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# MiR-503-5p functions as an oncogene in oral squamous cell carcinoma by targeting Smad7

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**Summary.** Background. Oral squamous cell carcinoma (OSCC) is a common oral malignancy. Previous studies indicated that the level of miR-503-5p was upregulated in OSCC tissues. However, the mechanism by which miR-503-5p regulates the proliferation and invasion of OSCC cells remains unclear. Therefore, this study aimed to investigate the role of miR-503-5p during the progression of OSCC. Methods. The level of miR-503-5p in Tca8113 cells was detected using RT-qPCR assay. In addition, CCK-8, transwell assays and flow cytometry assays were conducted to detect cell viability, migration, invasion and apoptosis, respectively. Meanwhile, the dual luciferase reporter assay was applied to explore the interaction between miR-503-5p and Smad7 in Tca8113 cells. Results. Overexpression of miR-503-5p significantly promoted the proliferation, migration and invasion of Tca8113 cells, while downregulation of miR-503-5p markedly inhibited proliferation, migration and invasion of cells. In addition, knockdown of miR-503-5p obviously induced the apoptosis of Tca8113 cells via increasing the levels of Bax and cleaved caspase 3, and decreased the expression of Bcl-2. Moreover, SMAD family member 7 (Smad7) was identified as a direct binding target of miR-503-5p in Tca8113 cells. Overexpression of miR-503-5p significantly downregulated the levels of Smad7 and E-cadherin, but upregulated the levels of N-cadherin and MMP-9 in Tca8113 cells. Conclusion. These results indicated that miR-503-5p might act as an oncogene in OSCC cells by targeting Smad7. Therefore, miR-503-5p might act as a novel and potential therapeutic target for the treatment of OSCC.

**Key words:** Oral squamous cell carcinoma, miR-503-5p, Oncogene, Smad7, Apoptosis

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## Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common human cancers with a propensity for local spread and distant metastasis (Parkin et al., 2005; Murphy et al., 2016). In addition, OSCC is a major concern with an incidence of nearly 300,000 new cases worldwide annually (Sajan et al., 2019). Previous studies indicated that betel quid, alcohol, smoking, and HPV infection are the vital risk factors for OSCC (Hashibe et al., 2009; Wen et al., 2010). Moreover, OSCC is characterized by a propensity for lymph node metastasis (Zhang et al., 2019). A high proportion of patients with OSCC are diagnosed at the advanced stages, with positive distant metastasis and lymph node metastasis (Chen et al., 2019a). The treatment options for patients with OSCC include standard surgery, chemotherapy and radiotherapy (Li et al., 2019c). However, these therapeutic methods generally lead to poor prognosis of patients with OSCC, and accompany unfavorable clinical outcome (Cervino et al., 2019; Li et al., 2019c). Therefore, it is urgent to identify novel biomarkers and explore the underlying mechanisms of metastasis in

order to promote the development of new therapies for patients with OSCC.

MicroRNAs (miRNAs) are a kind of short noncoding RNAs, which act as key regulators in multiple pathological processes (Liu et al., 2019; Xian et al., 2019). MiRNAs could directly bind to 3' untranslated region (UTR) of mRNAs to regulate gene expression (Cai et al., 2009). Additionally, miRNAs exhibit vital roles in tumorigenesis via functioning as oncogenes or tumor suppressor genes (Chen et al., 2019a). Accumulating evidence has demonstrated that miRNAs play important roles in the initiation and progression of OSCC (Dumache, 2017; Xu et al., 2019). For instance, overexpression of miR-1254 markedly suppressed the proliferation and invasion of OSCC cells (Chen et al., 2019a). Moreover, downregulation of miR-543 could induce the apoptosis of OSCC cells by targeting CYP3A5 (Wang et al., 2019a).

Chen et al indicated that miR-503 plays an oncogenic role in the progression of retinoblastoma (Cheng and Liu, 2019). Meanwhile, Li et al indicated that miR-503 acts as a tumor suppressor in gastric cancer, which could inhibit cell proliferation and invasion (Li et al., 2019b). Zheng et al indicated that the expression of miR-503-5p was significantly upregulated in OSCC tissues (Zheng et al., 2018). However, the functional role and molecular mechanisms of miR-503-5p in OSCC cells remain unclear. Therefore, this study aimed to investigate the function of miR-503-5p during the tumorigenesis of OSCC.

## Material and methods

### *Cell culture and cell transfection*

Human tongue squamous cell carcinoma cell line Tca8113 was obtained from Conservation Genetics CAS Kunming Cell Bank (Yunnan, China). Human tongue squamous cell carcinoma cell line CAL 27 was purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Human immortalized oral epithelial cell line HIOEC was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific), antibiotics (100 U/ml penicillin and 100 $\mu$g/ml of streptomycin) in a humidified incubator containing 5% $CO_2$ at 37°C.

The control mimics (NC), miR-503-5p mimics, and miR-503-5p inhibitor were synthesized and obtained from GenePharma (Shanghai, China). When Tca8113 or CAL 27 cells reached 80% confluence, cells were transfected with control mimics (NC), miR-503-5p mimics, or miR-503-5p inhibitor with Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocol.

