**Original Article**

*MiR-149-5p regulates cisplatin chemosensitivity, cell growth, and metastasis of oral squamous cell carcinoma cells by targeting TGFβ2*

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**Abstract:** Background: Oral squamous cell carcinoma (OSCC) is a public health problem worldwide. MicroRNAs, acting as either oncogenes or tumor suppressors, have gathered much attention. The aim of this study was to characterize the role of miR-149-5p in drug resistance, cell growth, and metastasis and its underlying mechanism in oral squamous cell carcinoma. Methods: The expressions of miR-149-5p and TGFβ2 were measured by quantitative real-time polymerase chain reaction. The survival rate of cells treated with different concentrations of CDDP was checked by CCK-8. The cell proliferation and apoptosis was determined by CCK-8 and flow cytometry, respectively. Cell migration and invasion were examined using transwell assay. The interaction of miR-149-5p and TGFβ2 was predicted by online software Targetscan and confirmed by luciferase reporter assay. The protein expression of TGFβ2, p-SMAD2 and p-SMAD3 was quantified using western blot. Results: The expression of miR-149-5p was obviously decreased in OSCC tissues and cell lines, and its expression was lower in a cisplatin resistant cell line (CAL-27/CDDP) than that of a normal OSCC cell line (CAL-27). CCK-8 assay suggested that miR-149-5p increased drug sensitivity in CAL-27 and CAL-27/CDDP cells. miR-149-5p attenuated proliferation, migration and invasion, and promoted apoptosis of CAL-27 and CAL-27/CDDP cells. In addition, TGFβ2 was up-regulated in OSCC cells at both mRNA and protein levels. Moreover, miR-149-5p promoted cisplatin chemosensitivity and regulated cell proliferation, apoptosis, migration and invasion by targeting TGFβ2 in CAL-27 and CAL-27/CDDP cells. Conclusion: miR-149-5p regulates cisplatin chemosensitivity, cell growth, apoptosis and metastasis by targeting TGFβ2. miR-149-5p/TGFβ2 axis has potential for therapy of OSCC.

**Keywords:** miR-149-5p, TGFβ2, oral squamous cell carcinoma, cisplatin chemosensitivity, proliferation, apoptosis, metastasis

**Introduction**

Oral squamous cell carcinoma (OSCC) is an important public health problem worldwide [1], accounting for 95% of all head and neck tumor forms [2]. In the past few years, the incidence of OSCC has exceeded 50%, ranking it as the sixth most common cancer in the world [2, 3]. Recently, strategies for diagnosis and treatment have been greatly optimized; however, the prognostic effects of recurrent and distant metastatic oral cancer are still unsatisfactory [4, 5]. According to statistics, the overall 5-year survival rate of OSCC varies from 50% to 60% [6], thus, more effective treatment is needed. However, the tolerance of cancer cells to anticancer drugs is a serious obstacle to effective treatment. Cisplatin is a major adjunct chemotherapeutic agent and is an integral part of topical therapy in advanced OSCC [7, 8]. Therefore, cisplatin resistance is one of the most important problems in treatment.

Currently, research progress on microRNAs has accelerated, and there is increasing evidence that miRNAs are involved in the regulation of a variety of biologic processes [9]. MiRNAs are a class of small non-coding RNAs with 22 nucleotides in length. Typically, miRNAs differentially regulate gene expression by binding to the 3’
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untranslated region (3'-UTR) of the target mRNA, resulting in mRNA degradation or translational inhibition [10]. Previous studies have reported that miRNAs play important roles in the regulation of gene expression and may dysregulate disease in many ways, including metabolic diseases, infectious diseases, cancers and drug resistance in cancers [11-14]. For example, miR-4513, acting in an oncogenic role, regulates the proliferation and apoptosis of oral squamous cell carcinoma cells by targeting CXCL17 [15]. Overexpression of SOX4 reversed the inhibited effects of miR-199a-5p on cell migration and invasion, and miR-199a-5p may deplete the migration and invasion of OSCC cells by targeting the EMT-related transcription factor SOX4 [16]. MiR-375 inhibits cell growth and enhances radiosensitivity of OSCC by targeting IGF-1R, suggesting that miR-375 may be a potential therapeutic target for OSCC patients [17]. MiR-149-5p is one of miRNAs and its roles in various cancers have been reported before. However, the potential function of miR-149-5p in OSCC and its role in drug resistance are still uncertain.

