

## Original Article

# miRNA-126b regulated apoptosis of the human tongue carcinoma cell line Tca8113-P60 via P38 signaling pathway

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**Abstract:** Human tongue carcinoma threatens human health and life severely. MicorRNA can regulate multiple cellular events, while it remains unclear whether miRNA-126b influences human tongue carcinoma. Our study focused on the potential effect of miRNA-126b on human tongue carcinoma in Tca8113-P60 cell model. MiRNA-126b and control microRNA were synthesized with routine protocol. MiRNA-126b and control microRNA were transfected into human tongue carcinoma cell line Tca8113-p60 with liposome transfection reagent. Cell growth of Tca8113-P60 was determined with MTT assay. Apoptosis of Tca8113-P60 was examined by Annexin V staining by flow cytometry. Expression of p38 was examined by Western Blot. Further, transfected Tca8113-P60 cells were treated with inhibitor of p38 signaling pathway (SB203580) for examination of cell growth and apoptosis. Compared with control microRNA, miRNA-126b not only significantly suppressed cell growth of Tca8113-P60 ( $P=0.0051$ ), but also remarkably enhanced apoptosis of Tca8113-P60 ( $P=0.021$ ). Moreover, p38 signaling pathway inhibitor abrogated apoptosis-promoting effect of miRNA-126b on Tca8113-P60 ( $P=0.0041$ ). In conclusion, MiRNA-126b induced apoptosis of human tongue carcinoma Tca8113-P60 cells via p38 signaling pathway and might be a potential therapeutic target for the treatment of human tongue carcinoma.

**Keywords:** miRNA-126b, p38 signaling pathway, tongue carcinoma, apoptosis

## Introduction

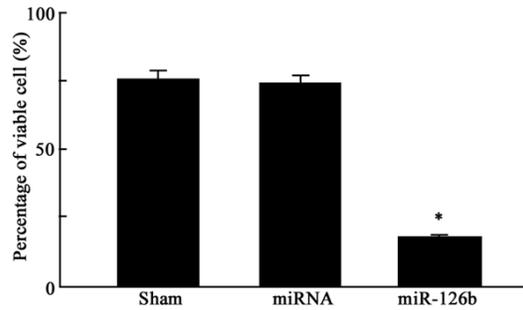
Tongue carcinoma is a severe oral carcinoma in global scale, which seriously influences health and life quality of patients [1, 2]. With the process of industrialization and changes of dietary structure, morbidity and five year mortality rate of human tongue carcinoma trend to increase gradually [3-5]. Clinical treatments for human tongue carcinoma include surgery, radiotherapy and chemotherapy [6, 7]. Although current therapies play important roles in treatment of human tongue carcinoma, disadvantages or shortcomings still limit their clinical application. Thus, therapy with promising efficacy was warranted in the treatment of human tongue carcinoma.

With more and more studies focused on novel targets for cancer, efficacy of molecule-target-

ed therapies are remarkably improved. Moreover, molecule-targeted therapies are expected to play pivotal roles in tongue carcinoma due to fewer side effects [2, 3]. However, the choice of target molecules was emphasis and difficulty in molecular targeted therapy [4, 5]. Accordingly, our study investigated a potential target for the treatment of human tongue carcinoma.

As an important member of RNA family, microRNA was widespread across different species [8, 9]. MiRNA was an endogenous regulator with multiple functions, including cell cycle regulation, organogenesis and tissue differentiation [10-12]. MiRNA was a promising target for cancer treatment with various advantages, such as low molecular weight and easily degradable [13, 14]. Previous studies demonstrated that miRNA-126b could effectively inhibit cell growth

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**Figure 1.** Percentage of viable cell in miRNA-126b transfection group was decreased. \* $P < 0.05$  versus sham group.

and enhance apoptosis in neuroglioma cells or breast cancer cells [8]. However, it remains unclear whether miRNA-126b influences human tongue carcinoma. Therefore, our study postulated miRNA-126b might be a potential protective factor against human tongue carcinoma.