### *Real-time reverse transcriptase quantitative PCR (RT-qPCR)*

Total RNA was extracted from Tca8113 and CAL 27 cells using TRIzol reagent (Thermo Fisher Scientific) respectively. Reverse transcription for miR-503-5p was performed by using the PrimeScript RT Master Mix (TaKaRa, Otsu, Shiga, Japan). The RT-qPCR was performed by the SYBR® Premix Ex Taq™ II kit (TaKaRa Bio, Otsu, Shiga, Japan) on a 7900HT system (Applied Biosystems, CA, USA) according to the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of application at 95°C for 30 sec, 60°C for 30 sec. The primers used were as follows: U6: Forward, 5'-CGCTTCGGCAGCACATATAC-3'; Reverse, 5'-AAATATGGAACGCTTCACGA-3'. MiR-503-5p: Loop primer: 5'-GTCGTATCCAGTGCAG GGTCCGAGGTATTCGCACTGGATACGACCTGCA GAA-3'; Forward: 5'-TGCGCTAGCAGCGGGA ACAGTTC-3'; Reverse: 5'-CCAGTGCAGGGT CCGAGGTATT-3'. The relative level of miR-503-6p normalized to U6 using the $2^{-\Delta\Delta Ct}$ method.

### *Cell counting Kit-8 (CCK-8) assay*

Cell counting kit-8 (Dojindo, Kumamoto, Japan) was applied to determine the cell viability according to the manufacturer's protocol. Tca8113 and CAL 27 cells were plated onto a 96-well plate respectively at the density of $5.0\times10^3$ cells/well and incubated at 37°C overnight. After that, cells were transfected with NC, miR-503-5p mimics, or miR-503-5p inhibitor for 72 h. Later on, 10 µL CCK-8 reagent was added into each well, and the plate was then incubated for 2 h at 37°C. The absorbance was detected at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

### *Immunofluorescence staining*

Tca8113 and CAL 27 cells were plated onto a 96-well plate respectively at the density of $5.0\times10^3$ cells/well and incubated at 37°C overnight. After that, cells were transfected with NC, miR-503-5p mimics, or miR-503-5p inhibitor for 72 h. Later on, cells fixed by 4% paraformaldehyde were incubated with rabbit monoclonal antibody anti-Ki67 (1:100, Abcam Cambridge, MA, USA) overnight at 4°C. Then, cells were incubated with anti-rabbit IgG secondary antibody (1:1000, Abcam) for 1 h at room temperature. After staining with DAPI for 5 min, cells were observed with a fluorescence microscope (Olympus BX53 Tokyo, Japan).

### *Transwell assays*

The migration and invasion abilities of Tca8113 cells were detected by transwell chamber assay. The transfected cells ($3\times10^4$ cells/well) in serum-free DMEM medium (100 $\mu$L) were plated onto the upper chamber in

24-well plates. In parallel, the lower chamber of each well was incubated with 600 μL of DMEM containing 10% FBS.

After 24 h of incubation at 37°C, the non-migrated and non-invaded cells on the upper surface were removed. Then, the migrated and the invaded cells on the lower surface were fixed with 4% paraformaldehyde for 15 min, followed by staining with 0.5% crystal violet for 30 min. Five fields of migrating and invading cells in each well were observed under a microscope (Olympus CX23 Tokyo, Japan). For cell invasion, the transwell inserts (Corning, New York, NY, USA) were pre-coated with 20 μg/μL of Matrigel (Sigma Aldrich, St. Louis, MO, USA).

### *Flow cytometry assay*

Cell apoptosis kit with Annexin V FITC and PI (Sigma-Aldrich, St. Louis, MO, USA) was used to examine the apoptosis rate according to the manufacturer's protocol. The cells were collected and then resuspended in 100 $\mu$L of binding buffer. After that, cells were stained with 5 $\mu$l annexin V-FITC and propidium iodide (PI) in the dark at 37°C for 30 min. Later on, flow cytometry (FACScan™; BD Biosciences, Franklin Lake, NJ, USA) was applied to analyze the apoptosis rate using CellQuest™ software version 5.1 (BD).

### *Western blot assay*

Tca8113 cells were lysed in RIPA lysis buffer (KeyGEN, Nanjing, China), and BCA Assay kit (Solar life science, Beijing, China) was used to detect protein concentration. Equal amounts of protein (30 μg) were separated by 10% SDS-PAGE, and proteins were then shifted onto polyvinylidene difluoride membrane (PVDF, Thermo Fisher Scientific). 5% nonfat dried milk in TBST was used to block the PVDF membrane at room temperature for 1 h. Later on, PVDF membrane was incubated at 4°C overnight) with the following primary antibodies: anti-Bax (1:1000, Abcam Cambridge, MA, USA), anti-cleaved caspase 3 (1:1000, Abcam), anti-Bcl-2 (1:1000, Abcam), anti-Smad7 (1:1000, Abcam), anti-E-cadherin (1:1000, Abcam), anti-N-cadherin (1:1000, Abcam), anti-MMP9 (1:1000, Abcam) and anti-β-actin (1:1000, Abcam). Then, the membrane was incubated with HRP-labeled goat anti-rabbit secondary antibody (1:5000, Abcam) for 1h at room temperature. Enhanced chemi-luminescence (ECL) reagent (Thermo Fisher Scientific) was used to visualize the protein bands according to the manufacturer's protocol. Image J Software was used to quantify the intensity of the bands. β-actin was used as an internal control.

