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Original Article

MiR-222 regulates the progression of oral squamous cell carcinoma by targeting CDKN1B

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Abstract: Objective: The purpose of this study was to establish a causal relationship between microRNA (miR-222) and oral squamous cell carcinoma (OSCC). Methods: The cell viability of each treatment group was measured by MTT. The effects of miR-222 on cell metastasis and apoptosis were measured by transwell and flow cytometry. The targeting relationship between miR-222 and CDKN1B was verified by dual-luciferase reporter gene assay and Western blot. Cell derived xenograft was further constructed to verify the effect of miR-222 on tumor growth by observing tumor weight and volume. The proliferation of tumor tissue was determined by hematoxylin-eosin staining and immunohistochemical staining. Results: Compared with those in adjacent tissues and normal cells, the levels of miR-222 in OSCC tissues and cells were significantly increased ($P<0.05$). The miR-222 mimic group promoted tumor cell proliferation, migration and cell cycle and inhibited cell apoptosis significantly ($P<0.05$). The up-regulation of CDKN1B expression inhibited cell viability, migration and invasiveness and promoted the apoptosis of OSCC ($P<0.05$). The dual-luciferase reporter gene assay found that miR-222 was targeted to CDKN1B and could inhibit fluorescence activity ($P<0.05$). *In vivo* assays showed that miR-222 could promote tumor growth through CDKN1B ($P<0.05$). Conclusion: MiR-222 was significantly upregulated in OSCC tissues and cells and regulated tumor progression by targeting CDKN1B.

Keywords: Oral squamous cell carcinoma, miR-222, CDKN1B, tumor progress

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor that occurs in the oral cavity and is dominated by squamous cells. Cancer cells can exist in multiple organs, such as the gums, hard palate, tongue, buccal mucosa and lips. About 50% of the most harmful tumors are head and neck squamous cell carcinoma. According to different tissue sources, they can be divided into cheek cancer and tongue cancer, among which tongue cancer is the most commonly seen in clinical practice. With the development of the lesions, it can cause functional disturbances in eating, chewing, speech, swallowing and breathing, seriously affecting the patient's health and quality of life [1]. In the past few decades, the survival rate of patients with OSCC has not improved significantly, the incidence rate has increased year by year, and the mortality rate of the average annual diagnosed cases is still about 50% [2]. At present, the main treatment methods for OSCC are surgery combined with adjuvant radiotherapy and chemotherapy. However, due to the easy occurrence of cell invasion and metastasis, the 5-year survival rate is low and the prognosis is poor [3, 4]. The reason for the poor prognosis can be attributed to the incomplete understanding of its pathogenesis. Therefore, in-depth exploration of the pathogenesis of OSCC is of great significance to improve the prognosis and treatment effect of patients.

Recently, many researchers have focused on the involvement of abnormally expressed microRNAs (miRNAs) in regulating the occurrence and development of OSCC, and many novel advances have been made [5]. MiRNAs are short-chain RNAs with a length of 19-22 nucleotides, which participate in the regulation of the occurrence and development of various

diseases including tumors, diabetes, obesity and cardiovascular diseases by targeting and regulating the level of mRNA [5]. Studies have shown that miR-144-3p could affect OSCC progression by inhibiting the expression of EZH2 [6]. Another study showed that tumor fibroblasts could deliver miR-382-5p into OSCC cells via exosome delivery to affect their proliferation and migration [7]. The expression of microRNA-222 (miR-222) was significantly enhanced in various tumors, but its role and specific mechanism in OSCC are not yet clear. The miRNA is abnormally expressed in a variety of malignant tumor tissues and plays an important role in regulating the proliferation and invasion of tumor cells [8]. Recent study showed that miR-222 significantly increased the ability of tongue squamous cell carcinoma to proliferate, migrate and invade [9]. Similarly, it has been found that miR-222 is significantly upregulated in oral cancer tissues and is able to promote tumor cell proliferation and migration, but its detailed mechanism remains unclear [10]. Therefore, miR-222 is expected to become an early screening marker and therapeutic target for OSCCs.

Cyclin-dependent kinase inhibitor B (CDKN1B) gene-encoded protein p27 is an inhibitor of cell cycle, which can control the cell cycle in G1 phase by blocking the activation of Cyclin complex progress [10]. Polyak first discovered that p27 is a thermostable protein in 1994, and found in the study that it has functions such as promoting apoptosis and regulating cell differentiation [11]. Initial studies have shown that CDKN1B gene mutations are significantly associated with breast cancer metastasis [13]. Numerous databases have predicted the target genes of miR-222 in renal cell carcinoma, with CDKN1B being the most widely reported [12]. In addition, there are survey data showing that CDKN1B may be involved in the regulation of tumor progression as a gene therapy target of OSCC and a biomarker for early screening [13]. However, the specific molecular mechanism of CDKN1B in regulating the biological behavior of OSCC and the role of its interaction with miR-222 in OSCC remain unclear. Thus, we carried out *in vitro* and *in vivo* experiments using molecular biology to verify miR-222's role in OSCC and its mechanism in order to provide novel evidence to support the treatment.

Materials and methods

Clinical samples and cells

Twenty pairs of OSCC and paracancerous tissue samples were collected from patients diagnosed between May 2015 and July 2018. The design and conduct of this study have been approved and documented by the Clinical Research Ethics Committee of Xiangya Stomatological Hospital, Central South University. All OSCC cell lines were obtained from Procell Company (Wuhan, China). We cultured tumor cells in DMEM medium (Sevier, China) containing 10% fetal bovine serum (FBS), penicillin (100 IU/ml) and streptomycin (100 mg/ml) (Sevier, China), in incubator with 5% CO_2 at 37°C ambient temperature.

Microarray analysis

A mRNA microarray analysis was conducted on tumor tissues and adjacent tissues from 5 patients. Difference analysis was performed using R 3.4.1, with Adj-P<0.05 and log2 (Fold change) >2 as screening condition. The top five differentially expressed mRNAs were selected and displayed in the form of a heat map.

Real-time quantitative PCR (qRT-PCR)

The level of miR-222 was determined using the miScript SYBR Green PCR Kit (Qiagen, USA). Total RNA and miRNA in tissues and cells were extracted using TRIZOL reagent (Invitrogen, USA) and miRcute miRNA kit (Tiangen, China) for subsequent detection. RNA was reverse transcribed to cDNA by PrimeScript® 1st Strand Synthesis Kit (TaKaRa, China). qRT-PCR detection was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA). In this study, U6 and GAPDH were selected as reference genes for miRNA and mRNA expression, respectively. Gene expression was relative quantified using the $2^{-\Delta\Delta Ct}$ method. Primers for all genes were purchased from Shanghai Sangon Biology Co., Ltd. The primer sequences are shown in **Table 1**.

