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Original Article

Predictive value of miR-27b as a potential biomarker for head and neck squamous cell carcinoma

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Abstract: Deregulation of miRNAs has been shown to involve in many human diseases including cancer. The aim of this study was to elucidate the role of miR-27b in head and neck squamous cell carcinoma (HNSC). Real-time PCR was performed to compare the expression level of miR-27b in HSNC tissues and adjacent normal tissues. Then its clinical significance in HSNC was further evaluated. MTT assay, cell counting assay and invasion assay were used to assess the effects of miR-27b upregulation or downregulation on the proliferation and invasion of HNSC cells. The expression level of miR-27b was significantly reduced in the HNSC tissues (P<0.01). In addition, HNSC patients in the low miR-27b expression group suffered worse long term overall survival compared with those in the high miR-27b expression group (P=0.045). Moreover, downregulation of miR-27b promoted the proliferation and invasion of HNSC cells, while upregulation of miR-27b led to opposite findings (P<0.05, P<0.01). The results demonstrated that miR-27b played a tumor-suppressive role in HSNC, which provide new insights into the molecular mechanisms accounting for the HNSC initiation and progression.

Keywords: miR-27b, prognosis, proliferation, invasion, head and neck squamous cell carcinoma

Introduction

Head and neck squamous cell carcinoma (HNSC), accounting for about 90% head and neck cancer, encompasses epithelial malignancies of several organs including nasal cavity, paranasal sinuses, oral cavity, tongue, pharynx, and larynx [1, 2]. Although great progress has been made for the treatment of this disease, the five year overall survival of HNSC remains unchanged at about 50% in the past decades [3]. Therefore it is necessary to further investigate the molecular mechanisms that drive the initiation and progression of this deadly cancer.

MicroRNAs (miRNAs) are a class of highly conserved small non-coding RNAs with lengths of approximately 18-25 nucleotides [4, 5]. They regulate gene expression through binding to the 3'-untranslated region (UTR) of target messenger RNA (mRNA), leading to translational repression or degradation. miRNAs have been shown to play important roles in regulating many biological processes including but not limited to proliferation, migration, differentiation, survival and development [6]. Deregulation of miRNAs implicates in a number of human diseases including cancer [7-9]. Summerer et al reported that plasma miR-186-5p could discriminate HNSC patients from healthy individuals with high sensitivity and specificity. They also demonstrated that high expression of miR-142-3p, miR-186-5p, miR-195-5p, miR-374b-5p and miR-574-3p in the plasma was associated with poorer prognosis of HNSC, indicating that this miRNA signature can be used for monitoring therapy responses [10]. The expression level of miR-196a was significantly upregulated in HNSC cells. Downregualtion of miR-196a suppressed the proliferation, migration and invasion capacity of cancer cells, and *vice versa.* In addition, annexin A1 (ANXA1) was shown to be a downstream target of miR-196a, indicating miR-196a might exert its oncogenic activities through regulating ANXA1 [11].

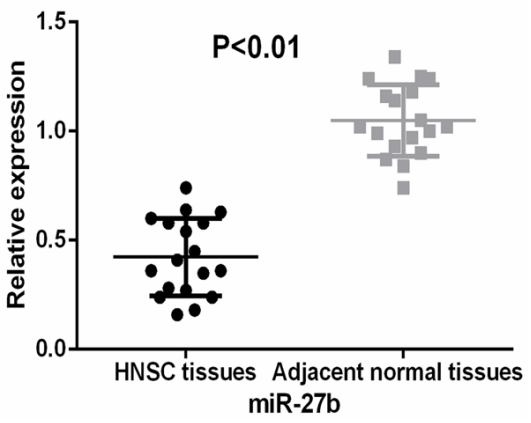

Figure 1. miR-27b was downregulated in HNSC tissues.

Aberrant expression of miR-27b has been reported in some types of cancers such as breast cancer, lung cancer and prostate cancer [12-14]. However, its role in HNSC is poorly known. Thus the purpose of this study was to evaluate the biological function and clinical significance of miR-27b in HNSC.