### *Dual-luciferase reporter assay*

Online bioinformatics tool TargetScan was applied to find target genes of miR-503-5p. Fragments of Smad7 3'-UTR containing wild-type (WT) miR-503-5p binding sequences and 3'-UTR containing mutant (MT) miR-503-5p binding sequences were synthesized by GenePharma and inserted into the Luciferase reporter gene to establish two reporter plasmids: Smad7-3'-UTR-WT and Smad7-3'-UTR-MT plasmids, respectively. The cells were co-transfected with miR-503-5p mimics, and Smad7-3'-UTR-WT or Smad7-3'-UTR-MT plasmids using Lipofectamine 2000. Forty-eight hours later, the luciferase activity was measured using the Dual-Luciferase Reporter Assay Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. Renilla luciferase activity was used as endogenous control.

### *Statistical analysis*

All experiments were expressed as mean ± standard error (SD). CCK-8 assay was performed in quintuplicate. Cell transfection, RT-qPCR, immunofluorescence staining, flow cytometry, western blot, transwell migration and invasion assays and Dual-luciferase reporter assay were repeated triply. Graphs were generated using GraphPad Prism software (version 7.0, La Jolla, CA, USA). One-way analysis of variance (ANOVA) and Tukey's tests were carried out for multiple group comparisons. P value $<0.05$ was considered as statistically significant.

## Results

### *Overexpression of miR-503-5p promoted the proliferation of OSCC cells*

Knowing that the level of miR-503-5p was upregulated in OSCC tissues (Zheng et al., 2018). We then investigated the role of miR-503-5p on OSCC cells. As shown in Fig. 1A, the level of miR-503-5p in Tca8113 cells was significantly upregulated compared with the human immortalized oral epithelial cell line (HIOEC cell). In addition, miR-503-5p mimics significantly upregulated the level of miR-503-5p in Tca8113 cells, whereas miR-503-5p inhibitor markedly downregulated the level of miR-503-5p in cells (Fig. 1B). CCK-8 assay results indicated that upregulation of miR-503-5p obviously promoted the viability of Tca8113 and CAL 27 cells, while downregulation of miR-503-5p notably inhibited the cell viability (Fig. 1C,D). Moreover, Ki67 immunofluorescence assay indicated that the Ki67 positive cell rate in Tca8113 cells transfected with NC, miR-503-5p mimics or miR-503-5p inhibitor were approximately 43, 75 and 17%. This data indicates that overexpression of miR-503-5p significantly promoted the proliferation of Tca8113 cells, whereas downregulation of miR-503-5p markedly suppressed cell proliferation (Fig. 1E,F). Furthermore, overexpression of miR-503-5p notably promoted the proliferation of CAL 27 cells, while knockdown of miR-503-5p exhibited the opposite effect (Fig. 1G,H). These

data suggested that overexpression of miR-503-5p could promote the proliferation of OSCC cells.

### *Overexpression of miR-503-5p increased the migration and invasion of OSCC cells*

To investigate the role of miR-503-5p during the migration and invasion of OSCC cells, transwell assays were applied. As shown in Fig. 2A-D, overexpression of miR-503-5p significantly increased the migration ability of Tca8113 and CAL 27 cells. In contrast, downregulation of miR-503-5p obviously inhibited the migration ability of Tca8113 and CAL 27 cells (Fig. 2A-D). Meanwhile, overexpression of miR-503-5p notably increased the invasion ability of Tca8113 cells, while downregulation of miR-503-5p markedly reduced the