Western blot

A total protein extraction kit (Sevier, China) was used to measure the total protein content of each group of tissues and cells. By using the BCA protein assay kit (Sevier, China), the pro-

Table 1. Primer sequence of the genes

Gene	Primer	Sequence
MiR-222	forward	5'-CGCAGATCTTTTCTTCCACAGAT-3'
	reverse	5'-GGGGATCCTCTCAGGACACTGAAGC-3'
CDKN1B	forward	5'-CTAACTCTGAGGACACGCATT-3'
	reverse	5'-TTCTTCTGTTCTGTTGGCTCTT-3'
U6	forward	CTCGCTTCGGCAGCACA
	reverse	AACGCTTCACGAATTTGCGT
GADPH	forward	5'-GGGTGTGAACCACGAGAAAT-3'
	reverse	5'-ACTGTGGTCATGAGCCCTTC-3'

tein concentrations in the samples were normalized. Tissue and cells from each treatment group were lysed in standard lysis buffer. An equal amount of cell lysate (20 μg) was loaded on an SDS-PAGE gel (Shanghai Beyotime, China), dissolved and transferred onto a nitrocellulose membrane. Membranes were incubated overnight with primary antibodies (anti-CDKN1B, 1:1000 and anti-GAPDH, 1:2000) (Abcam) diluted in wash buffer TBS-T. Subsequently, the membrane was incubated with a horseradish peroxidase (HRP)-lagged secondary antibody (Anti-Mouse IgG H&L, Abcam) for one hour at room temperature. Blots were detected using chemiluminescence reagent (Millipore, USA) and autoradiography.

Transient transfection

For transient transfection, a miR-222 mimic (5'-AGCUACAUUGGCUACUGGGU-3'), a miR-222 inhibitor (5'-ACCCAGUAGCCAGAUGUAGCU-3') and its negative control (5'-UUUGUACUACACAAAAGUACUG-3') (Genepharm, China) were transfected into tumor cells by Lip3000 Transfection Regent (Invitrogen, USA).

Dual-luciferase reporter gene detection

The interaction between miR-222 and CDKN1B 3'-UTR was verified by dual-luciferase gene verification reporter experiment. The wild-type full-length 3'-UTR and mutant 3'-UTR of CDKN1B mRNA were amplified and ligated to pMIR-GLO Vectors (Ambion, USA) and named as wild-type WT-CDKN1B pMIR-GLO vector group and mutant MUT-CDKN1B pMIR-GLO vector group. Cells were co-transfected with 200 nmol/L miR-222 mimic and 100 ng plasmid, and cultured in 24-well plates for 48 h. Cell lysates were collected, and Renilla and firefly luciferase fluorescence intensities were analyzed in 3 replicates using the Dual-Luciferase reporter assay kit (Abcam, USA) according to the protocol.

Cell viability assays

Cell viability was detected using MTT kit (Shanghai Beyotime, China). First, we dissolved 25 mg of MTT with 5 ml of MTT solvent to prepare a 5 mg/ml MTT solution. It was used immediately after preparation, or stored directly at -20°C away from light. The cells of each treatment group were seeded in a 96-well plate, and 100 μL of 2,000 cells were added to each well. After the relevant treatment, 10 μl of MTT solution was added to each well, and the cells were incubated for 4 hours in a cell culture incubator. Then, 100 microliters of Formazan lysis solution was added to each well, mixed properly, and continued to incubate in the cell incubator until the formazan was completely dissolved (observed under an ordinary optical microscope). Finally, absorbance was measured at 570 nm.

Wound healing assay

Cells were seeded into 6-well dishes and incubated for 48 hours. A sterile pipette tip was used to create a scratch on the cell monolayer. After washing with PBS, the cells of all treatment groups were incubated in DMEM containing 10% FBS for 48 hours, and the healing was observed under an inverted microscope and calculated by subtracting the width of healed scratch from the corresponding width of the original scratch.

Cell cycle detection

The cell cycle of each treatment group was detected by Cell Cycle Analysis Kit (Shanghai Beyotime, China). First, trypsinized cells were washed twice with PBS and then fixed with 70% ethanol overnight at 4°C. Propidium Iodide (PI) was added to the cells in a volume of 20 μg/mL, and the cells were then incubated at room temperature for 20 minutes. Finally, cell cycle analysis was performed by flow cytometry (FACS flow cytometry, USA).

Apoptosis detection

The cells of each treatment group were digested with EDTA-free trypsin. When the cells could

be gently pipetted down with a pipette or pipette tip, the previously collected cell culture medium was added, and all adherent cells were removed by gently pipetting. The sample was collected again into a centrifuge tube and centrifuged at 1,000 rpm for 5 min. The supernatant was carefully aspirated, and about 50 μl of culture medium was left to avoid aspirating the cells. About 1 ml of PBS pre-cooled at 4°C was added for washing, then centrifugation was again performed to pellet the cells. The supernatant was carefully removed. Cell apoptosis in each treatment group was detected by ANNEXIN V-FITC/PI Kit (Solebo, China) according to the protocol. Analysis was performed using FACS Diva software.

Cell invasion assay

For invasion assays, transwell chambers were individually pretreated with Matrigel (Nowa, China). While the bottom chamber contained FBS-containing DMEM medium, the top chamber contained serum-free medium. Using a cotton swab, the remaining cells on the surface were removed from the incubation at 37°C after 48 hours. Further, the cells on the bottom surface of the well plate were fixed with 4% formaldehyde by staining with 0.1% crystal violet, and the images were examined with a microscope (Nikon, Japan).

Animal experiments

Thirty 5-week-old male BALB/c nude mice were obtained from Beijing Weitong Lihua Laboratory Animal Co., Ltd. (Weitong Lihua, China). 5.0×10^6 TCA-83 cells stably expressing miR-222 mimics or negative controls were injected subcutaneously into the skin below the front legs of nude mice. Tumor formation in the mice was observed within 21 days, and the animals were guaranteed adequate food and water in the SPF animal room throughout the feeding period. Mice were then sacrificed (by sodium pentobarbital overdose with an anesthetic dose of 1,800 mg/kg), and the volume and weight of each tumor were determined. The tumor volume was generally measured once a week, and vernier calipers were used to measure the longest and shortest parts of the tumor. V (mm^3) = $1/2a \times b^2$ ('a' is the long axis, 'b' is the short axis).

Hematoxylin and eosin (H&E) staining

Mouse tumor tissue sections were taken, routinely deparaffinized, and stained with hematoxylin for 5 minutes. After staining, sections were destained by treating with 1% hydrochloric acid for 10 s. Subsequently, 0.25% eosin dye was added, and the sections were counterstained for 5 minutes. The tumor tissue was observed with a light microscope.

Ki-67 staining of tumor tissue

Tumor tissue sections of mice were taken after the above-mentioned dewaxing and transparent operation, and the tumor tissue was stained with Ki-67 immunohistochemical kit (Sangon Biotech, China) according to the manufacturer's method and observed by light microscope.

