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 dysregulated 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 \( \text{miR-27a-3p} \) in the prognosis of oral cancer was still unclear.

In this study, we mainly focused on the serum expression levels of \( \text{miR-27a-3p} \) 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) | *miR-27a-3p* expression | \( \chi^2 \) | \( P \) values |
|-------------------------------|---------------|-------------------------|--------------|----------------|
|                               |               | Low (n = 75)            | High (n = 61) |                |
| Age(year)                     |               |                         |              |                |
| < 60                          | 70            | 39                      | 31           | 0.019          | 0.891          |
| \( \geq 60 \)                 | 66            | 36                      | 30           |                |
| Gender                        |               |                         |              |                |
| Male                          | 75            | 45                      | 30           | 1.592          | 0.207          |
| Female                        | 61            | 30                      | 31           |                |
| TNM stage                     |               |                         |              |                |
| I-II                          | 62            | 28                      | 34           | 4.594          | 0.032          |
| III-IV                        | 74            | 47                      | 27           |                |
| T stage                       |               |                         |              |                |
| T1-T2                         | 71            | 32                      | 39           | 6.098          | 0.014          |
| T3-T4                         | 65            | 43                      | 22           |                |
| Lymph node metastasis         |               |                         |              |                |
| negative                      | 68            | 31                      | 37           | 5.024          | 0.025          |
| positive                      | 68            | 44                      | 24           |                |
| Differentiation               |               |                         |              |                |
| Moderate + high               | 70            | 36                      | 34           | 0.806          | 0.369          |
| poor                          | 66            | 39                      | 27           |                |
| Smoking                       |               |                         |              |                |
| non-smoker                    | 71            | 38                      | 33           | 0.159          | 0.690          |
| 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^−ΔΔCT 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 ± 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 χ² 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 classes (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, \(P = 0.038\)).

| Variable                  | HR     | 95%CI        | \(P\) value |
|---------------------------|--------|--------------|-------------|
| Low *miR-27a-3p* expression | 0.462  | 0.223–0.957  | 0.038       |
| High *miR-27a-3p* 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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**References**

1. Rajwar YC, Jain N, Bhatia G, Sikka N, Garg B, Walia E: *Expression and Significance of Cadherins and Its Subtypes in Development and Progression of Oral Cancers: A Review*. Journal of clinical and diagnostic research: JCDR 2015, 9(5):ZE05-07.

2. Siegel RL, Miller KD, Jemal A: *Cancer statistics, 2015*. CA: a cancer journal for clinicians 2015, 65(1):5-29.

3. Genden EM, Ferlito A, Bradley PJ, Rinaldo A, Scully C: *Neck disease and distant metastases*. Oral oncology 2003, 39(3):207-212.

4. Choi S, Myers JN: *Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy*. Journal of dental research 2008, 87(1):14-32.

5. de Vicente JC, Rodriguez-Santamarta T, Rosado P, Pena I, de Villalain L: *Survival after free flap reconstruction in patients with advanced oral squamous cell carcinoma*. Journal of oral and maxillofacial surgery: official journal of the American Association of Oral and Maxillofacial Surgeons 2012, 70(2):453-459.

6. Sun ZJ, Zhang L, Hall B, Bian Y, Gutkind JS, Kulkarni AB: *Chemopreventive and chemotherapeutic actions of mTOR inhibitor in genetically defined head and neck squamous cell carcinoma mouse model*. Clinical cancer research: an official journal of the American Association for Cancer Research 2012, 18(19):5304-5313.
7. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M et al: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. The New England journal of medicine 2005, 353(17):1793-1801.

8. Peng F, Xiong L, Tang H, Peng C, Chen J: Regulation of epithelial-mesenchymal transition through microRNAs: clinical and biological significance of microRNAs in breast cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 2016, 37(11):14463-14477.