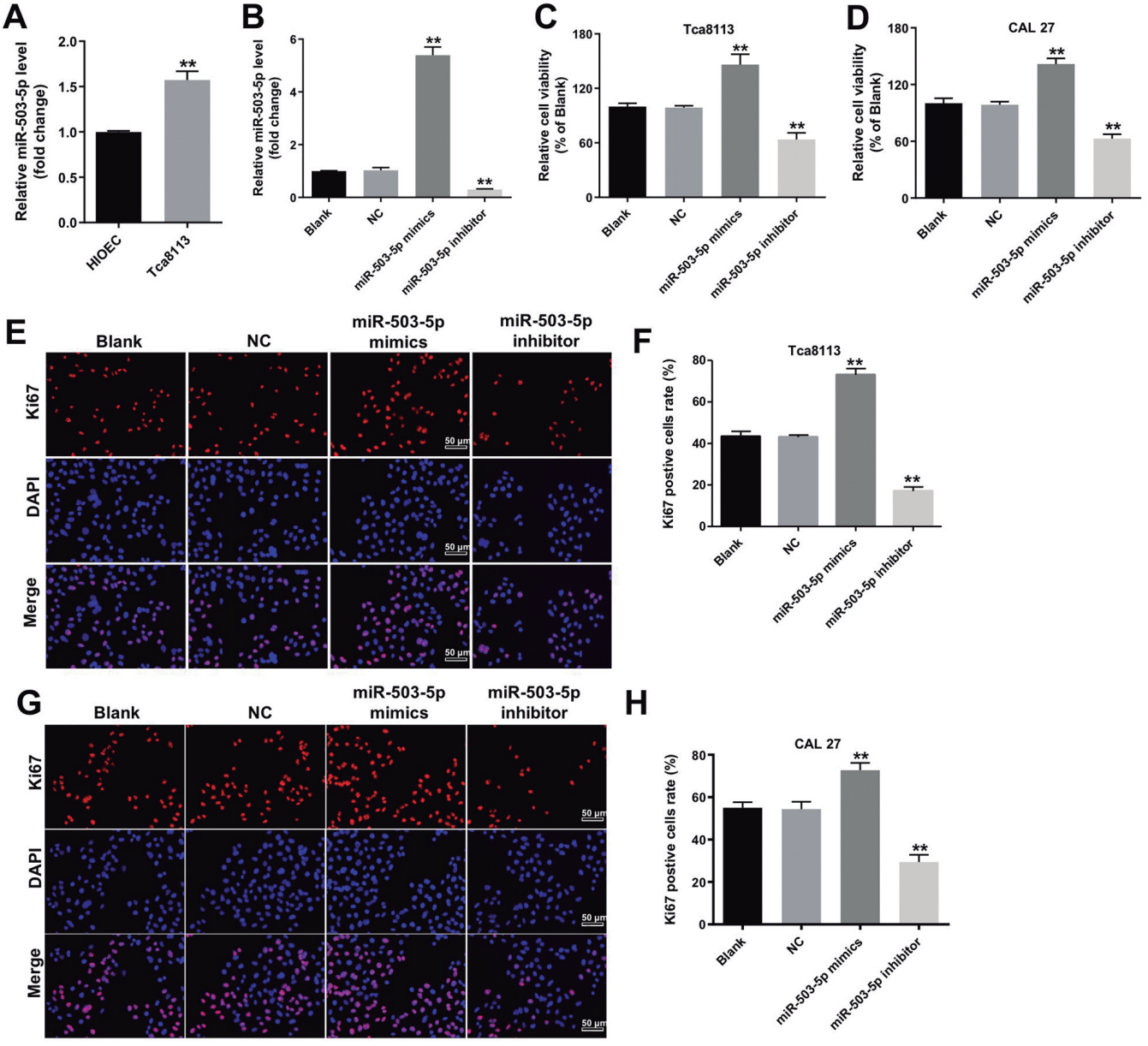


**Fig. 1.** Overexpression of miR-503-5p promoted the proliferation of OSCC cells. **A.** The levels of miR-503-5p in HIOEC and Tca8113 cells were investigated via RT-qPCR, and the experiment was repeated triply. **B.** Tca8113 cells were transfected with NC, miR-503-5p mimics or miR-503-5p inhibitor for 72 h. RT-qPCR was used to detect the level of miR-503-5p in Tca8113 cells, and the experiment was repeated triply. **C.** Tca8113 cells or CAL 27 cells **(D)** were transfected with NC, miR-503-5p mimics or miR-503-5p inhibitor for 72 h. Cell viability was determined using CCK-8 assay, the experiment was performed in quintuplicate. **E, F.** Quantification of Ki67 level by immunofluorescence assay in Tca8113 cells. The experiment was repeated triply. **G, H.** Quantification of Ki67 level by immunofluorescence assay in CAL 27 cells. The experiment was repeated triply. **P<0.01, compared with the NC group.

invasion ability of cells (Fig. 2E,F). All these data illustrate that overexpression of miR-503-5p could promote the migration and invasion of OSCC cells, while downregulation of miR-503-5p reduced the migration of cells.

### *Downregulation of miR-503-5p induced the apoptosis of OSCC cells*

Next, to explore the effect of miR-503-5p on the apoptosis of Tca8113 cells, flow cytometry assay was