Materials and methods

Study population

The study was approved by the Institutional Research Ethics Committee of Changzhou Second People's Hospital. Informed consent was obtained from the patients and their relatives prior to the use of these clinical materials for research purposes. Eighteen paired HNSC and adjacent non-tumor tissues were collected. All tissue samples were flash-frozen in liquid nitrogen immediately after collection and stored at -80°C until use. Both tumor and adjacent normal specimens were histologically confirmed by two independent experienced pathologists. No patients received chemotherapy or radiotherapy before receiving the surgery.

Cell culture

The HNSC cell line Tca8113 was purchased from the Culture Collection of Chinese Academy of Science (Shanghai, People's Republic of China). Tca8113 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, Mo., USA) supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA). Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Cell transfection

Tca8113 cells were transfected with miR-27b inhibitor/mimic or a non-specific control miRNA (Sigma-Aldrich). Transfections were performed using Lipofectamine™ 2000 (Invitrogen) according to manufacturer's instructions.

Real-time PCR

Total RNA was isolated from HNSC tissues, adjacent non-tumor tissues and Tca8113 cells using Trizol (Takara, Dalian, China) according to the manufacturer's instructions. RNA was quantified using a NanoDrop 2000 (Nano-drop Technologies, Wilmington, DE, USA) and stored at -80°C. All-in-One™miRNA qRT-PCR Detection Kit (GeneCopoeia, Rockville, MD, USA) was used to quantify the expression level of miR-27b according to the protocols. Real-time PCR was performed on a real-time PCR ABI 7500 Fast system (Applied Biosystems, Foster City, CA, USA). U6 small RNA was used as an internal control for normalization.

MTT assay

The cells were seeded at a density of 3000 cells/well in a 96-well plate and detected at the different indicated time points with 20 μl of MTT (Sigma-Aldrich) for 4 h at 37°C. The culture medium was removed and 200 μL DMSO (Sigma-Aldrich) was added. Absorbance was measured at 570 nm and all experiments were performed in triplicate.

Cell counting assay

Cells were plated in 6-well plates, and counted with a hemocytometer at different time points. Trypan blue exclusion test was performed to determine the number of viable cells.

Invasion assay

Invasion assays were performed using Transwell invasion chambers (No. 354480; BD Biosciences, New York, NY, USA) according to the manufacturer's instruction. About 2×10^5 cells suspended in 100 μl serum-free medium were added to the upper chambers. The lower chambers contained 700 μl DMEM with 10% FBS. After 24 h of incubation, the cells that had invaded to the bottom surface of the chamber were fixed with 4% formaldehyde for 10 min and stained with 0.1% crystal violet for 5 min.