Statistical analysis

GraphPad software was used for data processing in this study, and measurement data were expressed in the form of mean ± standard deviation (SD). The comparison between two groups was analyzed by Student-t test, among three groups or more were analyzed by one-way ANOVA, and Tukey's method was used for pairwise comparison between groups afterwards. $P<0.05$ difference was statistically significant.

Results

Expression and effect of miR-222 on tumor tissues and cells

Our qRT-PCR analysis indicated that the tumor tissue expressed miR-222 at a greater level than the control tissue (**Figure 1A**, $P<0.05$). In addition, miR-222 was downregulated in all four tumor cells compared to that in HIOEC (oral epithelial cells) (**Figure 1B**). Of these tumor cell lines, TCA-83 was selected for further experiments due to its most pronounced upregulation. We further constructed a miR-222 interference model and detected the expression of miR-222 in each treatment group by qRT-PCR. We found that miR-222 mimics could overexpress miR-222, and miR-222 inhibitor could inhibit the expression of miR-222 (**Figure 1C**). In terms of cell proliferation, cell viability was significantly enhanced in transfected miR-222 mimics at 72 and 96 h, while miR-222

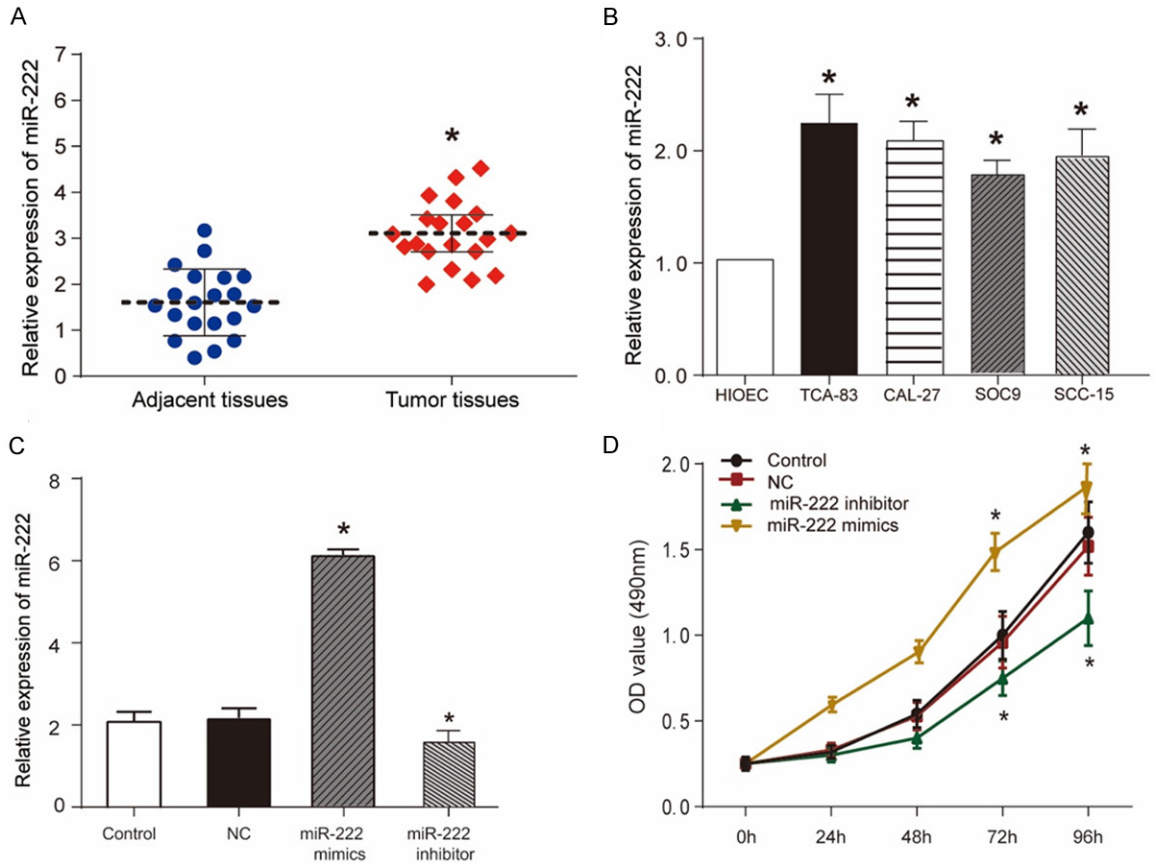


Figure 1. Expression of miR-222 in tumor tissues and cells and its effect on tumor cells. A. Expression of miR-222 measured by qRT-PCR (n=20); B. Levels of miR-222 in four oral squamous cell carcinoma cell lines compared with the normal cell line HIOEC; C. Expression of miR-222 measured by qRT-PCR; D. Cell viability after miR-222 treatment measured by MTT assay. *indicates vs. NC or HIOEC group, P<0.05.

inhibitor transfection was able to inhibit cell progress (**Figure 1D**).

Effect of miR-222 on tumor cell progress

Further clone formation experiments showed that the number of cell colonies in the miR-222 mimic group was increased, while the number of cell colonies in the miR-222 inhibitor group was significantly reduced (**Figure 2A**, P<0.05). In addition, the wound healing test results showed that the cell migration ability of the miR-222 mimic group was significantly stronger, while the cell migration ability of the miR-222 inhibitor was relatively weak (**Figure 2B**, P<0.05). The transwell invasion assay showed that the number of invasive cells in the miR-222 mimic group was up-regulated, while the number of invasive cells in the inhibitor was down-regulated (**Figure 2C**, P<0.05). In addition, we also performed FCM assay to determine the effect of miR-222 overexpression on the cell cycle and apoptosis of tumor cells. From the perspective of cell cycle, the proportion of arrested cells in G0/G1 phase was significantly increased in the miR-222 inhibitor group, thereby the number of cells in S phase was reduced (**Figure 3A**, P<0.05). In contrast, the cell cycle distribution of the miR-222 mimic group presented opposite results (P<0.05). In addition, the FCM assay showed that the apoptosis rate of the miR-222 mimic group was significantly down-regulated, while the apoptosis rate of the miR-222 inhibitor was increased (**Figure 3B**, P<0.05). Consequently, miR-222 mimics were found to promote OSCC cell viability and migration abilities and inhibit tumor cell apoptosis.

