microRNA-101 Represents an Innovative Bio-Marker in Oral Cancer Detection

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Primary research

Keywords: Oral cancer, MiR-101, Diagnosis, Serum

Posted Date: July 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-47875/v1>

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Abstract

Background: It had been suggested that microRNA-101 (*miR-101*) was involved in carcinogenesis and progression of various human tumors. The purpose of this study was to investigate the diagnostic value of *miR-101* in oral cancer.

Methods: Quantitative real-time polymerase chain reaction (QRT-PCR) was used to detect the serum mRNA level of *miR-101* in 130 oral cancer cases and 82 healthy individuals. The association of *miR-101* with clinical characteristics of oral cancer patients was estimated using chi-square test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic significance of *miR-101* in oral cancer.

Results: Compared with healthy controls, serum *miR-101* level was significantly down-regulated in oral cancer patients ($P<0.001$). Furthermore, low expression of *miR-101* was closely associated with histological grade ($P=0.037$), TNM stage ($P=0.018$) and lymph node metastasis ($P=0.023$). ROC curve analysis indicated that serum *miR-101* could effectively distinguish oral cancer patients from healthy controls with an area under the curve (AUC) of 0.878 (95%CI=0.834-0.922, $P<0.001$). The cutoff level of *miR-101* expression for oral cancer diagnosis was 1.46, with the sensitivity of 82.9% and the specificity of 74.6%.

Conclusions: Decreased expression of *miR-101* is correlated with the aggressive progression of oral cancer. Serum *miR-101* may be a promising bio-marker for early diagnosis of oral cancer.

Background

Oral cancer is a most common malignant tumor, with increasing mortality in the world [1]. Smoking and drinking are identified as the main risk factors of oral cancer [2]. Despite of the advanced therapeutic strategies, the 5-year overall survival rate of oral cancer patients is still not high and the prognosis remains poor [3]. Delay in diagnosis may be responsible for the dismal outcomes of oral cancer patients [4]. Currently, the most common approaches for early detection of oral cancer include conventional clinical examination, as well as open biopsy [5]. However, the application value of these methods are limited, and many oral cancer patients develop to advanced stages when initially diagnosed, leading to high death rate [6]. In order to improve the survival, it is necessary to identify novel and reliable bio-markers for the diagnosis and prognosis of oral cancer patients. Accumulating evidences have suggested that molecular bio-markers may be ideal candidates for early diagnosis of oral cancer [5].

As a class of non-coding RNAs, microRNAs (miRNAs) had been proven to be involved in a series of biological processes, including cell proliferation, differentiation and apoptosis [7]. Currently, growing evidences have indicated that miRNAs could be employed as novel bio-markers for non-invasive detection of various diseases, especially tumors [7, 8]. Among these miRNAs, *miR-101* is identified as a tumor suppressor, and its abnormal expression was observed in a variety types of human tumor, such as heaptatocellular carcinoma, osteosarcoma, etc [9, 10]. It was reported that *miR-101* could inhibit tumor

cell proliferation and invasive capacity, thus played an inhibitory role in development of cancer [11, 12]. Given its functional roles in tumorigenesis, *miR-101* was confirmed as a bio-marker for the diagnosis, therapy and prognosis of human cancer [13, 14]. In oral cancer, a study of Wu et al. mentioned that enhanced *miR-101* expression could suppress cell proliferation, invasion, migration, and induce cell apoptosis in vitro. Animal experiments demonstrated that *miR-101* played inhibitory role in tumor progression via mediating tumor growth and metastasis [15]. However, little is known about the diagnostic significance of *miR-101* in oral cancer patients.

In this study, we investigated the relative mRNA expression levels of *miR-101* in serum samples collected from the oral cancer patients and healthy controls. The relationship between *miR-101* expression and clinical characteristics of patients was analyzed. Then we also estimated the diagnostic value of *miR-101* in oral cancer.

Materials And Methods

Study subjects and sample

A total of 130 patients who were pathologically diagnosed with oral cancer at Chinese PLA General Hospital were enrolled in this study. None of patients had received surgery, radiotherapy or chemotherapy before sampling. The histopathological diagnoses of patients were confirmed by experienced pathologists. Besides, 82 healthy individuals served as healthy controls. The healthy controls and oral cancer patients were matched in gender and age. This study was approved by the Ethical Committee of the hospital and written informed consent was obtained from each participant in advance.

