Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

MiR-92b as a marker for TPF induced chemotherapy response prediction and prognosis evaluation in with advanced oral squamous cell carcinoma patients

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Received February 14, 2020; **Accepted** May 9, 2020; **Published** June 5, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.3.4

Abstract: This study aimed to explore the clinical value of miR-92b in advanced oral squamous cell carcinoma (OSCC) and to observe the relationship between miR-92b and TPF induced chemotherapy and prognosis. Totally 114 patients with advanced OSCC admitted to our hospital were selected as the study subjects, all of whom received docetaxel, cisplatin and 5-fluorouracil (TPF) induction chemotherapy. In addition, another 80 healthy subjects who underwent physical examination in our hospital from the same period were enrolled. The serum expression of miR-92b was detected by qRT-PCR. Serum miR-92b was up-regulated in patients with advanced OSCC, and its expression was associated with higher TNM staging and lymph node metastasis. The receiver operating characteristic (ROC) curve revealed that the area under the curve (AUC) of serum miR-92b for the diagnosis of advanced OSCC was 0.931. After treatment, the miR-92b expression was significantly reduced, and the ROC curve showed an AUC value of 0.889 for predicting treatment sensitivity of serum miR-92b. What's more, Logistic indicated that TNM staging, lymph node metastasis and serum miR-92b expression were independent risk factors affecting the treatment efficacy. Survival analysis demonstrated that OSCC patients with high miR-92b expression had poorer OS and DFS compared to patients with low miR-92b expression. Multivariate Cox regression exhibited that TNM staging, lymph node metastasis, and serum miR-92b expression were self-regulating risk factors for overall survival (OS) and disease-free survival (DFS) in patients with advanced OSCC. MiR-92b is up-regulated in patients with advanced OSCC, which can be used as a marker for induction chemotherapy and prognosis evaluation of advanced OSCC.

Key words: Advanced oral squamous cell carcinoma; MiR-92b; Induction chemotherapy; Prognosis.

Introduction

Oral squamous cell carcinoma (OSCC) is one of the subtypes of head and neck squamous cell carcinoma and the sixth most common malignancy (1). It has a large invasion range and is characterized by local anatomical structure invasion, which is prone to regional lymphatic metastasis (2). Despite the advances in surgical techniques and other anti-cancer methods in the past few decades, the prognosis of OSCC patients remains poor, with a high local recurrence rate and a 5-year survival of 50% (3). The low cure rate and high mortality of this disease pose a global public health problem, bringing a huge economic burden to individuals and the social economy (4).

With good efficacy, surgical resection is the preferred management for patients with early OSCC (5). As to advanced OSCC patients, the mainstream treatment is comprehensive therapy, and the effects of remission induction, surgical treatment as well as postoperative radiotherapy have been widely acknowledged, among which preoperative induction chemotherapy is a research hotspot (6). Docetaxel, cisplatin and 5-fluorouracil (TPF) induction chemotherapy are considered to able to improve the survival rate of a variety of malignant tumors, and are also common chemotherapy for advanced OSCC (7). In the beginning, TPF chemotherapy has a positive response, but some patients still present chemical resistance and relapse, which leads to a serious threat to the survival rate and quality of life of the patients (8). Therefore, the evaluation of chemotherapy effect and survival rate in patients with advanced OSCC will help to optimize treatment strategies.

MicroRNA (miRNA) is a highly conserved non-coding small molecule RNA composed of 20-24nt, which plays an important regulatory role in tumor proliferation, apoptosis and differentiation (9). The expression of miRNA in the blood is a biomarker closely related to the diagnosis, treatment and prognosis of malignant tumors (10, 11). Several studies have shown that the miR-17 and miR-92 families exert an essential effect on cisplatin resistance and can predict the clinical response of non-small cell lung cancer to platinum-based chemotherapy (12). MiR-92b is one of the mature miRNAs of miR-17-92 cluster and paralogs, which is not only highly expressed in many malignant tumors but also participates in biological functions such as tumor proliferation, apoptosis and differentiation (13, 14). Previous studies have displayed that miR-92b is up-regulated in OSCC tissues and can promote tumor growth *in vivo* or *in vitro* (15). Therefore, miR-92b may play an important part in the occurrence and development of OSCC. However, the role of miR-92b in the evaluation of TPF induction chemotherapy and prognosis evaluation in patients with

advanced OSCC remains a subject of investigation.