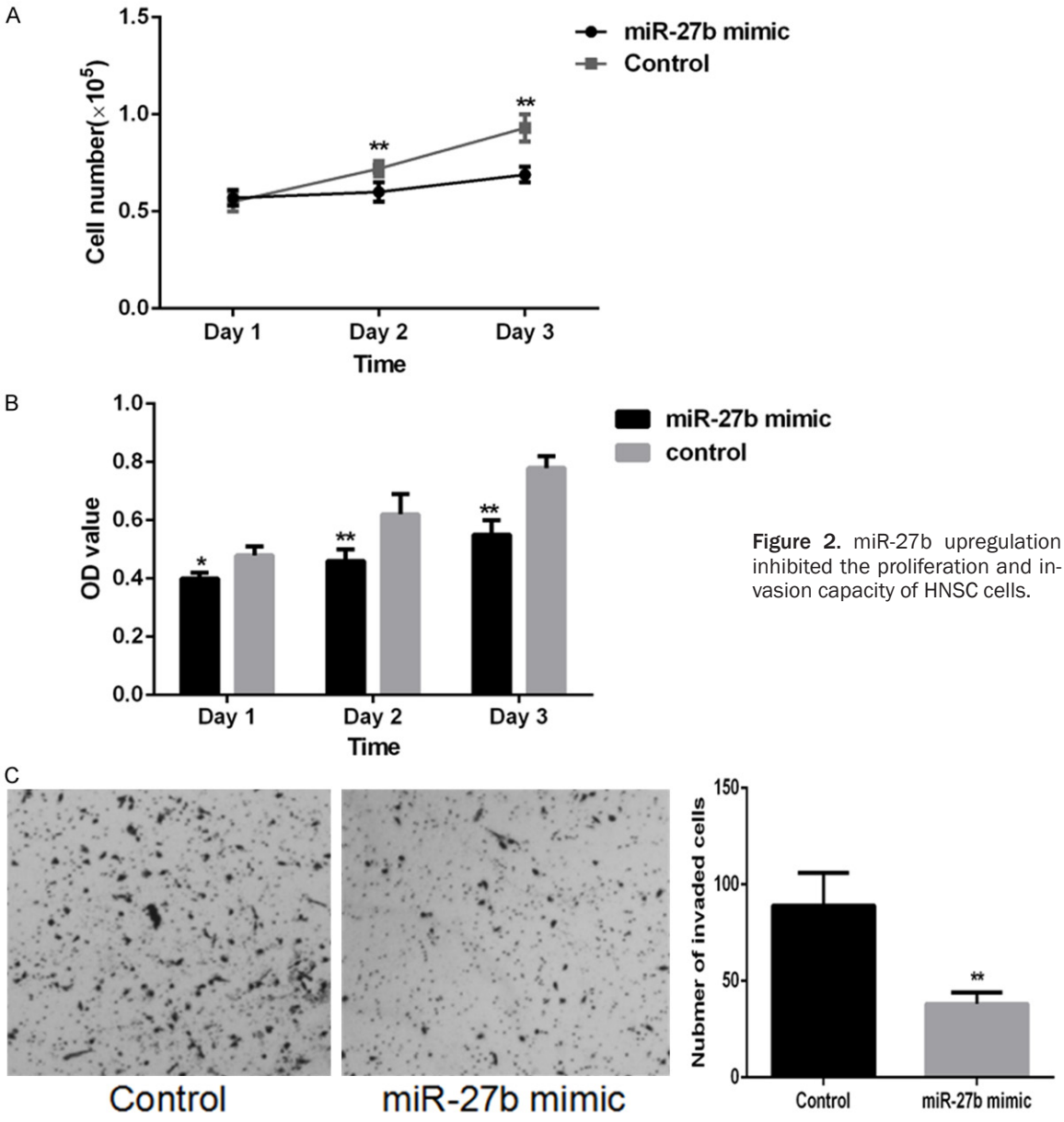

Figure 2. miR-27b upregulation inhibited the proliferation and invasion capacity of HNSC cells.

Cells that had passed through the membrane were counted under a microscope in four random fields at 20× magnification.

Statistical analysis

All the experimental data were presented as the means ± standard deviation (SD). Two-tailed Student's t-test was used for the data analysis. The cutoff point optimization of TCGA patient cohort was calculated with X-tile software. Kaplan-Meier method in combination with the log-rank test was performed for the long term overall survival analysis. Statistical analyses were performed using GraphPad PRISM 6.0 software (GraphPad, La Jolla, CA, USA) and SPSS version 21.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Result

miR-27b was downregulated in HNSC tissues

Our results showed that the expression level of miR-27b was significantly reduced in HNSC tissues compared with the adjacent normal tissues ($P<0.01$) (**Figure 1**).