MiR-222 targets and downregulates the expression of CDKN1B

MiR-222 was tested by predicting its target sites using the TargetScan database, which

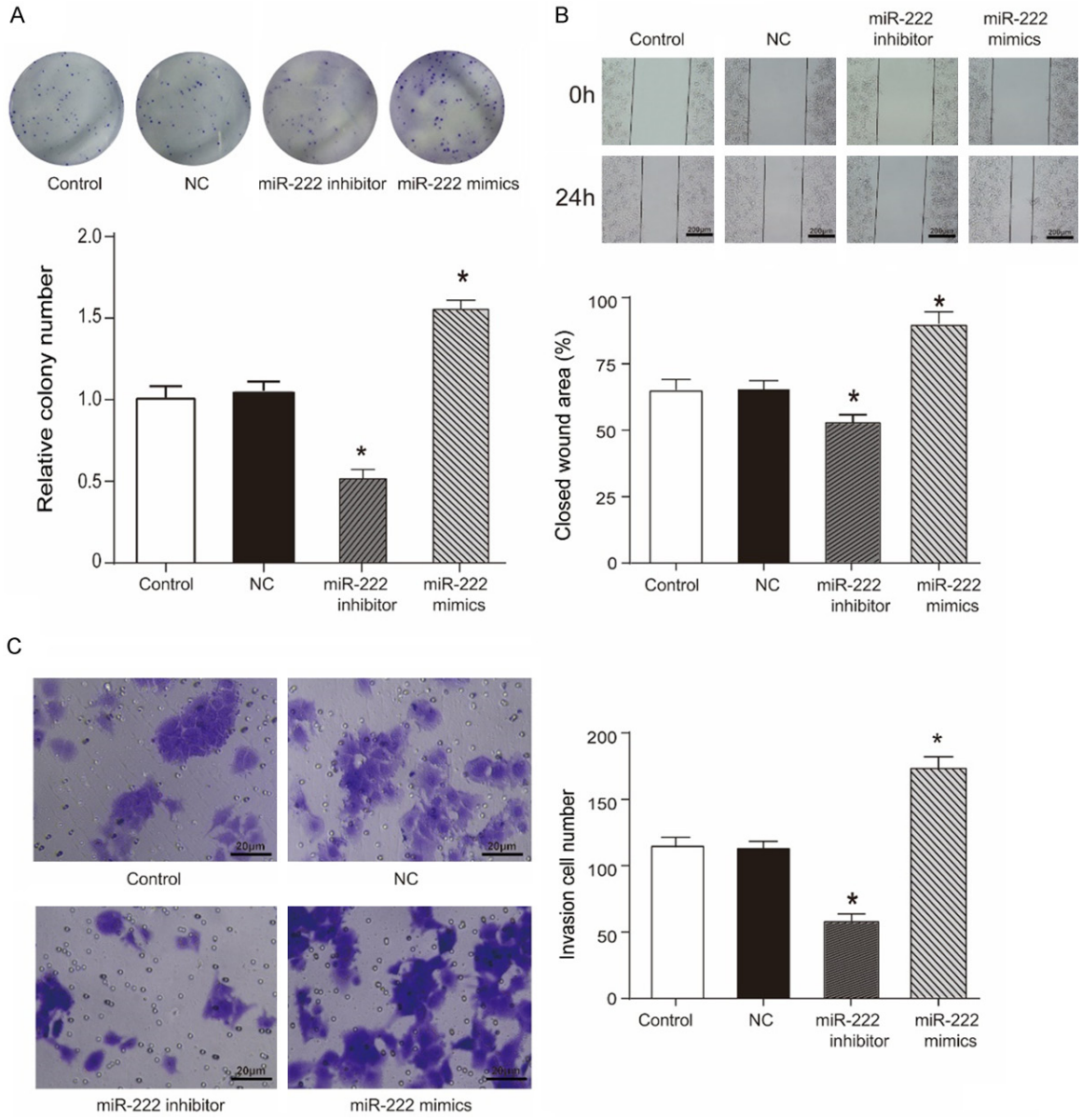
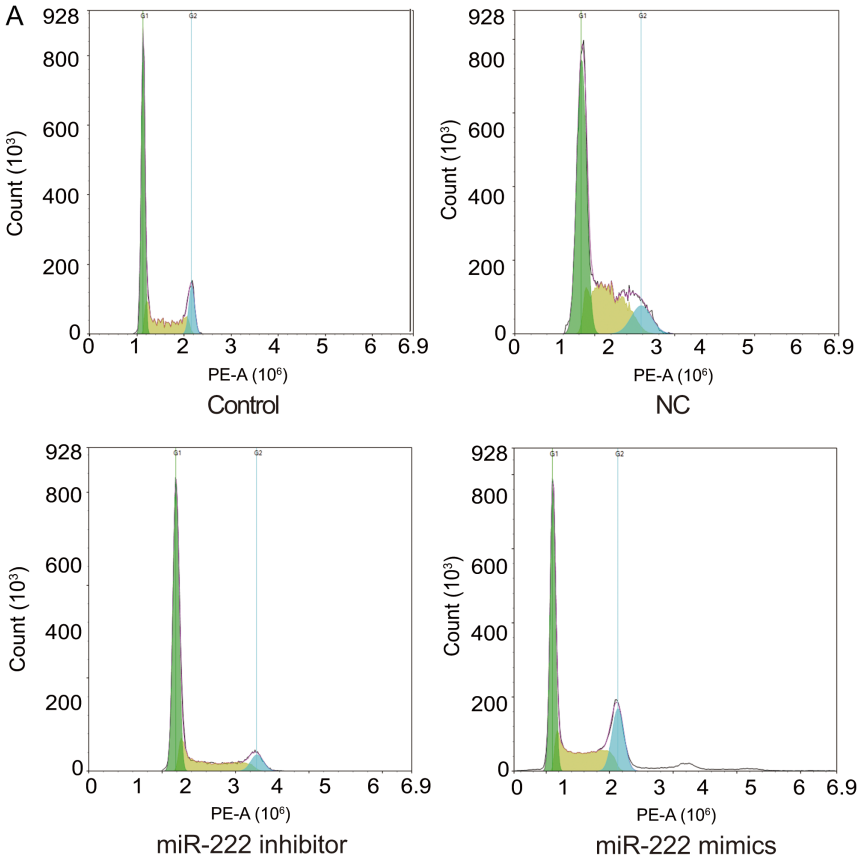
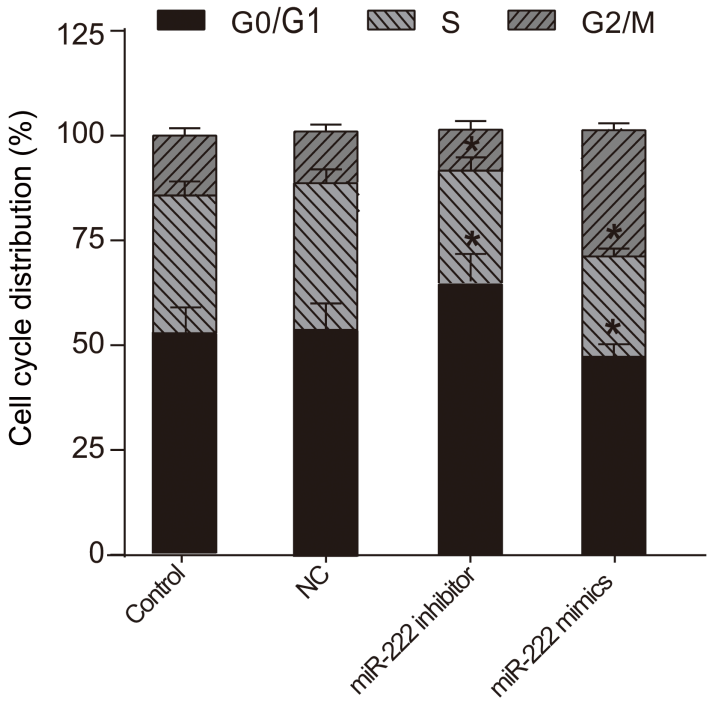