In this study, the serum expression of miR-92b in patients with advanced OSCC was detected, so as to explore the role of miR-92b in the treatment and prognosis of patients.

Materials and Methods

General information

From March 2012 to January 2014, 114 patients with advanced OSCC were enrolled, including 84 males and 30 females, aged 30-85 years. Inclusion criteria: Patients confirmed as OSCC by pathological histology (16), who had complete clinical data with an ECOG score of 0-2 points, and those without previous surgery or chemoradiotherapy. Exclusion criteria: Patients complicated with other malignant tumors, pulmonary metastasis, autoimmune diseases, endocrine systemic diseases, severe cardiovascular and cerebrovascular diseases, treatment contraindications, who were unable to cooperate with the treatment for mental disorders, or those withdrew from the experiment or lost to follow-up. In addition, 80 healthy subjects in the same period were selected, including 46 males and 34 females, aged 35±76 years. The study was approved by the Medical Ethics Committee of our hospital and conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from every study subject.

Treatment method

Induction chemotherapy (17): d_1: intravenous infusion of docetaxel (Qilu Pharmaceutical Co., Ltd., China, H20041129) at 75mg/m^2; $d_{1\text{-}3}$: intravenous infusion of Cisplatin (Qilu Pharmaceutical Co., Ltd., China, H20023461) at 75mg/m^2; $d_{1\text{-}5}$: intravenous infusion of 5-fluorouracil (Shanghai Xudong Haipu Pharmaceutical Co., Ltd., China, H31020593) at 750mg/m^2. The administration was conducted once every 3 weeks for a total of 2 cycles. Surgery was performed at least 2 weeks after the induction was completed. Follow-up treatment: radical surgery was performed on the primary lesion, followed by appropriate reconstruction, and then the total neck dissection was carried out. Postoperative radiotherapy: Radiotherapy was started 5 weeks after surgery, and standard conformal or intensity-modulated radiotherapy was given at a dose of 1.8 or 2GY/d for 5 days per week for a total of 6 weeks, with a total dose of 54-60Gy.

Efficacy evaluation

The efficacy was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) of the World Health Organization (WHO) (18). The efficacy evaluation and the corresponding symptoms were as follows: Complete response (CR): complete disappearance of the lesion. Partial response (PR): 30% reduction in volume after induction chemotherapy. Progressive disease (PD): baseline lesion length increased by at least 20% or new lesions were generated. Stable disease (SD): the length and diameter of the baseline lesions were reduced to less than PR or increased to less than PD. Toxicity was evaluated according to CTCAE version 3.0. Based on the therapeutic effect, we defined CR/PR as treatment sensitivity and SD/PD as treatment insensitivity.

Follow-up

Patients were followed every 3 months for the first 2 years and every 6 months for the ensuing 3-5 years. The overall survival (OS) was defined as the time from random date to death (19). While disease-free survival (DFS) was defined as calculated from the randomized date to the date of recurrence or death from any cause (20).

Detection Methods

An amount of 4mL peripheral venous blood samples were taken from the subjects and placed in a centrifuge tube. Then the samples were centrifuged at 1500×g for 10min at 4℃, and the collected serum was stored for later use. Total RNA was extracted from serum using a Tempus Blood RNA Tube (Applied Biosystems, CA, USA, 4342792), and the concentration of RNA was measured by a Nanodrop ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA). cDNA production and miR quantification were conducted by TaqMan MicroRNA (Applied Biosystems, CA, USA, A25576). Data analysis was performed on an ABI 7300 real-time PCR system (Applied Biosystems, CA, USA). qPCR amplification conditions: 94 °C for 5min, 94°C 30s, 55 °C for 30s, 72 °C for 30s, totaling 40 cycles. The average expression level of miR-92b was normalized by U6 using $^{2\text{-}\Delta Ct}$, in triplicate for each sample.