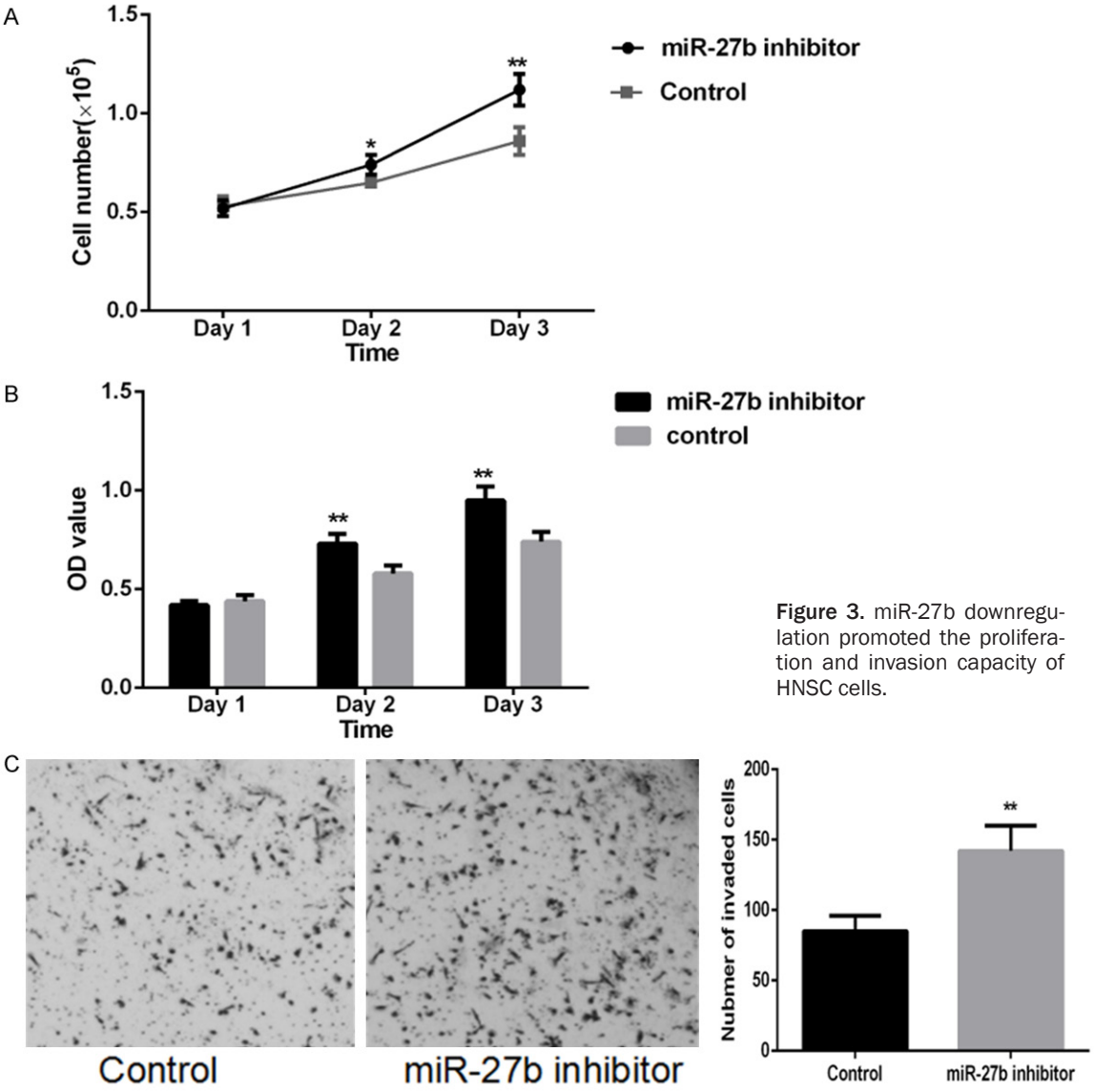

Figure 3. miR-27b downregulation promoted the proliferation and invasion capacity of HNSC cells.

miR-27b upregulation inhibited the proliferation and invasion capacity of HNSC cells

Both MTT assay and cell counting assay demonstrated miR-27b mimic suppressed the proliferation of HNSC cells at different time points (*P<0.05, **P<0.01) (**Figure 2A**, **2B**). The transwell invasion assay showed that the number of cancer cells that invaded through the membrane was significantly lower in miR-27b mimic treated group in compared with the control (P<0.01) (**Figure 2C**).

miR-27b downregulation promoted the proliferation and invasion capacity of HNSC cells

Both MTT assay and cell counting assay demonstrated miR-27b inhibitor treatment clearly enhanced the prolife capacity of HNSC cells at different time points (*P<0.05, **P<0.01) (**Figure 3A**, **3B**). The number of cancer cells that invaded through the membrane was significantly higher in miR-27b inhibitor treated group in compared with the control (P<0.01) (**Figure 3C**).