Figure 2. Overexpression of miR-222 inhibits cell viability and metastatic ability. A. Colony formation in each treatment group; B. The migration of each treatment group; C. The number of cell invasion in each treatment group measured by Transwell (× 200). *indicates vs. NC group, P<0.05.

was used to verify relationships between miR-222 and CDKN1B. We found that some potential binding sites for miR-222 were present in the 3'untranslated region (URT) of CDKN1B, suggesting that CDKN1B is one of the direct targets of miR-222 (**Figure 4A**). The relationship of miR-222 and CDKN1B was further verified by dual luciferase reporter gene, and it was found that it was the target gene of miR-222 (**Figure 4B**, P<0.05). In addition, we also found that CDKN1B was lowly expressed in tumor tissues based on microarray analysis (**Figure 4C**). At the same time, qRT-PCR also confirmed the phenomenon of low expression of CDKN1B in tumor tissues and cancer cells (**Figure 4D**, **4E**, P<0.05). qRT-PCR and Western blotting showed that the miR-222 mimic could inhibit the expression of CDKN1B, while its overexpression model could successfully induce the high expression of CDKN1B (**Figure 4F**, **4G**, P<0.05).


A
Count (10³)
PE-A (10⁶)
Control
NC
miR-222 inhibitor
miR-222 mimics


G0/G1
S
G2/M
Cell cycle distribution (%)
Control
NC
miR-222 inhibitor
miR-222 mimics

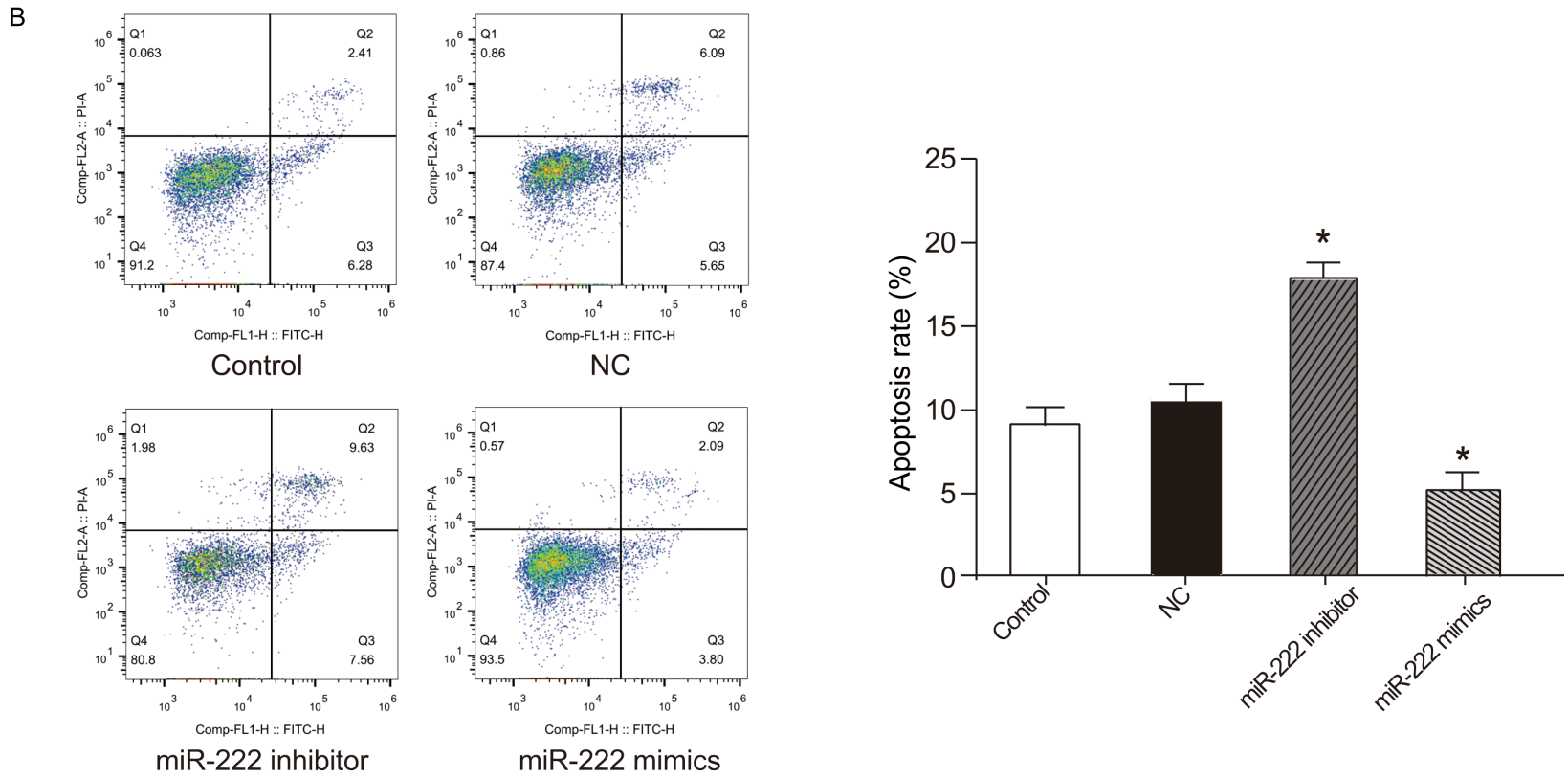


Figure 3. Overexpression of miR-222 in tumor cells inhibits cell cycle arrest and apoptosis. A. Cell cycle; B. Cell apoptosis. *indicates vs. NC group, P<0.05.