Statistical methods

Statistical analysis of the data was performed by IBM SPSS 20.0 statistical software (International Business Machines Corporation, Armonk, NY, USA). Normal distribution test was applicable for all the data, those following to normal distribution were expressed as mean ± standard deviation (Meas±SD). The measurement data in line with the normal distribution was analyzed by t-test of independent samples, and the paired t-test was applied for comparison between groups before and after treatment. In addition, the receiver operating characteristic (ROC) analysis was adopted to calculate the area under the curve (AUC), and the optimal cutoff value was obtained by the Youden index to calculate the sensitivity and specificity. Risk factors of chemotherapy in patients were determined by Logistic univariate and multivariate regression. Survival curves were evaluated by Kaplan-Meier and analyzed by the log-rank test. The Cox proportional hazard model for survival-related factors was applied to calculate HR and identify factors that influence survival.

Results

MiR-92b was up-regulated in patients with advanced OSCC

To detect the miR-92b expression in patients with advanced OSCC, we used qRT-PCR to detect the serum miR-92b in OSCC patients and healthy subjects. The results showed that, compared with healthy subjects, the expression level of miR-92b in OSCC patients was significantly up-regulated. Further analysis of the clinicopathological features of miR-92b and OSCC patients revealed that the up-regulation of miR-92b expression

Table 1. Relationship between miR-92b and clinicopathological features of OSCC patients.

Clinicopathologic features	n	miR-92b	t	P
Gender			0.842	0.402
Male	84	1.59±0.29		
Female	30	1.54±0.26		
Age (years)			1.550	0.124
<60	52	1.53±0.25		
≥60	62	1.61±0.31		
Smoking			1.253	0.213
Non-smoker	49	1.53±0.23		
Smoker	65	1.60±0.32		
Drinking			0.707	0.481
Non-drinker	37	1.55±0.28		
Drinker	77	1.59±0.29		
Tumor site			0.107	0.915
Tongue	38	1.58±0.28		
Other sites	76	1.57±0.28		
TNM staging			2.740	0.007
III	41	1.48±0.23		
IV	73	1.63±0.30		
Histologic differentiation			1.058	0.292
Good/moderate	58	1.55±0.25		
Poor	56	1.60±0.31		
Lymph node metastasis			2.378	0.019
No	31	1.47±0.22		
Yes	83	1.61±0.30		

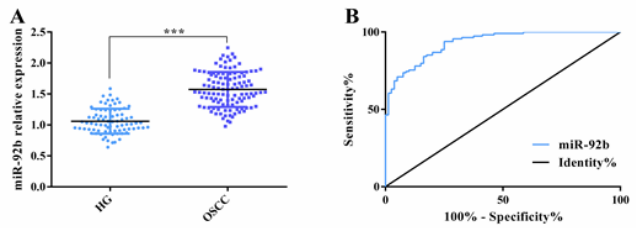

Figure 1. Serum miR-92b expression level in patients with advanced OSCC. A. Compared with the healthy subjects, miR-92b was significantly up-regulated in OSCC patients. B.ROC curve of serum miR-92b for OSCC diagnosis. Note: *** indicates P<0.001.

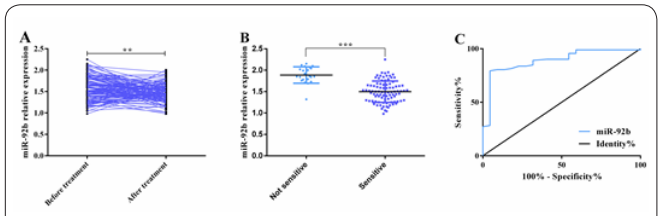

Figure 2. Expression of serum miR-92b before and after treatment in OSCC patients and its predictive value for treatment efficacy. Expression of miR-92b in OSCC patients before and after treatment. A. Expression of miR-92b in the serum of OSCC patients with and without treatment sensitivity. B. ROC curve of serum miR-92b in predicting the therapeutic sensitivity of OSCC patients. Note: ** indicates P<0.01, and *** indicates P<0.001.

level predicted a higher TNM staging and was related with lymph node metastasis. The ROC curve analysis indicated that the AUC of serum miR-92b for diagnosis of advanced OSCC was 0.931, the optimal cut-off was 1.18, the sensitivity was 96.86%, and the specificity was 78.00% (Figure 1, Table 1).