Transforming growth factor-β2 (TGFβ2) is an exercise-induced adipokine that regulates metabolism [18], which belongs to the members of a superfamily of conserved cytokines [19]. TGFβs were discovered in the early stage of 1980s, containing three orthologs in mammals (TGFβ1, TGFβ2, and TGFβ3) [20]. Recent studies have shown that TGFβs are effective stimulating factors for inflammation, fibrosis, and activation of certain matrix metalloproteinases (MMPs), which play a wide range of regulatory roles in biology, including fibrosis, proliferation, and drug resistance [21-23]. For example, TGFβ1 knockdown enhances chemosensitivity of cisplatin-resistant lung cancer cells through suppressing the expression of drug-resistant proteins [23]. TGFβ2 is a pleiotropic cytokine that acts on a wide range of diseases and regulates a variety of biologic processes, including cell cycle, cell differentiation, cell growth, cell death, ECM deposition and tissue [24, 25]. Previous research provided us with a reference, for instance, TGFβ2 treatment increased the mRNA and protein expression of the SRGN by targeting the SRGN relative promoter domain in TNBC cells, which claimed that SRGN interacted with TGFβ2 and regulated TNBC metastasis via the autocrine and paracrine routes [26]. In glioma, TGF-β2 could initiate autophagy through Smad and non-Smad pathway to promote glioma cell invasion [27]. High expression of HIF1α/TGFβ2/GLI2 is strongly associated with relapse after chemotherapy in patients with colorectal cancer, and activation of GLI2 induced by HIF-1α and TGF-β2 promotes chemoresistance in a hypoxic tumor microenvironment [28]. Unfortunately, there is little report of TGFβ2 in OSCC, therefore, it is important to determine the underlying role of TGFβ2 in OSCC cells and OSCC resistant cells.

In this study, we measured the expression of miR-149-5p in OSCC tissues and cell lines, investigated the role of miR-149-5p of cisplatin chemosensitivity in OSCC. The putative target gene (TGFβ2) of miR-149-5p was predicted and confirmed in our study, aiming to provide a potential mechanism and therapy strategies for OSCC.

Materials and methods

Sample tissues and cell lines

A total of 34 pairs of OSCC tissues and adjacent normal tissues were collected from Department of Dental Center, Sir Run Run Shaw Hospital, affiliated with the Zhejiang University School of Medicine. All samples were frozen in liquid nitrogen immediately once dissecting and then were stored at -80°C until further use. Informed consents have been signed by all subjects before clinical surgery. Collection and usage of the samples were reviewed and approved by Department of Dental Center, Sir Run Run Shaw Hospital, affiliated with the Zhejiang University School of Medicine.

Human OSCC cell lines SCC-9 and CAL-27 were purchased from American Tissue Culture Collection (ATCC; Manassas, VA, USA). Human oral keratinocyte cell line HOK was purchased from BeNa Culture Collection (BNCC; Suzhou, China). Cisplatin [cis-diamminedichloroplatinum (II), CDDP] was purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA) and dissolved in phosphate-buffered saline (PBS). In accordance with previously described methods, cisplatin resistant cell line CAL-27/CDDP was developed from its parental cell line CAL-27 by giving gradually incremental doses of cisplatin administration in cell culture medium (29). All cell lines were cultured in Dulbecco’s Modified Eagle Medium (Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine
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serum (FBS; Life Technologies) at 37°C in a humidified with 5% CO₂ incubator.

Cell transfection

miRNA mimics or inhibitor targeting miR-149-5p (miR-149-5p mimics or miR-149-5p inhibitor) and their corresponding negative controls (miR-NC or anti-miR-NC) were obtained from RIBOBIO (Guangzhou, China). TGFβ2 overexpression vector pcDNA3.1-TGFβ2 (TGFβ2) and pcDNA3.1 empty vector (Vector) were synthesized by GenePharma (Shanghai, China). All transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cells were harvested at 24 h after transfection for the following experiments.