5 mL blood samples were collected from oral cancer patients and healthy controls after fasting for one night. Then the samples were centrifuged at 3000 rpm for 10 min and the serum samples were stored at -80°C . The detail clinicopathologic characteristics of patients were shown in **Table 1**.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs, including miRNAs, were isolated from sera using miRNeasy mini kit (Qiagen, Valencia, CA, USA). The purity of the RNA was measured via OD260/280 value using NanoDrop 2000 Spectrophotometer (Thermo Scientific, Worcester, MA, USA). MiScript Reverse Transcription Kit (Qiagen, Valencia, CA, USA) was used for reverse transcription. The relative expression level of *miR-101* was estimated by qRT-PCR, which was conducted according to miScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA). *U6* served as internal control. The sequences of primers for *miR-101* and *U6* were as follows: *miR-101*, forward-5'-CGGCGGTACAGTACTGT- GATAA-3', and reverse-5'-CTGGTGTCTGGAGTCGGCAATT-3'; *U6*, forward-5'-CTCGCTTCGGCAGCACA-3', and reverse-5'-AACGCTTCACGAATTTGCGT-3'. $2^{-\Delta\Delta\text{Ct}}$ method was utilized to calculate the relative quantification of *miR-101* expression. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were performed with SPSS 18.0 software and GraphPad Prism 5.0 software. The data were expressed as mean $\pm$ standard deviation (SD). Student's t-test was used to evaluate the difference between two groups. The relationship between *miR-101* expression and clinical characteristics of patients was analyzed via χ^2 test. A receiver operating characteristic (ROC) curve was applied to assess the diagnostic value of *miR-101* in oral cancer patients. $P < 0.05$ was considered statistically significant.

Table 1
Relationship between *miR-101* expression and clinical characteristics of patients with oral cancer

Clinical Features	Cases (n = 130)	<i>MiR-101</i> expression		χ^2	<i>P</i>
		High (n = 59)	Low (n = 71)		
Age (years)				0.136	0.712
≤ 55	66	31	35		
> 55	64	28	36		
Gender				0.654	0.419
Male	82	35	47		
Female	48	24	24		
Smoking				0.911	0.340
No	85	36	49		
Yes	45	23	22		
Drinking				1.521	0.218
No	65	33	32		
Yes	65	26	39		
T classification				2.857	0.091
T1-T2	71	37	34		
T3-T4	59	22	37		
Histological grade				4.342	0.037
Well/Moderate	73	39	34		
Poor	57	20	37		
TNM stage				5.568	0.018
I-II	69	38	31		
III-IV	61	21	40		
Lymph node metastasis				5.143	0.023
Negative	81	43	38		
Positive	49	16	33		

Results

Characteristics of study subjects

As shown in **Table 1**, there were 48 female and 82 male oral cancer patients with the mean age of 55.36 ± 15.66 years (range, 39-72 years) in this study. 45 oral cancer patients had smoking history and 65 patients had drinking history. T classification of 71 cases was at T1-T2 and T classification of 59 cases was at T3-T4. 73 patients had well/moderate histological grade and 57 patients had poor grade. 69 patients were at stage I-II, while 61 cases were at stage III-IV. Lymph node metastasis was observed in 49 patients.

Serum *miR-101* was down-regulated in oral cancer patients

The relative mRNA expression levels of *miR-101* in serum samples of oral cancer patients and healthy individuals were detected via qRT-PCR. The result showed that serum *miR-101* level was significantly lower in oral cancer patients than that in healthy controls ($P < 0.001$, **Figure 1**).

Relationship between *miR-101* expression and clinical features of oral cancer patients

To investigate the effect of *miR-101* on the development of oral cancer, 130 patients were divided into two groups (high *miR-101* expression group and low *miR-101* expression group) according to the mean value of *miR-101* expression. Then the correlation between *miR-101* expression and clinical characteristics of patients was analyzed. The outcome indicated the decreased expression of *miR-101* was closely associated with advanced histological grade ($P = 0.037$), TNM stage ($P = 0.018$) and positive lymph node metastasis ($P = 0.023$) (**Table 1**). However, there was no obvious relationship between *miR-101* expression and age, gender, smoking, drinking or T classification ($P > 0.05$, **Table 1**).

The diagnostic value of *miR-101* in oral cancer

ROC curve was conducted to estimate the diagnostic value of *miR-101* in oral cancer patients. It demonstrated that the area under the curve (AUC) was 0.878 (95%CI=0.834-0.922, $P < 0.001$) with a sensitivity of 82.9% and a specificity of 74.6%, suggesting the high diagnostic value of serum *miR-101* for oral cancer (**Figure 2**). The cutoff value of *miR-101* expression for oral cancer diagnosis was 1.46.