MiR-92b was down-regulated after treatment in OSCC patients

After treatment, there were 35 cases of CR, 57 cases of PR, 17 cases of SD, and 5 cases of PD among all the 114 patients with advanced OSCC. The main adverse reactions during treatment were nausea, vomiting, hair loss, leukopenia and other mild adverse reactions. Remission was observed after active symptomatic treatment. To observe the relationship between miR-92b and the therapeutic efficacy of OSCC patients, qRT-PCR was applied to distinguish the expression level of miR-92b in the serum of OSCC patients before and after treatment. The data showed that the expression of miR-92b was significantly decreased after treatment. In addition, we observed the relationship between serum miR-92b before treatment and treatment sensitivity. The results indicated that the expression of serum miR-92b in patients with treatment sensitivity was significantly lower than that in patients without. What's more, the ROC curve for the therapeutic effect of serum miR-92b in OSCC patients was constructed, from which we could see that the AUC value of serum miR-92b in predicting the therapeutic effect was 0.889, the optimal cut-off value was 1.70, the sensitivity was 79.35%, and the specificity was 95.45%. (Figure 2)

Logistic regression analysis of the factors influencing the therapeutic effect

The median value of miR-92b (1.53) was used as the segmentation point to compare the clinicopathological features of OSCC patients and the difference between

Table 2. Clinicopathological features of OSCC patients and the association between serum miR-92b and treatment sensitivity.

Factors	n	Sensitive (n=92)	Insensitive (n=22)	χ^2	P
Gender				0.426	0.514
Male	84	69 (75.00)	15 (68.18)		
Female	30	23 (25.00)	7 (31.82)		
Age (years)				2.091	0.148
<60	52	45 (48.91)	7 (31.82)		
≥60	62	47 (51.09)	15 (68.18)		
Smoking				0.487	0.485
Non-smoker	49	41 (44.57)	8 (36.36)		
Smoker	65	51 (55.43)	14 (63.64)		
Drinking				1.177	0.278
Non-drinker	37	32 (34.78)	5 (22.73)		
Drinker	77	60 (65.22)	17 (77.27)		
Tumor site				0.704	0.401
Tongue	38	29 (31.52)	9 (40.91)		
Other sites	76	63 (68.48)	13 (59.09)		
TNM staging				5.902	**0.015**
III	41	38 (41.30)	3 (13.64)		
IV	73	54 (58.70)	19 (86.36)		
Histologic differentiation				2.298	0.130
Good/moderate	58	50 (54.35)	8 (36.36)		
Poor	56	42 (45.65)	14 (63.64)		
Lymph node metastasis				4.512	**0.034**
No	31	29 (31.52)	2 (9.09)		
Yes	83	63 (68.48)	20 (90.91)		
miR-92b				8.111	**0.004**
<1.53	57	52 (56.52)	5 (22.73)		
≥1.53	57	40 (43.48)	17 (77.27)		

Table 3. Analysis of multivariate logistic regression.

Variables	B	S.E	Wals	P	OR	95% CI
Gender	0.609	0.710	0.735	0.391	0.544	0.135-2.187
Age	0.324	0.791	0.168	0.682	0.723	0.153-3.408
Smoking	0.395	0.749	0.278	0.598	0.674	0.155-2.924
Drinking	0.521	0.861	0.367	0.545	0.594	0.110-3.208
Tumor site	0.974	0.868	1.258	0.262	2.648	0.483-14.509
TNM staging	2.674	0.964	7.696	**0.006**	14.492	2.192-95.819
Histologic differentiation	1.003	0.749	1.791	0.181	2.725	0.628-11.831
Lymph node metastasis	1.955	0.783	6.236	**0.013**	0.142	0.031-0.657
miR-92b	2.814	1.013	7.714	**0.005**	16.671	2.289-121.412

miR-92b and treatment effect, and it was found that there were differences in TNM staging, lymph node metastasis and serum miR-92b expression in OSCC patients with treatment sensitivity and insensitivity. We further performed multivariate Logistic regression analysis for these differences, and the data showed that TNM staging (P=0.006), lymph node metastasis (P=0.013) and serum miR-92b expression (P=0.005) were independent risk factors affecting the treatment efficacy. (Tables 2 and 3)

Up-regulation of miR-92b was associated with poor prognosis in patients with OSCC