RNA isolation and qRT-PCR analysis

Trizol reagent (Invitrogen) was used to isolate total RNA from specific tissues and cells according to manufacturer’s protocol. RNA samples were reverse-transcribed into cDNA using a TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) for miR-149-5p and PrimeScript RT reagent kit (Takara, Dalian, China) for TGFβ2. Then qRT-PCR was conducted using TaqMan MicroRNA assays (Applied Biosystems) for miR-149-5p and SYBR Green Master PCR mix (Applied Biosystems) for TGFβ2 on ABI 7900 system (Applied Biosystems). The expression levels of mature miRNA-149-5p and TGFβ2 were normalized by U6 and GAPDH, respectively. Expression was calculated using 2⁻△△Ct method. All procedures were performed following the manufacturer’s instructions. The primers used were as follows: GAPDH (Forward, 5'-ATCCATGGC-ACCGTACCGTGA-3' and Reverse, 5'-TTCCTCAATCGGTGACAGCCCA-3'); TGFβ2 (Forward, 5'-AGCAAGCTGAAGCTCACCAGT-3' and Reverse, 5'-TTGCCGATAGTACTTCGTCG-3'). The primers for miR-149-5p and U6 were purchased from RiboBio (Guangzhou, China).

CCK-8 assay

The cisplatin resistance and cell proliferation were quantified by CCK-8 assay. For cisplatin resistance detection, cells treated with different concentrations of cisplatin were planted into 96-well plates (3 × 10³). After culture for 48 h, cells were combined with 10 μL reagent of cell counting kit-8 (CCK-8) (Beyotime) for 2 h continuously. Finally, the absorbance value at 450 nm was measured by microplate reader (Bio-Rad, Hercules, CA, USA). Five replicate wells were used for each group. The IC50 of cisplatin was the cisplatin concentration reducing viability by 50%. As for cell proliferation, cells seeded in 96-well plates were cultured for 24 h, 48 h or 72 h, and then examined by CCK-8 as mentioned above.

Flow cytometry assay

Flow cytometry was carried out for apoptosis analysis by using Fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kits (Invitrogen). In briefly, CAL-27 and CAL-27/CDDP cells were incubated for 48 h after transfection. Afterwards, cells were trypsinized, washed with PBS, and re-suspended. Next, 5 μL Annexin V-FITC/PI were used to stain apoptotic cells for 15 min in the dark. Subsequently, the apoptotic cells (Annexin V-FITC- and PI+) were detected using flow cytometer (BD Biosciences, San Jose, CA, USA).

Transwell assay

Transwells were conducted to detect cell migration and invasion. After 48-h transfection, cells were harvested and re-suspended in serum-free medium. For migration, the cells were placed in the top chamber of a transwell (Corning, NY, USA) and the bottom chamber was filled with DMEM with 10% FBS. After 24-h incubation, the cells migrated to the lower surface of the membrane were fixed with 4% paraformaldehyde (PFA), stained with crystal violet, and photographed using a microscope (Tokyo, Japan) at × 400 magnification. The methods for invasion were similar to the above, except that the upper chamber needed to be coated with Matrigel (Corning) in advance.

Bioinformatic analysis and luciferase reporter analysis

Online software Targetscan was used to screen putative mRNAs binding to miR-149-5p and analyze the potential binding sites between miR-149-5p and 3’UTR of TGFβ2. The wild type and mutant sequences of 3’UTR of TGFβ2 (TGFβ2-WT and TGFβ2-MUT) containing miR-149-5p binding sites were inserted into the downstream portion of a dual-luciferase reporter vector. For luciferase assay, 3 × 10⁴ cells...
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CAL-27 cells were co-transfected with miR-149-5p mimics or miR-NC and TGFβ2-WT or TGFβ2-MUT, respectively. After transfection for 24 h, the luciferase activity was detected using Dual-Luciferase reporter assay system on a luminometer (Promega, Madison, WI, USA).