Apoptosis is also called programmed cell death, and inducing cancer apoptosis is the main anti-tumor strategy [15]. Many apoptosis-related signaling pathways are involved in tumor progress, including ERK, p38 and JNK. What's more, p38 is proved to influence diagnosis of oral carcinoma [16, 17]. Collectively, our study focused on whether p38 signaling pathway was regulated by miRNA-126b.

Human tongue carcinoma Tca8113-P60 was an acknowledged cell line which was used for several studies investigating tongue carcinoma cell function *in vitro* [14, 15]. Thus, our study used Tca8113-P60 as a cell model to investigate the role of miRNA-126b in tongue carcinoma.

### Materials and method

#### Reagents and cell line

DMEM (Dulbecco Modified Eagle Medium) and serum were purchased from Gibco Company (USA). Inhibitor of p38 signaling pathway (SB-203580) and p38 activity assays were purchased from Sino-american biotechnology company (China). Antibiotics and other inorganic reagents were purchased from Beijing Dingguo Chansheng biotechnology co. Ltd. (China). MTT kit was purchased from Sangon biotech company (China). Caspase-activity assay kit and flow cytometry reagents FITC-annexin V

were purchased from Beyotime Biotechnology company (China). MiRNA-126b and mimics miRNA-126b (control microRNA) were synthesized with routine protocol by Beijing Sunbiotech co.,Ltd (China). The sequence of miRNA-126b was as follows: 5'-UCGUACCGUGAGUAAUAAUGCG-3'. The sequence of control microRNA was as follows: 5'-GGCTCGTACCGTGAGTAAT-3'.

Human tongue carcinoma cell line Tca8113-P60 was purchased from America microbiological culture collection center.

#### Cell culture of Tca8113-P60 and liposome transfection

Human tongue carcinoma Tca8113-P60 cells were treated and cultured as reported previously [17]. Briefly, Tca8113-P60 cells were rapidly transformed into water bath at 37°C after uncased from liquid nitrogen. Centrifuge cells at room temperature, 600 rpm, 4 min, and remove liquid supernatant and culture Tca8113-P60 cells in DMEM for 24 hours.

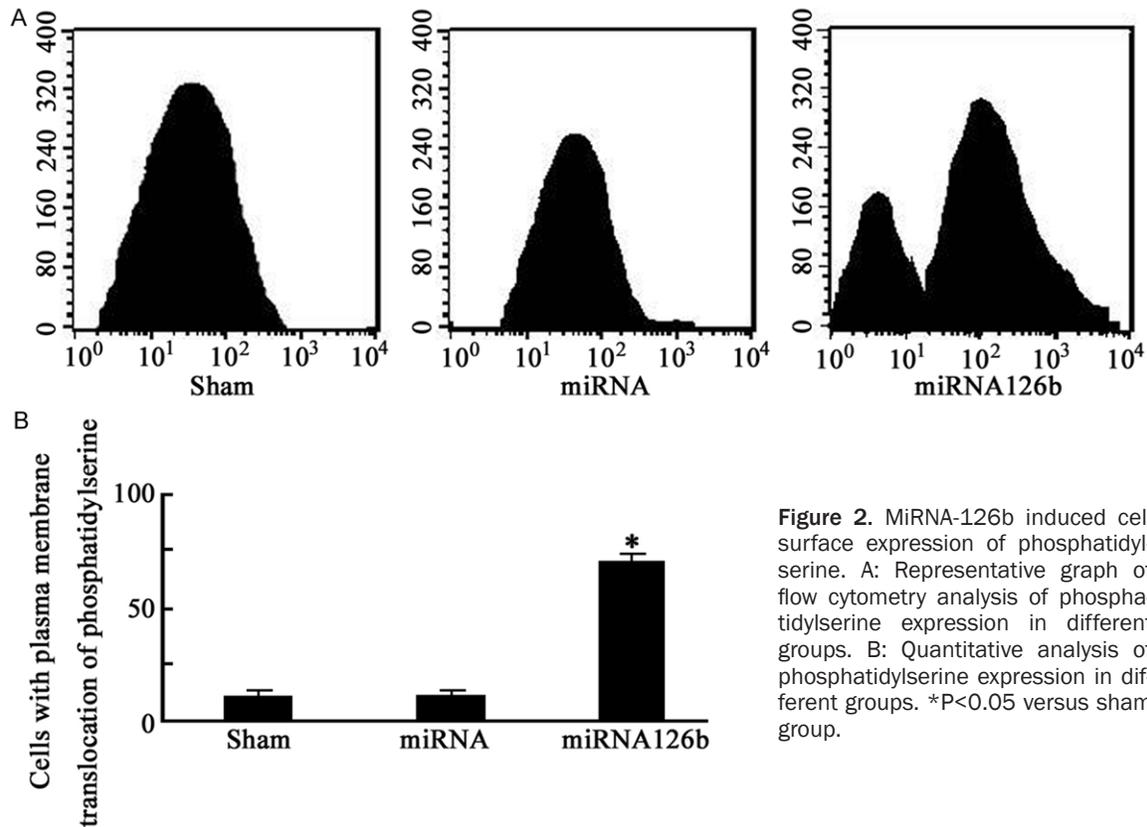
Liposome transfection was performed as reported previously [16]. Tca8113-P60 cells were planked for 12 hours before transfection. Add 3  $\mu$ l lipo2000 into 2  $\mu$ l miRNA-126b or control microRNA, and incubate for 2 min at room temperature to fully package miRNA-126b or control microRNA into liposome. Transfection complex was slowly added into Tca8113-P60 cells, and replace new medium after 12 hours transfection.