All the 114 patients with advanced OSCC were successfully followed up for 5 years. On the whole, the 5-year OS of the 114 OSCC patients was 59.65% (68/114) and the 5-year DFS was 51.75% (59/114). With the median value of miR-92b (1.53) as the segmentation point, we divided it into a high expression of miR-92b (≥1.53) and low expression of miR-92b (<1.53). Survival analysis exhibited that OSC patients with high miR-92b expression presented poorer OS (P=0.013) and DFS (P=0.005) compared to those with low miR-92b expression. As to OS and DFS, univariate Cox regression analysis indicated that TNM staging, lymph node metastasis and serum miR-92b expression were important prognostic factors for OSCC patients, and multivariate Cox regression analysis indicated that

Table 4. Uni and mult-ivariate Cox regression analysis of OS in OSCC patients.

Factors	Univariate Cox regression			Multivariate Cox regression		
	P value	HR	95CI%	P value	HR	95CI
Gender	0.684	1.146	0.593-2.214			
Age	0.833	1.065	0.594-1.908			
Smoking	0.453	1.254	0.694-2.268			
Drinking	0.442	1.286	0.677-2.444			
Tumor site	0.670	1.143	0.617-2.119			
TNM staging	**0.021**	2.220	1.127-4.374	**0.031**	2.165	1.090-4.298
Histologic differentiation	0.457	1.246	0.698-2.227			
Lymph node metastasis	**0.004**	5.542	1.718-17.882	**0.027**	4.044	1.227-13.326
miR-92b	**0.009**	2.250	1.226-4.132	**0.031**	1.985	1.066-3.693
Effects of TPF chemotherapy	0.477	1.299	0.632-2.672			

Table 5. Univariate and multivariate Cox regression analysis of DFS in OSCC patients.

Factors	Univariate Cox regression			Multivariate Cox regression		
	P value	HR	95CI%	P value	HR	95CI%
Gender	0.205	1.423	0.825-2.455			
Age	0.611	1.138	0.692-1.870			
Smoking	0.425	1.225	0.744-2.019			
Drinking	0.382	1.270	0.743-2.172			
Tumor site	0.365	1.281	0.749-2.192			
TNM staging	**0.005**	2.242	1.271-3.957	**0.024**	1.946	1.094-3.462
Histologic differentiation	0.213	1.367	0.836-2.235			
Lymph node metastasis	**0.021**	2.097	1.118-3.933	**0.040**	1.936	1.031-3.635
miR-92b	**0.002**	2.196	1.326-3.638	**0.012**	1.926	1.153-3.217
Effects of TPF chemotherapy	**0.038**	1.962	1.037-3.714	0.081	1.740	0.933-3.244

these clinical parameters were also independent risk factors for OS and DFS in OSCC patients. (Figure 3, Tables 4 and 5)

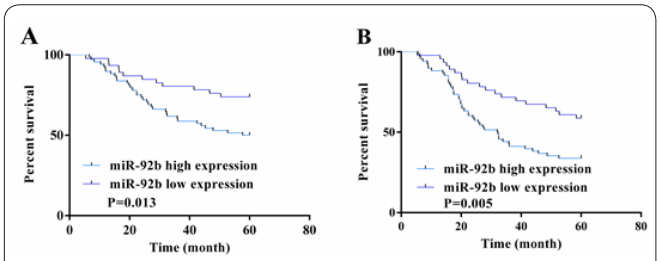

Figure 3. Prognostic value of miR-92b in patients with advanced OSCC. OSCC patients with high miR-92b expression presented poorer OS (A) and DFS (B) than those with low miR-92b expression.

Discussion

TPF neoadjuvant chemotherapy has proved to be beneficial to patients with advanced OSCC (21). Whereas, some studies have revealed that OSCC has a limited response to this treatment (22). These reports highlight the importance of identifying biomarkers associated with the implementation of TPF-induced chemotherapy regimens in OSCC patients. Here in our study, miR-92b was highly expressed in patients with advanced OSCC and had good diagnostic value for advanced OSCC. In addition, the expression of serum miR-92b was significantly decreased after treatment, and the risk of chemotherapy insensitivity was increased in OSCC patients with high miR-92b expression, which in turn predicted poor OS and DFS. Our study highlights the role of miR-92b as a biomarker for adjuvant chemotherapy and prognostic assessment, which has important implications for future medical applications.