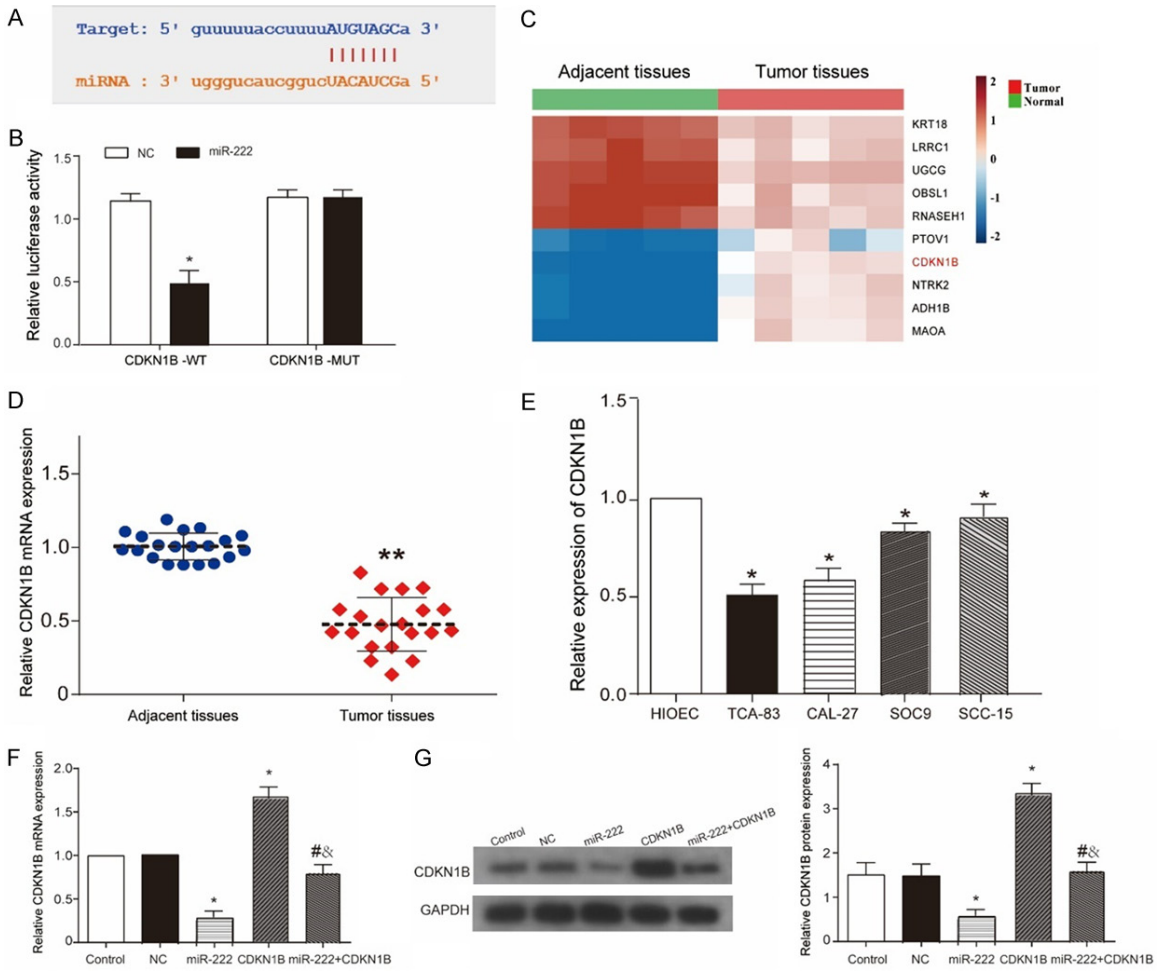


Figure 4. MiR-222 targets and downregulates the expression of CDKN1B. A. The potential binding site of miR-222 on the CDKN1B 3'untranslated region predicted by the TargetScan database; B. The luciferase activity of cells transfected with the 3'untranslated region wild-type CDKN1B vector in the miR-222 mimic group was significantly weaker than that in the NC group confirmed by the dual-luciferase reporter assay; C. Microarray analysis showed that CDKN1B was lowly expressed in tumor tissue; D, E. CDKN1B was lowly expressed in tumor tissue and cells examined by qRT-PCR; F, G. The mRNA and protein levels of CDKN1B in cells transfected with miR-222 mimics or CDKN1B measured by QRT-PCR and Western blot. *indicates vs. NC group, P<0.05, #indicates vs. miR-222 group, P<0.05, &indicates vs. CDKN1B group, P<0.05. CDKN1B, cyclin-dependent kinase inhibitor 1B.

Effect of miR-222 on tumor cells by regulating CDKN1B

As shown in **Figure 5A**, miR-222 mimic significantly promoted tumor cell proliferation, while CDKN1B overexpression inhibited cell growth (P<0.05). In addition, cell mobility and invasiveness were assessed by wound healing and transwell experiments. The cell migration assay showed that the migration ability of the miR-222 mimic group was significantly increased after 24 h of transfection with related reagents, while the wound healing results of the CDKN1B overexpression group was the opposite (**Figure 5B**, **5D**, both P<0.05). The results of transwell experiments showed that the invasive ability of cells in the miR-222 mimic group was significantly enhanced, while CDKN1B significantly inhibited cell invasiveness (**Figure 5C**, **5E**, P<0.05). Furthermore, our results showed that overexpression of both miR-222 and CDKN1B simultaneously reversed the changes in tumor regulation caused by a single treatment. It is indicated that CDKN1B inhibited cell proliferation and metastasis, while miR-222 could reverse the effects of CDKN1B on cells and promote tumor cell growth and migration by regulating CDKN1B.