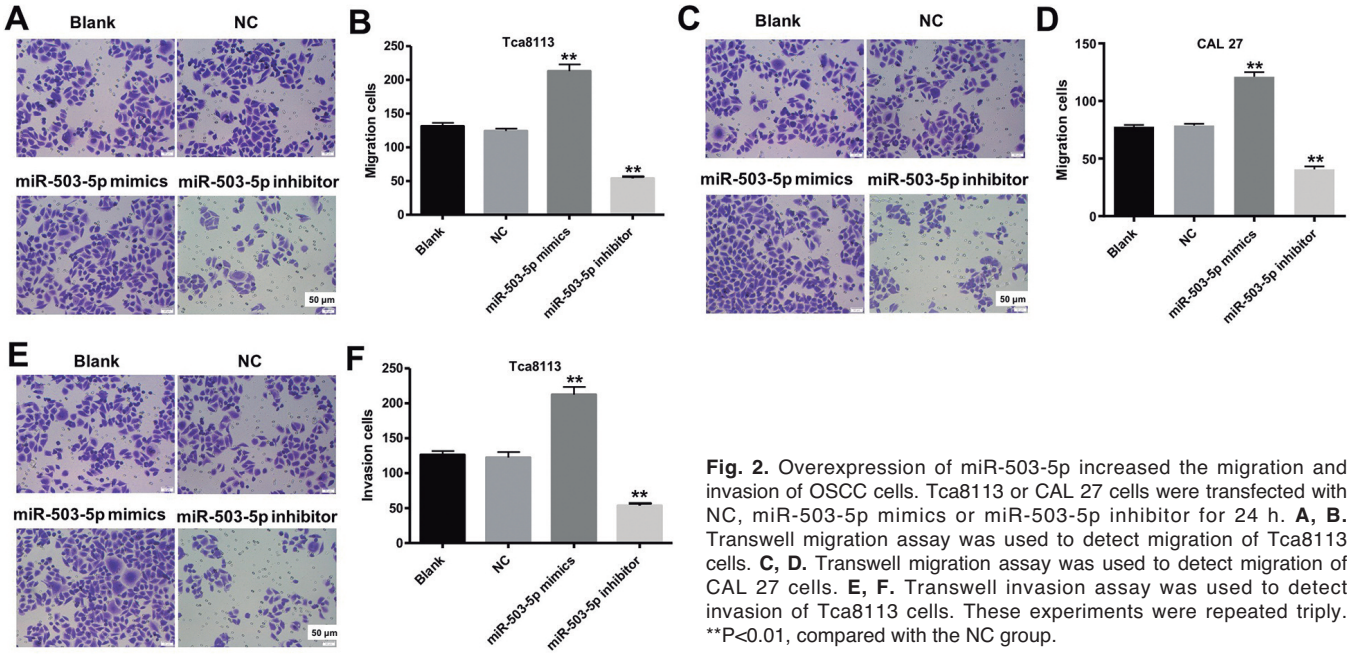


**Fig. 2.** Overexpression of miR-503-5p increased the migration and invasion of OSCC cells. Tca8113 or CAL 27 cells were transfected with NC, miR-503-5p mimics or miR-503-5p inhibitor for 24 h. **A, B.** Transwell migration assay was used to detect migration of Tca8113 cells. **C, D.** Transwell migration assay was used to detect migration of CAL 27 cells. **E, F.** Transwell invasion assay was used to detect invasion of Tca8113 cells. These experiments were repeated triply. **P<0.01, compared with the NC group.