9. Alipoor SD, Adcock IM, Garssen J, Mortaz E, Varahram M, Mirsaedi M, Velayati A: The roles of miRNAs as potential biomarkers in lung diseases. European journal of pharmacology 2016, 791:395-404.

10. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004, 116(2):281-297.

11. Shukla GC, Singh J, Barik S: MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. Molecular and cellular pharmacology 2011, 3(3):83-92.

12. Ameres SL, Zamore PD: Diversifying microRNA sequence and function. Nature reviews Molecular cell biology 2013, 14(8):475-488.

13. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, Lo YM: Detection and characterization of placental microRNAs in matenal plasma. Clinical chemistry 2008, 54(3):482-490.

14. Redova M, Svoboda M, Slaby O: MicroRNAs and their target gene networks in renal cell carcinoma. Biochemical and biophysical research communications 2011, 405(2):153-156.

15. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA: MicroRNAs--the micro steering wheel of tumour metastases. Nature reviews Cancer 2009, 9(4):293-302.

16. Zeng G, Xun W, Wei K, Yang Y, Shen H: MicroRNA-27a-3p regulates epithelial to mesenchymal transition via targeting YAP1 in oral squamous cell carcinoma cells. Oncology reports 2016, 36(3):1475-1482.

17. Scully C, Bagan J: Oral squamous cell carcinoma overview. Oral oncology 2009, 45(4-5):301-308.

18. Sun L, Liu L, Fu H, Wang Q, Shi Y: Association of Decreased Expression of Serum miR-9 with Poor Prognosis of Oral Squamous Cell Carcinoma Patients. Medical science monitor : international medical journal of experimental and clinical research 2016, 22:289-294.

19. Hunt S, Jones AV, Hinsley EE, Whawell SA, Lambert DW: MicroRNA-124 suppresses oral squamous cell carcinoma motility by targeting ITGB1. FEBS letters 2011, 585(1):187-192.

20. Wiklund ED, Gao S, Hulf T, Sibbritt T, Nair S, Costea DE, Villadsen SB, Bakholdt V, Bramsen JB, Sorensen JA et al: MicroRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma. PloS one 2011, 6(11):e27840.

21. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, Wong DT: Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clinical cancer research : an official journal of the American Association for Cancer Research 2009, 15(17):5473-5477.
22. Nakata W, Uemura M, Sato M, Fujita K, Jingushi K, Ueda Y, Kitae K, Tsujikawa K, Nonomura N: Expression of miR-27a-3p is an independent predictive factor for recurrence in clear cell renal cell carcinoma. *Oncotarget* 2015, *6*(25):21645-21654.

23. Xu W, Liu M, Peng X, Zhou P, Zhou J, Xu K, Xu H, Jiang S: miR-24-3p and miR-27a-3p promote cell proliferation in glioma cells via cooperative regulation of MXI1. *International journal of oncology* 2013, *42*(2):757-766.

24. Wu X, Bhayani MK, Dodge CT, Nicoloso MS, Chen Y, Yan X, Adachi M, Thomas L, Galer CE, Jiffar T et al: Coordinated targeting of the EGFR signaling axis by microRNA-27a*. *Oncotarget* 2013, *4*(9):1388-1398.

25. Kara M, Yumrutas O, Ozcan O, Celik OI, Bozgeyik E, Bozgeyik I, Tasdemir S: Differential expressions of cancer-associated genes and their regulatory miRNAs in colorectal carcinoma. *Gene* 2015, *567*(1):81-86.

26. Jiang X, Du L, Duan W, Wang R, Yan K, Wang L, Li J, Zheng G, Zhang X, Yang Y et al: Serum microRNA expression signatures as novel noninvasive biomarkers for prediction and prognosis of muscle-invasive bladder cancer. *Oncotarget* 2016, *7*(24):36733-36742.

27. Jiang X, Du L, Wang L, Li J, Liu Y, Zheng G, Qu A, Zhang X, Pan H, Yang Y et al: Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. *International journal of cancer* 2015, *136*(4):854-862.

**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 ± 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).