial role in patients with oral cancer and is associated with the development of oral cancer.

Furthermore, the prognostic value of *miR-27a-3p* was also been investigated in our study. Kaplan-Meier analysis result showed that patients with a low *miR-27a-3p* expression had poor overall survival (log rank test, $P < 0.05$), which suggested that *miR-27a-3p* expression was related to the prognostic value for patients with oral cancer. Then we used Cox regression analysis to further examine the prognostic value of *miR-27a-3p*. The results showed that the low *miR-27a-3p* expression was an independent and significant prognostic factor for oral cancer.

Conclusions

In conclusion, the down-regulated expression of *miR-27a-3p* was observed in oral cancer and its expression is influenced by some clinical factors in this study. The present evidence provides support for *miR-27a-3p* to be a potential prognostic marker. Although further studies are needed to confirm the results of our study, our findings may serve as the basis for future researches about the clinical value of *miR-27a-3p* in monitoring treatment efficacy and forecasting prognosis of oral cancer.

List Of Abbreviations

quantitative real-time polymerase chain reaction (qRT-PCR)

oral squamous cell carcinoma (OSCC)

microRNAs (miRNAs)

cell renal cell carcinoma (ccRCC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

he datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

L.W. design of the work; S.Y. the acquisition, analysis, H.S. interpretation of data; L.W. the creation of new software used in the work; S.Y. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

Figure 1

The relative miR-27a-3p expression in oral cancer tissues and adjacent normal tissue. The expression of miR-27a-3p in oral cancer patients tissues was significantly lower than that in adjacent normal tissues; All the data were presented as mean $\pm$ SD; ** compared with controls $p < 0.01$.

Figure 2

Association between miR-27a-3p and overall survival. Patients with high miR-27a-3p expression had a longer overall survival than those low expression (log rank test, $P < 0.05$).