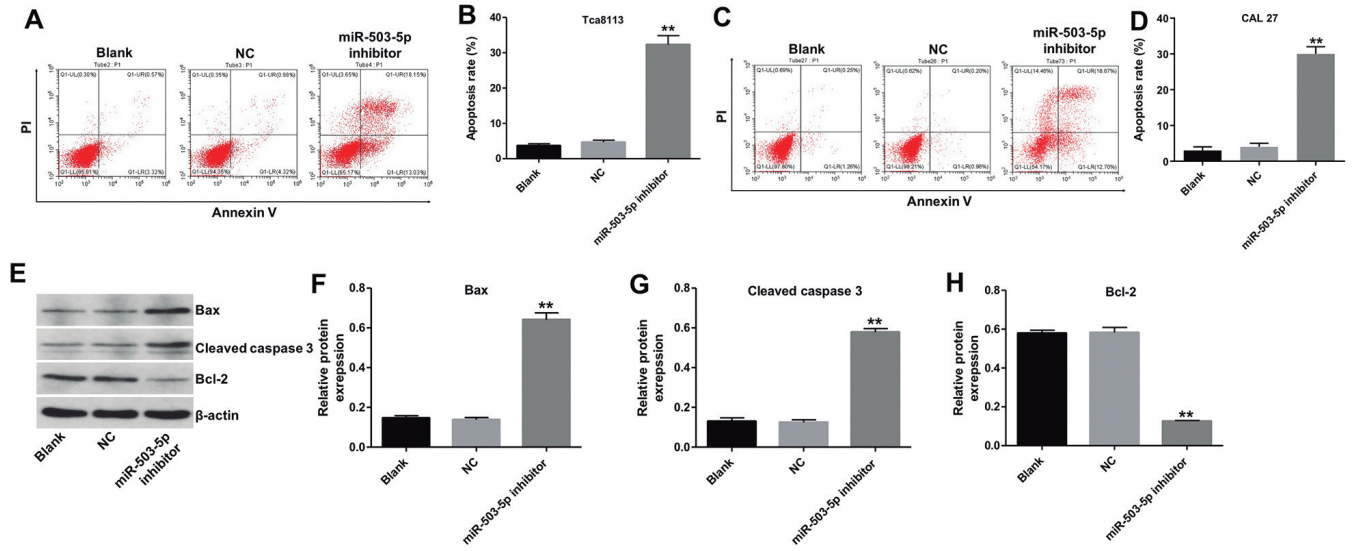


**Fig. 3.** Downregulation of miR-503-5p induced the apoptosis of OSCC cells. **A, B.** Tca8113 cells were transfected with NC, or miR-503-5p inhibitor for 72 h. Annexin V/PI double staining was applied to measure the apoptotic cells. **C, D.** CAL 27 cells were transfected with NC, miR-503-5p inhibitor for 72 h. Annexin V/PI double staining was applied to measure the apoptotic cells. **E.** Tca8113 cells were transfected with NC, or miR-503-5p inhibitor for 72 h. Expression levels of Bax, cleaved caspase 3 and Bcl-2 in Tca8113 cells were detected using western blotting. **F-H.** The relative expressions of Bax, cleaved caspase 3 and Bcl-2 in Tca8113 cells were quantified via normalization to β-actin. These experiments were repeated triply. **P<0.01, compared with the NC group.

applied. As indicated in Fig. 3A-D, downregulation of miR-503-5p markedly induced the apoptosis of Tca8113 and CAL 27 cells. In addition, the expressions of pro-apoptotic proteins Bax and cleaved caspase 3 were upregulated, whereas the expression of anti-apoptotic protein Bcl-2 was downregulated in Tca8113 cells following transfection with miR-503-5p inhibitor (Fig. 3E-H). These results suggest that downregulation of miR-503-5p could induce the apoptosis of OSCC cells.

### *Smad7 was a direct binding target of miR-503-5p*

Online bioinformatics tool TargetScan was applied to explore target genes of miR-503-5p. More than 5 potential binding targets of miR-503-5p were identified. The data illustrated that Smad7, proline rich 11 (PRR11), suppressor of cancer cell invasion (SCAI), and E2F transcription factor 3 (E2F3) might be the potential direct targets of miR-503-5p. In addition, these proteins have been implicated in the tumorigenesis of human cancers (Zhang et al., 2018; Shi et al., 2019a; Tong et al., 2020; Zhao et al., 2019). However, dual luciferase reporter assay revealed that Smad7 was closely associated with miR-503-5p mimics, and the data indicated that miR-503-5p mimics markedly reduced the luciferase activity of psiCHECK-2-Samd7-WT, but did not affect the luciferase activity of psiCHECK-2-Smad7-MT (Fig. 4A,B). These data indicate that Smad7 was a

