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MiR-92a regulates oral squamous cell carcinoma (OSCC) cell growth by targeting FOXP1 expression

Jun Guo[a,b], Ning Wen[a,*], Sefei Yang[a,*], Xiaohang Guan[b], Song Cang[b]

[a] Department of Stomatology, Chinese PLA General Hospital, Beijing, 100853, China
[b] Department of Orthodontics, Tianjin Stomatological Hospital (Hospital of Stomatology, Nankai University), Tianjin, 300041, China

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ABSTRACT

Increasing evidence indicates that microRNAs dysregulation contributes to the development and progression of various human cancers, including oral squamous cell carcinoma (OSCC). However, little is known about the potential role of microRNA-92a (miR-92a) in OSCC. Thus, the aim of this study was to investigate the effects of miR-92a expression on OSCC cell growth, apoptosis and tumorigenesis. Real-time quantitative polymerase chain reaction was used to detect the expression level of miR-92a in primary tumor tissues and OSCC cell lines. The effects of miR-92a on cell proliferation, cell cycle, apoptosis and tumorigenesis of OSCC cells were explored after miR-92a expression was increased or decreased in the UM1 and Tca-8113 cells, respectively. The 3′-untranslated region (3′-UTR) of FOXP1 combined with miR-92a was analyzed with dual-luciferase reporter assays. The level of miR-92a expression was significantly up-regulated in the OSCC tissues and cell lines. The up-regulation of miR-92a expression promoted UM1 cell proliferation, cell cycle progression *in vitro* and tumor growth in nude mice, but its expression reduction inhibited these processes and induced apoptosis in Tca-8113 cells. Additionally, miR-92a expression was inversely correlated with FOXP1 protein expression in the OSCC tissues and cell lines. Furthermore, FOXP1 was identified as a functional downstream target of miR-92a by directly targeting the 3′-UTR of FOXP1. These findings indicate that miR-92a may act as a tumor inducer in OSCC by suppressing FOXP1 expression, and it could serve as a potential therapeutic target for OSCC treatment.

1. Introduction

It is well-known that oral squamous cell carcinoma (OSCC) originates from the epithelium lining of the oral cavity, and more than 90% of all oral cancers are squamous cell carcinoma [1]. OSCC is the sixth leading solid tumor malignancy worldwide, and is the most common malignant epithelial neoplasm of the head and neck in terms of incidence and mortality [2]. Despite recent advances in diagnosis, surgery and chemotherapy strategy, the 5-year survival rates for OSCC remain quite low [3]. A growing body of evidence suggests that microRNAs (miRNAs) are aberrantly expressed in many human carcinomas and that they play key roles in the initiation, development and metastasis of human cancers, including OSCC [4–6].

miRNAs are a group of endogenous, non-coding, 18–24 nucleotide length single-strand RNAs that regulate the expression of other genes on a post-transcriptional level through mRNA degradation or translational repression [7]. Accumulating research indicates that dysregulation of miRNAs has been frequently found and closely associated with tumor initiation, promotion, and progression [8]. miRNAs can not only regulate the expression levels of oncogenes and tumor suppressor genes to influence the progression of cancer, but also function as oncogenes and tumor suppressor genes [9]. Recent studies have explored the utility of miRNAs as diagnostic biomarker and prognostic tools and as potential therapeutic targets. The expression level of miR-92a was found to be up-regulated in cervical cancer [10], hepatocellular carcinoma [11], osteosarcoma [12]; while its expression was obviously lower in plasma of patients with breast cancer or non-Hodgkin's lymphoma compared with healthy subjects [13–15]. However, the specific biological role of miR-92a in OSCC development still remains unclear.

Here, we investigated the expression level of miR-92a in OSCC tissues and cell lines, and explored the role and its associated molecular mechanism of miR-92a on OSCC cell growth by targeting the *FOXP1* gene.

Abbreviations: OSCC, oral squamous cell carcinoma; miR-92a, microRNA-92a; 3′-UTR, 3′-untranslated region; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, (3-[45-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide); DMEM, Dulbecco's modified eagle's medium

* Corresponding authors at: Department of the Stomatology, Chinese PLA General Hospital, Beijing, 100853, China.
E-mail addresses: wenning@fmmu.edu.cn (N. Wen), sfyang@ad.unc.edu (S. Yang).

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Table 1
Clinicopathological characteristics of patients with OSCC.

Characteristics	No. of patients (%)
Gender	
Male	23 (67.6)
Female	11 (32.4)
Age	
< 60	20 (58.8)
> 60	14 (41.2)
T stage	
T1/T2	26 (76.5)
T3/T4	8 (23.5)
TNM stage	
I + II	21 (61.8)
III + IV	13 (38.2)
Site of primary tumor	
Tongue	13 (38.2)
Oropharynx	2 (5.9)
Floor of mouth	2 (5.9)
Buccal mucosa	17 (50.0)
Histologic differentiation	
Well	12 (35.3)
Moderately	19 (55.9)
Poorly	3 (8.8)
Tumor size	
< 4 cm	29 (85.3)
> 4 cm	5 (14.7)
Lymph node metastasis	
Positive	8 (23.5)
Negative	26 (76.5)

2. Materials and methods

2.1. Human tissue specimens

Primary OSCC tissues and paired non-cancerous matched tissue samples were collected during tumor resection from 34 patients with OSCC and without chemotherapy or pre-operative radiation. The clinicopathological characteristics of patients are shown in Table 1. All tissue specimens were immediately frozen in liquid nitrogen after resection and stored at −80 ℃ until use for RT-PCR and western blot analysis. This study was approved by the Institutional Ethics Committee and Review Board of our hospital, and the informed consent was obtained from each patient.