Discussion

Oral cancer is a common malignancy with high incidence rate [16]. Early detection and timely treatments are pivotal for clinical outcomes of the patients. However, until now the pathology of oral cancer can not be completely explained. Diagnosis and prognosis evaluation remain a great challenge for oral cancer in clinic. Since the functional roles of miRNA in various biological processes, great progression has been made in understanding the etiology of cancer [17]. A growing body of evidences have demonstrated that miRNAs may be the reliable bio-markers for the detection of human cancers because they can present in body fluids in a stable form that could be easily detected [18].

MiR-101 represents a common member of miRNA family, which had been shown to be decreased and play an anti-tumor role in various cancers [19, 20]. Accumulating researches had reported that the abnormal expression of *miR-101* was involved in cell proliferation, colony formation, migration and apoptosis through targeting some genes [21, 22]. Given its significant functions in controlling the biological behaviors of tumor cells, *miR-101* was considered as a promising candidate bio-marker for therapy and prognosis of some cancers [23, 24]. For examples, the study of Li et al. reported that the expression of *miR-101* was decreased in laryngeal squamous cell carcinoma tissues, and showed negative association with high tumor grade, lymph node metastasis as well as advanced clinical stage [25]. Zhang et al. mentioned that the expression of *miR-101* was decreased in bladder transitional cell carcinoma tissues and closely correlated with tumor grade, clinical stage and lymph node metastasis of the patients. *MiR-101* might be a potential biomarker for prognosis evaluation in bladder cancer [26]. Nevertheless, the diagnostic value of *miR-101* in oral cancer patients remained poorly understood.

In the present study, we investigated the expression profile of *miR-101* in oral cancer, as well as its diagnostic performance in this disease. We found that the relative mRNA level of *miR-101* in serum samples of oral cancer patients was significantly down-regulated compared to that in healthy controls. Chi-square test results showed that patients with reduced *miR-101* expression were more likely to have poor histological grade, advanced TNM stage and positive lymph node metastasis. All the data revealed that *miR-101* was a tumor suppressor gene in oral cancer, and its down-regulation predicted aggressive tumor progression for oral cancer. These results were in accordance with the previous studies [25, 26]. However, Bao et al. reported that low *miR-101* expression was obviously associated with tumor size of gallbladder carcinoma patients, which was different from our result [27]. The divergences might be caused by the different types of malignancy, relatively small sample size as well as the different detection specimens.

Early detection is an effective approach to improve the clinical outcomes of oral cancer patients. In the current study, we estimated whether *miR-101* could be applied for the diagnosis of oral cancer. ROC curve analysis demonstrated that serum *miR-101* could be an effective bio-marker for distinguishing oral cancer patients from healthy individuals with satisfied AUC, sensitivity and specificity. Serum *miR-101* might be a non-invasive biomarker for patients with oral cancer. In addition to oral cancer, the diagnostic significance of serum *miR-101* was also confirmed in other types of tumor. Zhuang et al. reported that serum *miR-101* showed significantly different between hepatocellular carcinoma patients and healthy controls, which could act as a promising bio-marker for the diagnosis of the disease [28]. However, the precise molecular mechanism of *miR-101* in the progression of oral cancer is still poorly known. Further studies will be required.

Conclusions

In conclusion, serum *miR-101* is down-regulated in oral cancer and correlated with the development of this disease. Besides, serum *miR-101* may be a potential bio-marker for early diagnosis of oral cancer.

List Of Abbreviations

microRNA-101 (*miR-101*)

Quantitative real-time polymerase chain reaction (QRT-PCR)

Receiver operating characteristic (ROC)

area under the curve (AUC)

microRNAs (miRNAs)

standard deviation (SD)

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

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

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

Acknowledgements Not applicable.

Funding:

The 63rd batch of grants from China Post-Doctoral Science Fund (2018M633673).

The 11th batch of special grants from China Post-Doctoral Science Fund (2018T111137).

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Figures

Figure 1

Serum miR-101 expression levels in oral cancer patients and healthy individuals. The relative mRNA expression of miR-101 was significantly lower in serum of oral cancer patients than that in healthy controls ($P < 0.001$). *** indicated P less than 0.001.

Figure 2

ROC curve analysis for the diagnostic value of miR-101 in oral cancer. The curve demonstrated that serum miR-101 could be employed as biomarker for oral cancer diagnosis. The AUC was 0.878 with a sensitivity of 82.9% and a specificity of 74.6%, and the cut-off value was 1.46.