#### Examination of cell viability with MTT assay

Treatment of Tca8113-P60 cells was performed as reported previously [17]. MTT assay was performed as follows: Tca8113-P60 cells were cultured in 96-well plate for 24 hours. MTT reagents were added into 96-well plate and incubated for 2 minutes at room temperature. Add reaction solutions and keep reaction for 5 minutes at room temperature. Optical density at 560 nm was measured by a microplate reader.

#### Examination of apoptosis with flow cytometry

Treatment of Tca8113-P60 cells was performed as reported previously [17]. Cell groups were as follows: control microRNA transfection group, miRNA-126b transfection group, SB20-



**Figure 2.** MiRNA-126b induced cell surface expression of phosphatidylserine. A: Representative graph of flow cytometry analysis of phosphatidylserine expression in different groups. B: Quantitative analysis of phosphatidylserine expression in different groups. \*P<0.05 versus sham group.

3580+ control microRNA transfection group, SB203580+ miRNA-126b transfection group. Collect 3×10<sup>4</sup> Tca8113-P60 cells from each group. Add buffer solution and annexin V solution and incubate for 8 minutes at room temperature. Apoptosis of Tca8113-P60 cell was examined by a flow cytometer.

*Caspase-3 activity of Tca8113-P60 cell*

Treatment of Tca8113-P60 cells was performed as reported previously [17]. Cell groups were as follows: control microRNA transfection group, miRNA-126b transfection group, SB203580+ control microRNA transfection group, SB203580+ miRNA-126b transfection group. Collect 3×10<sup>4</sup> Tca8113-P60 cells from each group for Caspase-3 activity examination. All operations were performed according to Caspase-3 kit protocol.

*p38 activity of Tca8113-P60 cell*

Treatment of Tca8113-P60 cells was performed as reported previously [17]. Cell groups were as follows: control microRNA transfection group, miRNA-126b transfection group, SB20-

3580+ control microRNA transfection group, SB203580+ miRNA-126b transfection group. Collect 3×10<sup>4</sup> Tca8113-P60 cells from each group for p38 activity examination. p38 signaling pathway was detected with Caspase-3 kit and microplate reader. Western Blot was performed to examine the phosphorylation level of p38.