The association between miR-27b expression level and overall survival

The HNSC patients in the low miR-27b expression group had a significantly shorter long term overall survival than those in the high miR-27b expression group (P=0.045) (**Figure 4**).

Discussion

Head and neck cancer is the sixth most common cancer around the world and still a signifi-

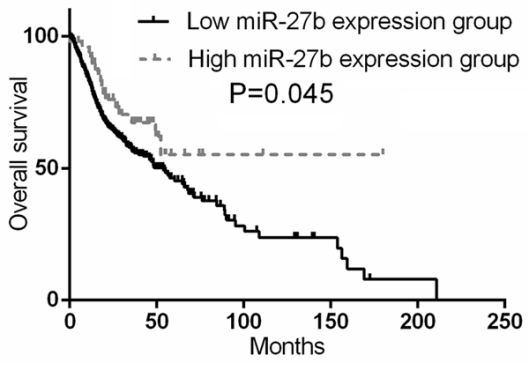

Figure 4. The association between miR-27b expression level and overall survival.

cant public health issue. Therefore, elucidating the molecular mechanisms for the initiation and progression of head and neck cancer is very important. The Cancer Genome Atlas (TCGA) project has genomic, epigenomic, transcriptomic, and proteomic data covering over 20 different cancer types. TCGA also has clinical data such as histopathology, clinical stage and overall survival. Analysis of these data provides valuable insights in determining the clinical significance of cancer.

Our results demonstrated that the expression level of miR-27b was reduced in HNSC tissues. In addition, miR-27b inhibition promoted the proliferation and invasion capacity of HNSC cells. Opposite findings were found when miR-27b was overexpression. Moreover, HNSC patients with lower miR-27b expression suffered a significantly worse long term overall survival compared to those with higher miR-27b expression, suggesting that miR-27b might act as a tumor suppressor in the progression of HNSC. Consistent with our findings, miR-27b expression level was significantly decreased in tissue and plasma samples from patients with oral squamous cell carcinoma tissue in comparison with the normal controls [15, 16]. Ye et al showed that miR-27b expression level was reduced in colorectal cancer tissues. Also miR-27b could suppress the oncogenic activities of colorectal cancer cells by targeting vascular endothelial growth factor C (VEGFC) both *in vitro* and *in vivo*, indicating that miR-27b act as a tumor suppressor in colorectal cancer [17]. Similarly, Jiang et al reported that the expression level of miR-27b was remarkably reduced in non-small cell lung cancer (NSCLC) cell lines. In addition, ectopic expression of miR-27b suppressed the proliferative and invasive capacity of NSCLC cells and *vice versa*. Moreover, Sp1 transcription factor was identified as a downstream target of miR-27b [18].

It is common to observe the phenomenon that a specific miRNA functions as an oncogene in some types of cancers, while plays a tumor suppressive role in other types of cancers. Downregulation of miR-27b was significantly associated with tamoxifen resistance in breast cancer cells. In addition, its downregulation played an important in the generation of breast cancer stem cells by activating ENPP1, suggesting miR-27b could promote the progression of breast cancer [19]. Similarly, miR-27b was upregulated in cervical cancer cell lines and tissues. miR-27b overexpression enhanced the oncogenic behaviors of cervical cancer cells, while inhibitory effects were observed when miR-27b was suppressed [20]. Overall speaking, the role of miR-27b is very complicate in cancers and its function might be closely correlated with tumor microenvironment.

A limitation of this current study is that the clinical sample size. Large cohort studies should perform to determine the clinical significance of miR-27b in HNSC. Also the molecular mechanisms responsible for the tumor suppressive role of miR-27b needed to be elucidated.

Conclusion

miR-27b is reduced in HNSC tissues and associated with worse long term overall survival of HNSC. In addition, miR-27b downregulation promotes the proliferation and invasive capacity of HNSC cells and *vice versa*. Collectively, miR-27b might be a promising target for HNSC therapy.

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Disclosure of conflict of interest

None.

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