**Western blot assay**

RIPA lysis buffer (Beyotime, Shanghai, China) was used to extract total proteins from specific cells. After measuring the concentration of proteins by Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidenedifluoride (PVDF) membranes (Millipore, Shanghai, China). Next, the membranes were blocked with Tris-buffered saline (TBS) buffer containing 5% non-fat milk for 2 h. Then, membranes were incubated with primary antibodies against TGFβ2 (ab36495; 1:1,000; Abcam, Cambridge, UK), GAPDH (ab37168; 1:1,000; Abcam), smad2 (ab17812; 1:1,000; Abcam), smad3 (ab122028; 1:1,000; Abcam), phosphorylated smad3 and phosphorylated smad2 (1:500; Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4°C. Next day, the membranes were washed with TBS with Tween-20 (TBST) for 3 times and were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody. Finally, enhanced chemiluminescence (ECL) (Beyotime) reagent was used to visualize the signal on the membranes.

**Statistical analysis**

Statistical analysis was performed using SPSS 23.0. The data are presented as the means ± SD. One-way ANOVA test and two-tailed Student’s t-test were used to identify variables in two groups or multiple groups. \( P < 0.05 \) was considered statistically significant. All experiments were repeated at least three times.

**Results**

The expression of miR-149-5p was significantly decreased in OSCC tissues, cell lines, and cisplatin-resistant OSCC cells

To explore the potential role of miR-149-5p in OSCC, the expression of miR-149-5p was measured in OSCC tissues and paired normal tissues. The results showed that miR-149-5p was significantly down-regulated in OSCC tissues compared with that in normal tissues (Figure 1A). In addition, the expression of miR-149-5p in OSCC cell lines (CAL-27 and SCC-9) was lower than that in human oral keratinocytes (HOK) (Figure 1B). Moreover, the data displayed that the expression of miR-149-5p in the cisplatin-resistant cells (CAL-27/CDDP) was much reduced compared to that in CAL-27 cells (Figure 1C). The data suggested that dysregulation of miR-149-5p might be associated with cisplatin resistance in OSCC.

miR-149-5p influenced the CDDP resistance of CAL-27 cells and CAL-27/CDDP cells

To test the correlation of miR-149-5p and CDDP resistance, miR-149-5p mimics or miR-NC were transfected in CAL-27 and CAL-27/CDDP cells. First, cell survival rate was measured in CAL-27 and CAL-27/CDDP cells after treatment with different concentrations of CDDP. The result showed that the IC50 of CDDP in CAL-27 (1.778) was significantly lower than that in CAL-27/CDDP cells (5.551) (Figure 2A). Then, the qRT-PCR...
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PCR showed that the expression of miR-149-5p in CAL-27 treated with 1 μg/mL CDDP was decreased compared with that in CAL-27 without CDDP treatment (Figure 2B). miR-149-5p was notably up-regulated both in CAL-27 and CAL-27/CDDP cells transfected with miR-149-5p mimics compared with control (Figure 2C). Next, CCK-8 suggested that overexpression of miR-149-5p significantly decreased the IC50 values of cisplatin compared with negative control in both CAL-27 and CAL-27/CDDP cells (Figure 2D and 2E). These data implied that miR-149-5p was able to sensitize OSCC cells to chemotherapy with cisplatin.

MiR-149-5p attenuated proliferation, migration, and invasion and promoted apoptosis of CAL-27 and CAL-27/CDDP cells

We further studied the effects of miR-149-5p on cell proliferation, apoptosis, migration, and invasion. CCK-8 assay showed that overexpression of miR-149-5p inhibited cell proliferation in CAL-27 and CAL-27/CDDP cells (Figure 3A and 3B). In addition, up-regulation of miR-149-5p significantly increased apoptosis compared with controls. Furthermore, we also assessed the role of miR-149-5p in cell migration and invasion. Transwell assay demonstrated that the number of migrated and invaded cells in CAL-27 and CAL-27/CDDP cells transfected with miR-149-5p mimics was very diminished compared with controls and miR-NC (Figure 3G and 3H). Collectively, our findings suggested that miR-149-5p negatively regulated proliferation, migration, and invasion and increased the cell apoptosis by functioning as a tumor suppressor of OSCC cells in vitro.