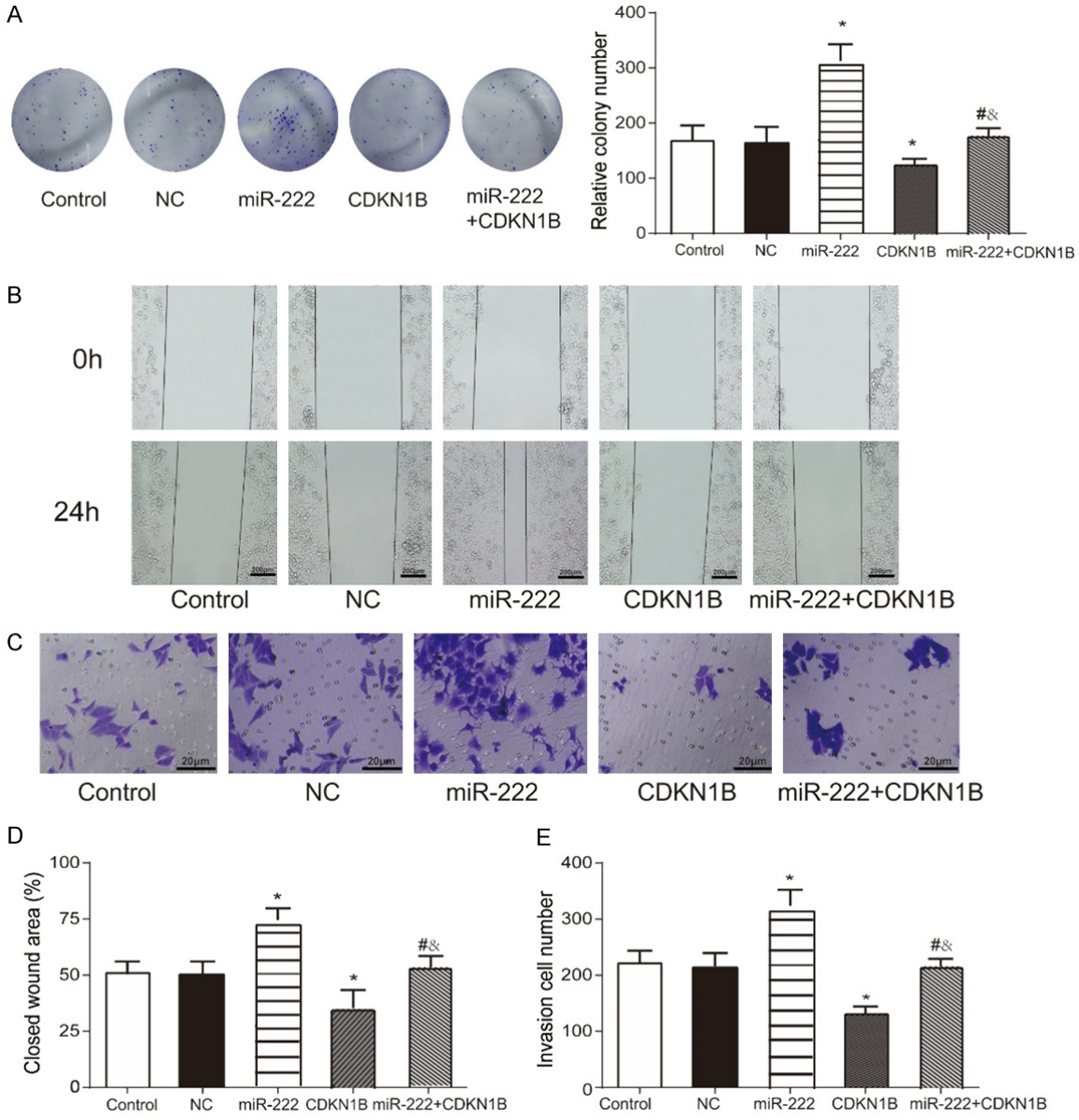


Figure 5. MiR-222 regulates the effect of CDKN1B on tumor cells. A. Colony formation in each treatment group; B. The migration of each treatment group (× 200); C. The number of cell invasion in each treatment group measured by Transwell (× 200); D. The wound area of each treatment group; E. The number of cell invasion in each treatment group measured by Transwell. *indicates vs. NC group, P<0.05, #indicates vs. miR-222 group, P<0.05, &indicates vs. CDKN1B group, P<0.05. CDKN1B, cyclin-dependent kinase inhibitor 1B.

MiR-222 affects tumor cell cycle by downregulating CDKN1B

As shown in **Figure 6**, the arrest in G0/G1 phase was significantly reduced following transfection with miR-222 mimics, whereas the arrest in S phase was significantly enhanced (P<0.05). This indicated that miR-222 mimics could promote the process of tumor cells. Conversely, upregulation of CDKN1B expression increased the number of cells arrested in the G0/G1 phase. Therefore, miR-222 can promote cell cycle progression by downregulating CDKN1B.

MiR-222 promotes tumor growth by targeting CDKN1B

As shown in **Figure 7A**, **7B**, tumor volume in mice was significantly increased after injection of miR-222 mimic-transfected cells. In addition, compared with the NC group, the tumor weight of the mice in the miR-222 mimic group was

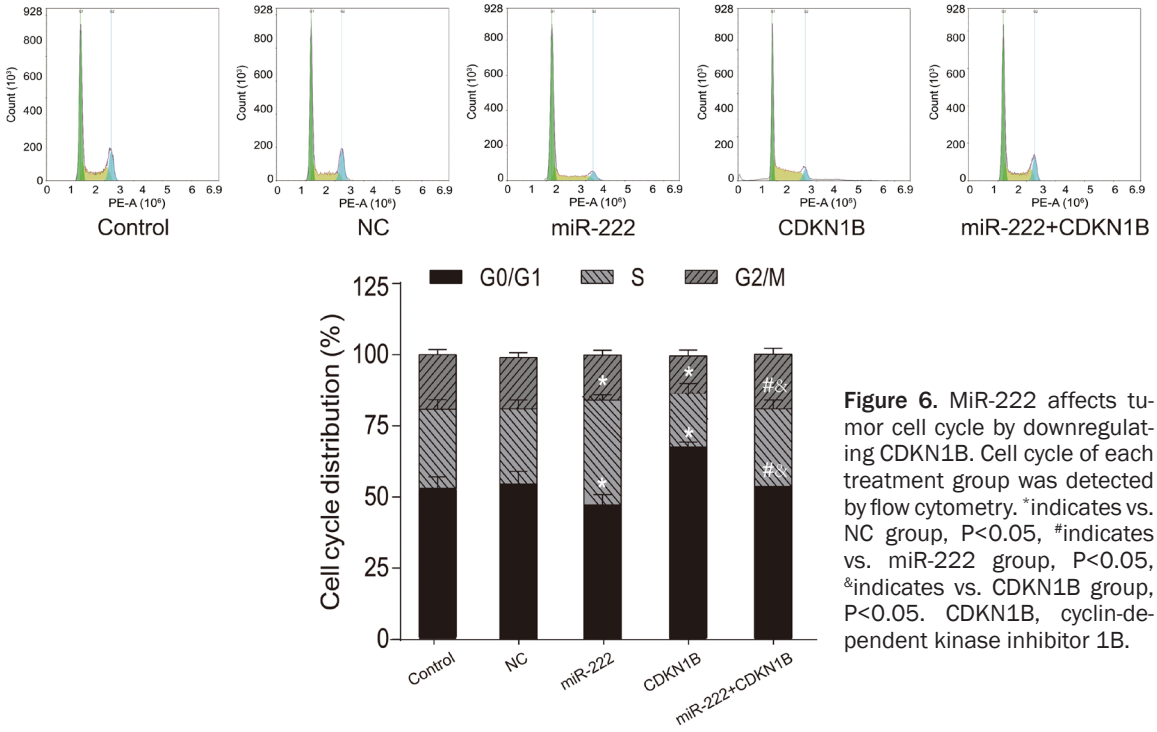


Figure 6. MiR-222 affects tumor cell cycle by downregulating CDKN1B. Cell cycle of each treatment group was detected by flow cytometry. *indicates vs. NC group, P<0.05, #indicates vs. miR-222 group, P<0.05, &indicates vs. CDKN1B group, P<0.05. CDKN1B, cyclin-dependent kinase inhibitor 1B.