Previous studies have confirmed that miRNAs are stable and easy to detect in the blood. The expression of miRNAs varies in different cancers, which is related to the diagnosis, severity and prognosis of various malignant tumors (23). Among them, miR-92b has been shown to be highly expressed in a variety of malignancies (including OSCC) in previous studies (24). As reported by Zhou (25), miR-92b was significantly up-regulated in osteosarcoma patients and associated with poor prognosis. Besides, according to Huang (26), miR-92b could target DAB2IP to promote EMT and thus to enhance migration and invasion of bladder cancer. Previous studies of miR-92b mostly focused on tissues or cells, the role of serum miR-92b in OSCC, however, remains poorly understood. In the present study, we verified the serum miR-92b expression in patients with advanced OSCC through qRT-PCR and found that the serum miR-92b was markedly up-regulated in OSCC patients compared with healthy subjects. ROC showed that the AUC of miR-92b in OSCC diagnosis was 0.931, which indicated that miR-92b could be used as an excellent diagnostic marker for OSCC patients. However, there are some certain deficiencies as our study did not include early-stage patients. We further analyzed the clinicopathological features of miR-92b and OSCC patients and observed that the upregulation of miR-92b

expression level indicated a higher TNM staging and was associated with clinical node metastasis, suggesting that miR-92b might be implicated in the progression of OSCC. According to the results of randomized phase III trial by Zhong (17), compared with patients without induction chemotherapy, patients receiving TPF chemotherapy did not significantly increase the overall OS of OSCC patients, but it was observed that chemotherapy had a trend of improving the survival rate and the risk of tumor recurrence in patients was significantly reduced. The TPF chemotherapy regimen, while not significantly improving the overall OS of OSCC patients, is effective in reducing the risk of recurrence. In the current study, the expression of miR-92b was remarkably decreased after TPF chemotherapy, and the AUC value of miR-92b for the diagnosis of chemotherapy sensitivity before treatment was 0.889, suggesting that the sensitivity of chemotherapy could be predicted by observing miR-92b. Further studies revealed an increased risk of chemotherapy-insensitive expression of high TNM staging, lymph node metastasis, and serum miR-92b (≥1.53). Therefore, observing the expression of miR-92b is conducive to predict the sensitivity of patients with advanced OSCC to implement TPF chemotherapy regimen. Previous studies have reported that miR-92b can affect the growth of non-small cell lung cancer cells and the chemical sensitivity of cisplatin by regulating PTEN (27). Combined with our study, miR-92b may have potential value in OSCC development and chemoresistance, but the mechanism is still unknown. Finally, we followed up OSCC patients for 5 years. The survival analysis demonstrated that OSCC patients with high miR-92b expression had poorer OS and DFS and that TNM staging, lymph node metastasis and serum miR-92b expression were important prognostic factors of OSCC patients, which suggested that detection of miR-92b was helpful for prognosis evaluation of OSCC patients. In the study of Ma (28), induction chemotherapy could reduce the incidence of distant metastasis in patients with head and neck squamous cell carcinoma, though failed to improve local control and survival rate. Yan (29) reported that the expression profile of miRNA in plasma changed during the development of OSCC and could be used as a monitoring indicator for postoperative OSCC recurrence. Serum miR-92b expression may be a biomarker for TPF chemotherapy and prognosis evaluation in OSCC patients, but the mechanism still waits for discussion (30-42).

In conclusion, miR-92b is up-regulated in patients with advanced OSCC and can be used as a marker for the diagnosis, treatment and prognosis of advanced OSCC. However, there are still some shortcomings in the study. The mechanism of miR-92b in OSCC cell resistance is still unclear. In addition, the sample size of the study is narrowed, which needs to be further expanded to validate our results.

Acknowledgements
Not applicable.

Funding
No funding was received.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
HX wrote the manuscript. JW and RL conceived and designed the study. QC and YD were responsible for the collection and analysis of the experimental data. YD and YX interpreted the data and drafted the manuscript. HX and JW revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Affiliated Stomatological Hospital of Nanchang University & Jiangxi Province Key Laboratory of Oral Biology Medicine, China. Patients who participated in this research signed informed consent and had complete clinical data. Signed written informed consent was obtained from the patients and/or guardians.

Consent for publication
Not applicable.

Conflict of interest
The authors declare that they have no competing interests.

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