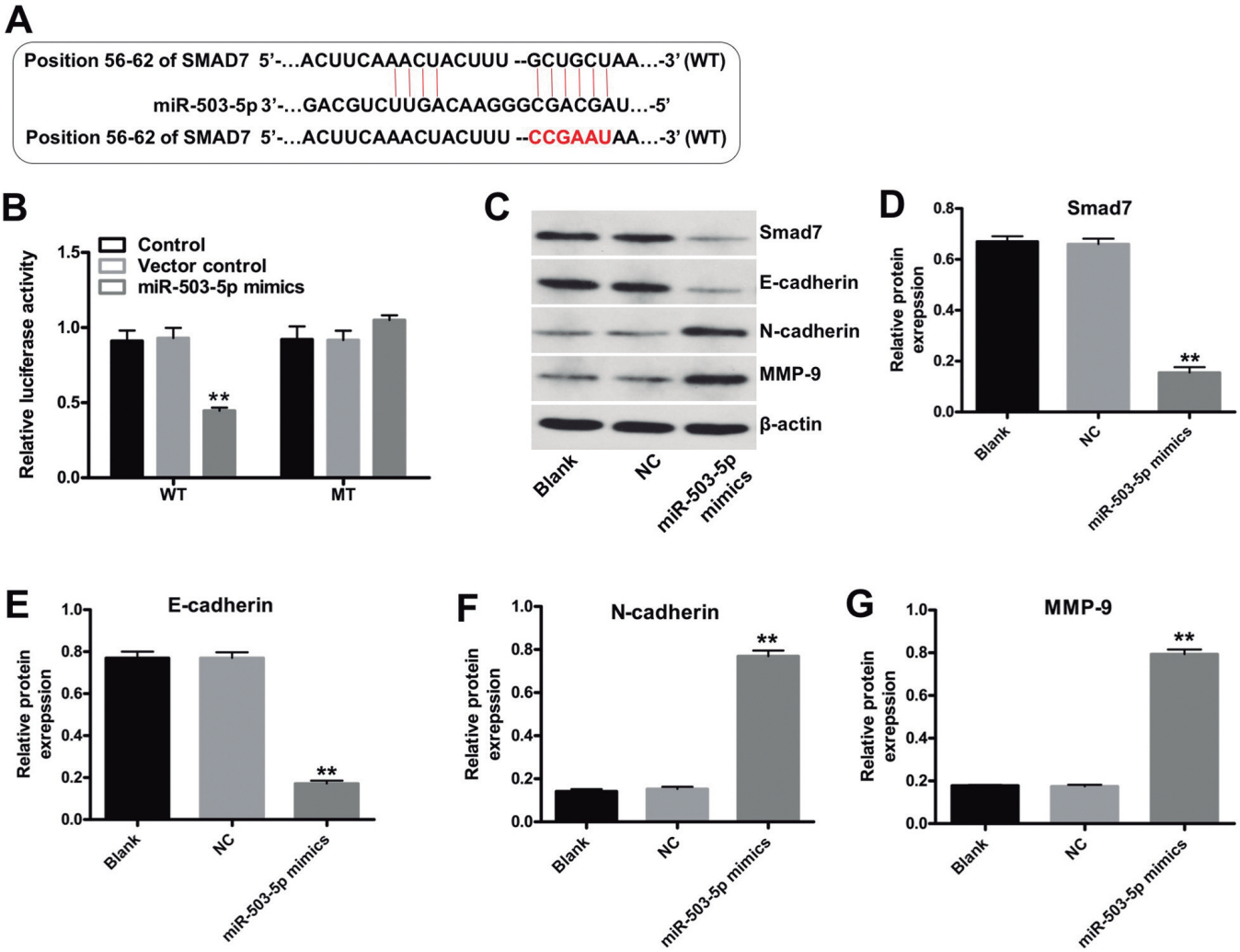


**Fig. 4.** MiR-503-5p functions as an oncogene via targeting Smad7. **A.** Sequence alignment of miR-503-5p with the putative binding sites within the WT or MT regions of Smad7. **B.** The luciferase activity in Tca8113 cells following co-transfecting with Smad7-WT/MT 3'-UTR plasmid and miR-503-5p mimics were detected using dual luciferase reporter assay. **C.** Tca8113 cells were transfected with NC, or miR-503-5p mimics for 72 h. Expression levels of Smad7, E-cadherin, N-cadherin and MMP-9 in Tca8113 cells were detected using western blotting. **D-G.** The relative expressions of Smad7, E-cadherin, N-cadherin and MMP-9 in Tca8113 cells were quantified via normalization to β-actin. These experiments were repeated triply. **P<0.01, compared with the NC group.

direct binding target of miR-503-5p.

### *MiR-503-5p functions as an oncogene via inhibiting Smad7*

It has been shown that Smad7 is an important regulator of cellular processes including invasion and apoptosis (Fu et al., 2019). Thus, western blot assay was applied to investigate whether miR-503-5p mimics would affect the levels of epithelial-to-mesenchymal transition (EMT) associated proteins in Tca8113 cells. As shown in Fig. 4D-G, upregulation of miR-503-5p markedly reduced the levels of Smad7 and E-cadherin, but increased the levels of N-cadherin and MMP-9 in Tca8113 cells. In contrast, downregulation of miR-503-5p significantly increased the levels of Smad7 and E-cadherin in Tca8113 cells (Fig. 5A-C). Meanwhile, knockdown of miR-503-5p slightly decreased the expression of N-cadherin in Tca8113 cells (Fig. 5A,D). These data indicate that miR-503-5p functions as an oncogene in Tca8113 cells via inhibiting Smad7 and promoting EMT.

## Discussion

Several miRNAs were found to be upregulated or downregulated in OSCC, and dysregulated miRNAs have been implicated in the tumorigenesis of OSCC (Shi et al., 2019b; Xu et al., 2019). Previous studies indicated that the level of miR-503-5p was upregulated in OSCC tissues (Zheng et al., 2018). Wei et al reported that overexpression of miR-503 could inhibit migration and invasion, and induce apoptosis in non-small-cell lung cancer cells (Wei et al., 2019b). In addition, Cheng et al studied whether downregulation of miR-503 could impede the proliferation and invasion of retinoblastoma (Cheng and Liu, 2019). However, the mechanism by which miR-503-5p regulates apoptosis and invasion of OSCC cells remains unclear. In this study, we found that miR-503-5p functions as an oncogenic miRNA in OSCC cells, and downregulation of miR-503-5p could inhibit the proliferation, migration and invasion of OSCC cells.

Previous studies indicated miR-503-5p as a tumor suppressor miRNA in hepatocellular carcinoma and lung cancer (Sun et al., 2017; Jiang and Li, 2019). In contrast, our data indicated that overexpression of miR-503-5p promoted the proliferation of Tca8113 and CAL 27 cells, while downregulation of miR-503-5p inhibited cell proliferation. It is possible that the difference might exist in the area of miR-503-5p expressing profiles in different diseases. In addition, downregulation of miR-503-5p suppressed the migration and invasion abilities of OSCC cells, and induced cell apoptosis. Taking the above data together, our data suggested that miR-503-5p may function as an oncogenic miRNA in OSCC cells, and indicate that downregulation of miR-503-5p