*Statistical analysis*

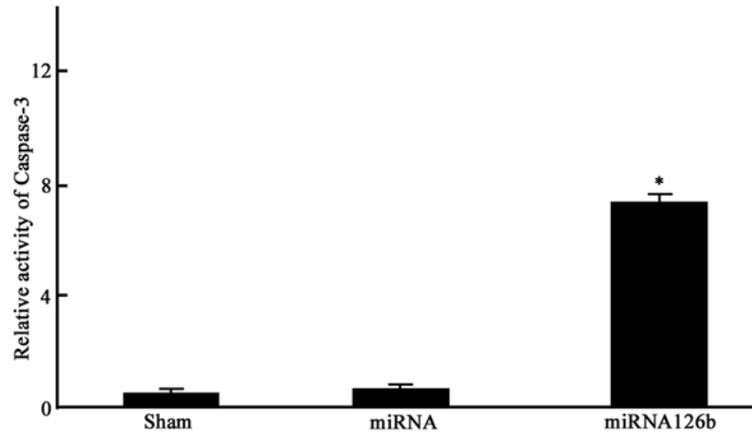
SPSS11.2 software was used for data processing. Measurement data with normal distribution were expressed as mean ± standard deviation (SD). Levene's test was performed to test normal distribution. Statistical significance between multiple treatment groups was analyzed by one-way ANOVA. P value < 0.05 was considered to be statistically significant.

**Results**

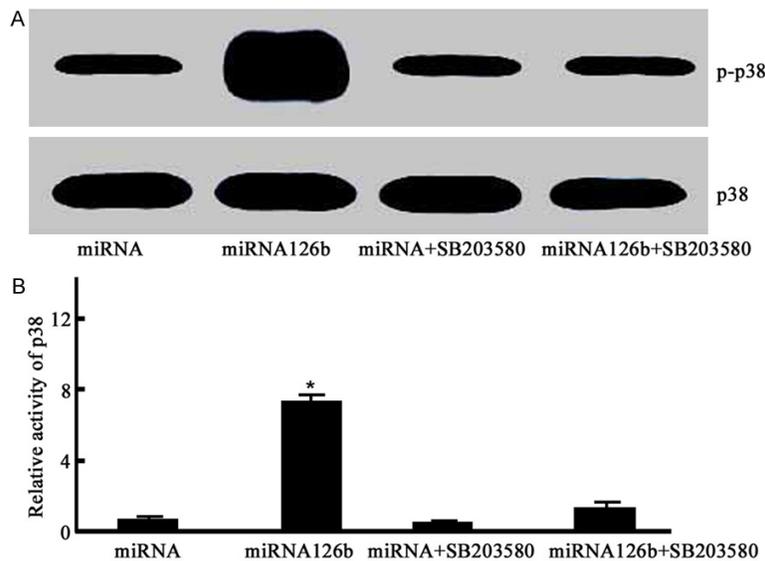
*MiRNA-126b remarkably reduced cell viability and growth of Tca8113-P60*

As showed in **Figure 1**, compared with sham group, Tca8113-P60 cell viability in miRNA-

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**Figure 3.** MiRNA-126b transfection increased Caspase-3 activity in Tca8113-p60 cells. \*P<0.05 versus sham group.



**Figure 4.** miRNA-126b increased activity of p38. A. Western Blot for p-p38 and p38 in four groups. B. Relative activity of p38 in four groups. \*P<0.05 versus SB203580+ miRNA-126b transfection group.

126b transfection group was significantly decreased ( $P=0.0051$ ), suggesting miRNA-126b remarkably reduced cell viability and growth of Tca8113-P60. No difference was detected between sham group and control miRNA group (Figure 1).

### *MiRNA-126b enhanced apoptosis of human tongue carcinoma cell line Tca8113-p60*

As showed in Figures 2, 3, compared with sham group or control miRNA group, miRNA-126b transfection significantly increased the expres-

sion of phosphatidylserine and Caspase-3 activity in Tca8113-p60 cells ( $P < 0.05$ ), suggesting miRNA-126b induced apoptosis of Tca8113-p60 cells.

### *miRNA-126b enhanced p38-dependent apoptosis of Tca8113-p60 cells*

Compared with control microRNA transfection group, miRNA-126b transfection group had a significant increase in the expression of phosphorylated p38 (p-p38, Figure 4A, 4B), which was verified by Western Blot, while inhibiting p38 signaling pathway abrogated activating effect of miRNA-126b on p-p38. No differences of the total expression of p38 were observed among four groups (Figure 4A). Moreover, inhibitor of p38 signaling pathway (SB203580) significantly decreased apoptosis-promoting effect of miRNA-126b on Tca8113-p60 cells (Figure 5), suggesting miRNA-126b enhanced apoptosis via activating p38 signaling pathway.