2.2. Cell culture

Human OSCC cell lines (HSC3, OC3, UM1, SSC-25, and Tca-8113) and a human normal oral keratinocyte (hNOK) cell line were obtained from Cell Bank of Chinese Academy of Science (Shanghai, China).Human OSCC cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Invitrogen, CA), 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 ℃ in a humidified atmosphere of 5% CO_2 and 95% air. hNOK was maintained in keratinocyte serum-free medium, supplemented with 1% keratinocyte growth factor plus epithelial growth factor mixture (Life Technologies, Carlsbad, CA, USA)

2.3. RNA extraction and quantitative real-time PCR

Total RNA was extracted from the collected tissue specimens and cultured OSCC cells or hNOK cells using TRIzol reagent (Invitrogen, Carlsbad, CA). The reverse transcription reaction of miR-92a and its control U6 was performed using the looped miRNA RT primer sets (RiboBio, Guangzhou, China) and a PrimeScript RT reagent kit (Takara, Tokyo, Japan). Additionally, FOXP1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were respectively reverse transcribed into cDNA using a reverse transcription kit in accordance with the manufacturers' instructions. The subsequent PCR amplification for miR-92a and U6 was carried out using a miScript SYBR Green PCR kit (Qiagen, Germany) on a 7500 real-time PCR system (Applied Biosystems, CA) with enzyme activation at 95 ℃ for 5 min, followed by 40 cycles at 95 ℃ for 5 s and 60 ℃ for 20 s, and a final extension at 65 ℃ for 15 s. In addition, the PCR reaction for FOXP1 and GAPDH was performed on a 7500 real-time PCR system using FastStart Universal SYBR Green Master (Tiangen Biotech Co., Beijing, China) with amplification profile as follows: 95 ℃ for 30 s, followed by 35 cycles at 94 ℃ for 10 s and 60 ℃ for 30 s, and a final extension at 72 ℃ for 20 s. The specific primers used for FOXP1 and GAPDH were as follows [16]: FOXP1 forward 5′-ACCGCTTCCATGGGAAATC-3′ and reverse 5′−CCGTTCAGCTCTTC CCGTATT-3′; GAPDH forward, 5′-GGTGGTCTCCTCTGA

CTTCAACA-3′ and reverse 5′-GTGGTCGTTGAGGGCAATG-3′. The expression level of miR-92a or FOXP1 relative to U6 or GAPDH was determined as the respective comparative threshold cycle values ($2^{-\Delta\Delta Ct}$).

2.4. Cell transfection

The miR-92a gain-of-function study was performed using miR-92a mimics (100 nM) (Ribobio Company, Guangzhou, China) and a scrambled miRNA served as its negative control (100 nM) (Ribobio) on the UM1 cells, and the loss-of-function study was conducted with miR-92a inhibitor (100 nM) (Ribobio) and a scrambled miRNA as its negative control (100 nM) (Ribobio) on the Tca-8113 cells. There was a blank control without any transfection for each cell line. The cells were transfected with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) in Opti-MEM medium according to the manufacturer's instructions, and followed by replacing with fresh complete medium. After 72 h, the transfected cells were harvested and subjected to qRT-PCR for miR-92a expression level analysis, cell proliferation assay, cell cycle assay, apoptosis analysis and western blot analysis.

2.5. In vitro cell proliferation assay

The cells were seeded into 96-well plates at a density of 5×10^3 cells/well, and cultured for 24, 48 and 72 h, respectively. Cell proliferation was then determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 20 μl MTT solutions (5 mg/ml) were added into each well for incubation of 4 h at 37 ℃, followed by addition with 150 μl dimethyl sulphoxide. The absorbance value was then measured by Microtiter plate reader (Promega, Fitchburg, WI) at 570 nm.

2.6. Cell cycle analysis

After transfection of 72 h, the cells were harvested and washed with PBS, followed by fixation with 75% ethanol for 1 h. After removal of 75% ethanol, the cells were incubated in cell cycle staining solution (50 μg/ml of propidium iodide) (Beyotime, China) for 30 min, and cell cycle distribution was measured by FACScan using a flow cytometer (BD, Franklin Lakes, NJ).

2.7. Apoptosis assay

Annexin V-FITC/propidium iodide (PI) apoptosis detection kit (Qcbio, Shanghai, China) was used to detect cellular apoptosis. After transfection of 72 h, the cells were collected and incubated with binding buffer containing Annexin V-FITC and PI following the instructions of manufacturer. Annexin V-$FITC^+/PI^-$ cells in the lower-right quadrant (Q4) stood for early apoptotic cells, whereas Annexin V-$FITC^+/PI^+$ cells in the upper-right quadrant (Q2) represented late apoptotic cells. Total apoptotic cells of each sample were considered to include the cells in the early and late stages of apoptosis. Apoptotic cells were then detected by flow cytometer (BD, Franklin Lakes, NJ), and apoptotic percentage was expressed as fold change normalized to blank control