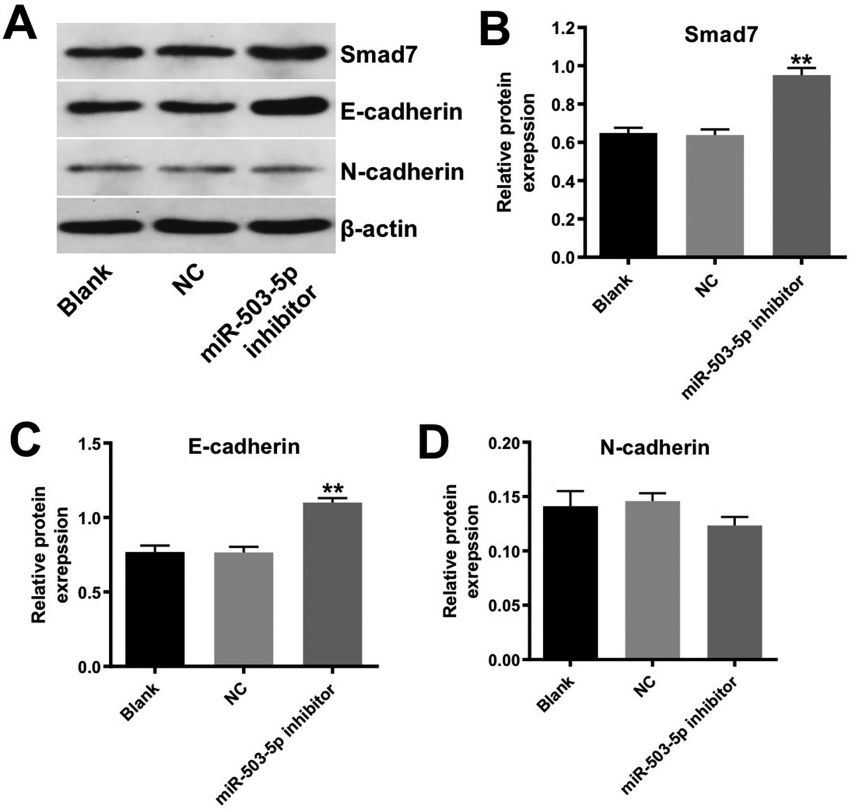


**Fig. 5.** Downregulation of miR-503-5p inhibited the growth of Tca8113 cells via upregulation of Smad7. Tca8113 cells were transfected with NC, or miR-503-5p inhibitor for 72 h. **A.** Expression levels of Smad7, E-cadherin, and N-cadherin in Tca8113 cells were detected using western blotting. **B-D.** The relative expressions of Smad7, E-cadherin, and N-cadherin in cells were quantified via normalization to β-actin. These experiments were repeated triply. **P<0.01, compared with the NC group.

inhibited the growth of OSCC cells.

MiRNAs were known to play an important role in tumorigenesis via repression target genes expression (Wang et al., 2019c). For instance, miR-125b markedly inhibited the proliferation of OSCC cells by targeting PRXL2A (Chen et al., 2019b). MiR-199a-5p significantly suppressed proliferation, but induced apoptosis of Tca8113 cells through targeting nuclear factor-κB (NF-κB) kinase β (IKKβ) (Wei et al., 2019a). Meanwhile, miR-182-5p markedly promoted the proliferation of OSCC cells via inhibiting CAMK2N1 (Li et al., 2018). In the present study, the data stored in online bioinformatics tool TargetScan indicated that Smad7 might be a potential binding target of miR-503-5p. In addition, luciferase reporter assay confirmed that Smad7 was a binding target of miR-503-5p in Tca8113 cells. Moreover, overexpression of miR-503-5p markedly downregulated the level of Smad7 in Tca8113 cells. Hu et al indicated that overexpression of miR-497 increased the invasion ability of OSCC cells through inhibition the level of Smad7 (Hu et al., 2016). Shuang et al found that knockdown of miR-503 could inhibit the proliferation and invasion in laryngeal squamous cell carcinoma cells via targeting programmed cell death protein 4 (Shuang et al., 2017). Meanwhile, miR-503 could induce mesenchymal stem cell differentiation by targeting Smad7 (Gu et al., 2018). These data illustrated that overexpression of miR-503-5p promoted the proliferation and invasion of Tca8113 cells via inhibiting Smad7.

Smad7 is a negative regulator of the transforming growth factor-β (TGF-β) signaling (Wang et al., 2019b). TGF-β signaling pathway is an important pathway of carcinogenesis, and is associated with metastasis in different malignancies (Huo et al., 2019). In addition, TGF-β is a key regulator of EMT, and EMT plays an important role in tumorigenesis via promotion cell proliferation and metastasis (Li et al., 2019a). Ryu et al indicated that upregulation of Smad7 could inhibit the metastasis of breast cancer cells via suppressing EMT (Ryu et al., 2019). During the EMT progress, cells loss of the epithelial marker E-cadherin, and increased the level of mesenchymal marker N-cadherin (Chen et al., 2017). In this study, overexpression of miR-503-5p significantly reduced the levels of Smad7 and E-cadherin, and increased the levels of MMP-9 and N-cadherin in Tca8113 cells. These data indicated that overexpression of miR-503-5p promoted the proliferation and invasion of Tca8113 cells by inhibiting Smad7 via activation of EMT.

### *Conclusion*

This study indicates that miR-503-5p functions as a tumor suppressor that inhibits the proliferation and invasion of OSCC cells, and suggests that miR-503-5p might serve as a potential biomarker for diagnose of OSCC. Therefore, miR-503-5p might be a therapeutic target for the treatment of OSCC.

*Conflicts of interest.* The authors declare no competing financial interests.

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