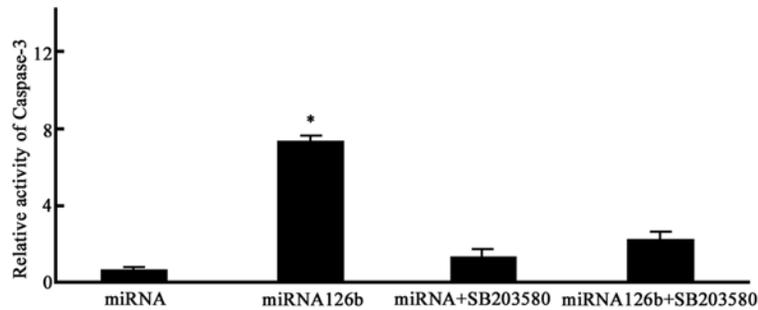
## Discussion

Previous studies proved that microRNA regulated multiple cellular events of cancer, including cell growth, cell cycle, cell apoptosis [9, 10]. Our

study demonstrated that miRNA-126b not only inhibited cell growth of Tca8113-p60, but also enhanced apoptosis via p38 signaling pathways, suggesting miRNA-126b could attenuate lesion of human tongue carcinoma.

Several studies showed the involvement of miRNA-126 in the pathogenesis of cancer either as a tumor suppressor or an oncogene depending on the specific type of cancer [18] as demonstrated aberrant expression of miRNA-126 in several cancers, such as colorectal cancer [19], gastric cancer [20], lung cancer

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**Figure 5.** Relative activity of Caspase-3 in four groups. \* $P < 0.05$  versus SB203580+ miRNA-126b transfection group.

cell lines [21], prostate cancer [22], breast cancer [23]. miRNA-126-mediated-inhibition of cancer progression might be through negative control of proliferation, migration, invasion and cell survival. While miRNA-126 may also support cancer progression through the promotion of blood vessel formation and inflammation at the site of activation [18]. Consistent with the role of miRNA-126 in the regulation of cell proliferation, in this study, we showed 1) miRNA-126b transfection significantly inhibited cell growth, promoted apoptosis and activated p38 signaling pathways; 2) SB203580, an inhibitor of p38 signaling pathways, attenuated apoptosis-promoting effect of miRNA-126b on Tca-8113-p60 cells. Based on these findings, we believed that miRNA-126b was a potential therapeutic target for the treatment of human tongue carcinoma.

Many apoptosis pathways have been elucidated in the field of tumor, including ERK, p38 and JNK [16, 17]. Given the previous report about relationship between p38 and tongue cancer [16], we chose p38 signaling pathway as a potential mechanism to investigate the effect of miRNA-126b on human tongue cancer. Furthermore, miRNA-126b indeed activated p38 signaling pathways, further supported by the inhibition experiment of p38. More specifically, when SB203580 inhibited p38 signaling pathways, apoptosis-promoting effect of miRNA-126b was abrogated, suggesting apoptosis-promoting effect of miRNA-126b was p38-dependent.

Besides p38 signaling pathway, several other targets of miRNA-126b have been identified in cancer or cancer cell lines, such as VEGF [21], IRS-1 [23], IGFBP2, PITPNC1, and MERTK [24],

whether other targets involve in the miRNA-126b-mediated apoptosis of tongue carcinoma cell lines remains unclear and requires further investigation. Moreover, some other limitations of our study warrant future exploration: 1) The exact molecular mechanism by how miRNA-126b activates p38 signaling pathways. 2) Downstream signal molecules of miRNA-126b remain unclear. 3) No clinical studies on

samples from patients with tongue carcinoma and also lack of quantitative analysis on relationship between p38 signaling pathways and the expression miRNA-126b.

In conclusion, miRNA-126b promoted apoptosis of human tongue carcinoma via activating p38 signaling pathways, suggesting miRNA-126b might be a novel therapeutic target in the treatment of human tongue carcinoma.

### Disclosure of conflict of interest

None.

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