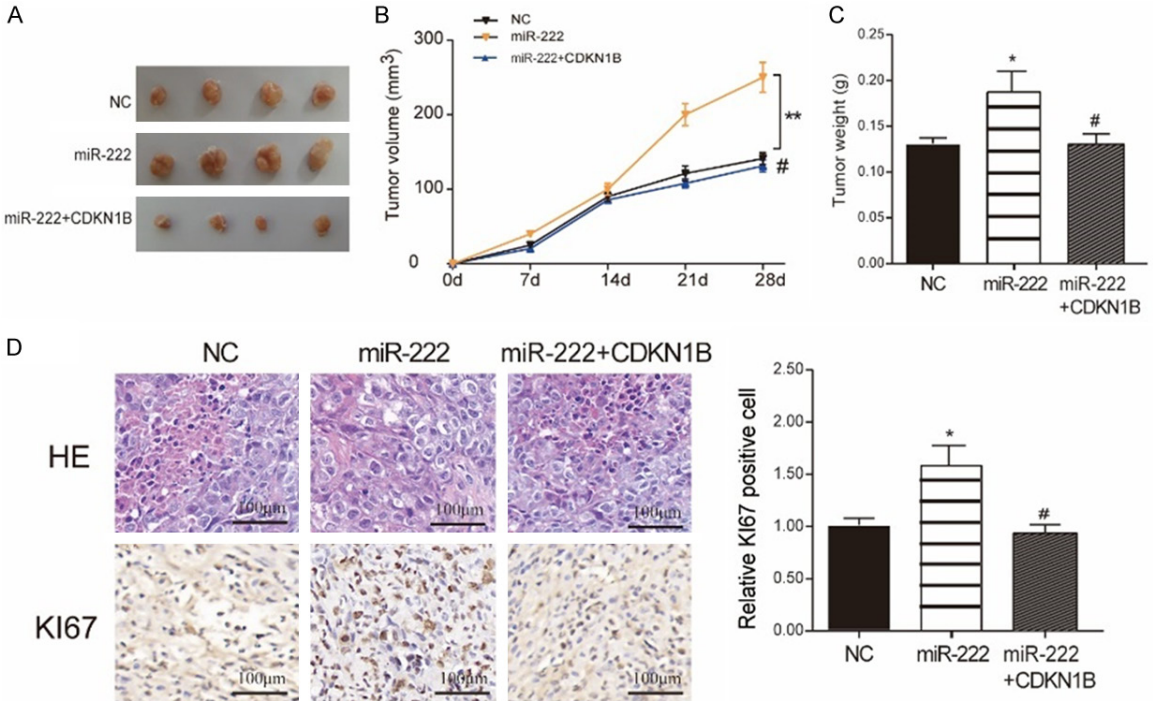


Figure 7. MiR-222 promotes tumor growth by targeting CDKN1B *in vivo*. (A) Tumor situation in each treatment group; (B, C) Tumor volume (B) and weight (C) in each treatment group; (D) H&E and KI67 staining results of tumor tissue, magnification 400 times; *indicates vs. NC group, P<0.05, #indicates vs. miR-222 group, P<0.05. CDKN1B, cyclin-dependent kinase inhibitor 1B.

also significantly heavier (**Figure 7C**, P<0.05). However, co-treatment of CDKN1B and miR-222 could reverse the regulation of miR-222 on OSCC progression. In addition, this phenom-

enon was confirmed in further histological H&E and KI67 staining (**Figure 7D**). We found that miR-222 promoted cell proliferation and increased the number of KI67-positive cells, which was reversed when CDKN1B was simultaneously overexpressed.

Discussion

OSCC is a common tumor of the head and neck. Although existing treatment modalities have been significantly improved by combined management of surgery, adjuvant chemoradiotherapy and gene-targeted therapy, the prognosis is still poor. The latest data show that the 5-year survival rate of patients with OSCC is only about 50%. These data suggest that the process of OSCC has seriously affected people's life health and quality of life [14]. The reason for the poor prognosis can be attributed to the incomplete understanding of its pathogenesis. Therefore, in-depth exploration of the pathogenesis of OSCC is of great significance to improve the prognosis and treatment effect of patients. In the present study, we first verified by qRT-PCR that the expression of miR-222 was significantly upregulated in OSCC tissues and cells. Further cell viability, wound healing, transwell and flow cytometry confirmed that it can affect tumor progression by regulating tumor cell proliferation and metastasis. Moreover, we identified CDKN1B as a possible target gene of miR-222 by using bioinformatics analysis, and this was verified by our dual-luciferase reporter assay. This finding may provide reliable data to support the selection of molecular markers and the development of targeted therapy for OSCC.

MiR-222 promotes osteosarcoma cell growth by inhibiting the expression of TIMP3 in osteosarcoma cells, according to a recent study [15]. MiR-222 expression has also been found to be increased in renal cell carcinomas and has been shown to influence the progression of this disease by promoting cell proliferation and inhibiting apoptosis [16]. Overexpression of miR-222 promoted progress of OSCC cells and inhibited apoptosis. It further affected OSCC metastasis by regulating tumor cell progress. These articles demonstrate the oncogenic function of miR-222 in OSCC, and miR-222 is expected to be a biomarker for early screening of OSCC and a target for therapy. Our results showed that the expression of miR-222 was significantly up-regulated in tumor tissues and cells. Further, the results of clone formation, cell cycle, scratch and transwell experiments showed that overexpression of miR-222 could promote cell proliferation, metastasis and invasion. These results demonstrate that miR-222 also has the potential to be an early screening marker and a therapeutic target in OSCC. However, its mechanism of promoting tumor proliferation still needs to be further explored.

It has been well established that miRNAs can promote proliferation, apoptosis, and metastasis by regulating target gene expression through competitive RNA adsorption (ceRNA) [17]. Current studies have shown that miR-222 regulates the progress and metastasis of tumor cells by inhibiting the level of TIMP3 [18]. Recent studies have found that CDKN1B may be a potential regulatory target of miR-222 in oral cancer [19, 20]. The role of miR-222 in oral cancer cells, however, has been less studied [21]. This study successfully verified CDKN1B as a direct target gene of miR-222 by dual-luciferase reporter and reversion experiments. According to the luciferase reporter gene analysis, the miR-222 mimic group's luciferase activity was decreased in TCA-83 cells. More importantly, cell viability, migration, and invasion were reduced and apoptosis was promoted by the overexpression of CDKN1B. Therefore, we believe that CDKN1B, as an effector target of miR-222, plays a regulatory role in OSCC cell proliferation, apoptosis, migration and invasion. However, although this study found the regulatory role of miR-222 in OSCC, its application value in clinical diagnosis and the specific pathways involved in the regulation of CDKN1B are still unclear, so we still need to further explore it.

In summary, we first found that miR-222 was upregulated in OSCC tissues and OSCC cells. Further assays confirmed that miR-222 could promote the progression of OSCC cells by regulating the expression of CDKN1B. These data provide new directions for biomarkers and targets for early diagnosis of OSCC.

Disclosure of conflict of interest

None.

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