TGFβ2 was a direct target of miR-149-5p in OSCC cells

To manifest the underlying mechanisms of the inhibited effects of miR-149-5p on cell growth and metastasis in OSCC, we screened the putative targets of miR-149-5p using bioinformatics tool Targetscan. The binding sites of miR-149-5p and 3’UTR of TGFβ2 are displayed in Figure 4A. Then the sequences containing the WT or MUT 3’UTR of TGFβ2 (TGFβ2-WT and TGFβ2-MUT) (Figure 4A) were inserted into luciferase reporter plasmid, and the fusion plasmid together with miR-149-5p mimics or miR-NC were cotransfected in CAL-27 cells. As shown in Figure 4B, when CAL-27 cells were transfected with the TGFβ2-WT 3’UTR, co-transfection of
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A

B

C

D

E

F

G

H
Figure 3. The role of miR-149-5p in cell proliferation, apoptosis, migration, and invasion was studied in CAL-27 and CAL-27/CDDP cells. (A and B) CCK-8 was used to measure cell proliferation in CAL-27 and CAL-27/CDDP cells transfected with miR-149-5p mimics at 24 h, 48 h, and 72 h after transfection. (C-F) Flow cytometry was performed to detect cell apoptosis in CAL-27 (C and D) and CAL-27/CDDP (E and F) cells transfected with miR-149-5p mimics. (G and H) Transwells were used to examine cell migration and invasion in CAL-27 and CAL-27/CDDP cells treated with miR-149-5p mimics. Blank control and miR-NC acted as a comparison. **P < 0.01.

Figure 4. Relationship between miR-149-5p and TGFβ2 in CAL-27 cells. (A) Putative binding sites of TUG1 and miR-133b are shown. (B) Luciferase activity was detected in CAL27 cells co-transfected with TGFβ2 WT or TGFβ2 MUT and miR-149-5p or miR-NC. (C) The mRNA expression of miR-149-5p was measured in HOK, CAL-27, and CAL-27/CDDP cells. (D and E) The mRNA expression (D) and protein expression (E) of miR-149-5p were measured in CAL-27...
miR-149-5p mimics significantly inhibited luciferase activity. In contrast, the influence of miR-149-5p mimics was not obvious in CAL-27 cells cotransfected with TGFβ2-MUT 3’UTR. Also, the mRNA expression level of TGFβ2 was greatly strengthened in CAL-27 and CAL-27/CDDP cells compared with that in HOK (Figure 4C). After treatment with 1 μg/mL CDDP, the relative mRNA expression (Figure 4D) and the protein expression level (Figure 4E) of TGFβ2 were rapidly enhanced in CAL-27 cells compared with those in untreated cells. Next, we investigated the effects of the expression of miR-149-5p on TGFβ2 mRNA expression in CAL-27. The data indicated that the mRNA expression of TGFβ2 sharply increased in the miR-149-5p inhibitor compared to the anti-miR-NC group. However, TGFβ2 mRNA expression was very weak in miR-149-5p mimics compared with that in miR-NC group (Figure 4F). Moreover, the protein levels of TGFβ2/SMAD signal pathway related proteins, p-SMAD2, P-SMAD3, and TGFβ2, were measured. The result showed that TGFβ2, p-SMAD2, and P-SMAD3 protein levels were significantly increased in the miR-149-5p inhibitor group compared with that in anti-miR-NC group. At the same time, their protein levels were markedly decreased in CAL-27 transfected with miR-149-5p mimics compared with miR-NC (Figure 4G). Additionally, the forced expression of TGFβ2 in OSCC tissues was negatively correlated with the expression of miR-149-5p (Figure 4H and I). that the above data suggest that miR-149-5p regulated the expression of TGFβ2 at the transcriptional and translational levels by directly binding to putative TGFβ2 3’UTR regions.

MiR-149-5p promotes cisplatin chemosensitivity by targeting TGFβ2 in CAL-27 and CAL-27/CDDP cells

To verify the interaction of miR-149-5p and TGFβ2 on cisplatin resistance, CAL-27 and CAL-27/CDDP cells were transfected with miR-149-5p mimics, miR-NC, miR-149-5p mimics + vector or miR-149-5p mimics + TGFβ2. The transfection efficacy was validated at the transcriptional level and protein level. As exhibited in Figure 5A-C, the mRNA expression and protein expression of TGFβ2 were decreased in two cell lines transfected with miR-149-5p mimics compared to miR-NC. On the contrary, the mRNA expression and protein expression of TGFβ2 were recovered in the two cell lines transfected with miR-149-5p mimics + TGFβ2 compared to miR-149-5p mimics + vector. In addition, gain-of-function experiments showed that overexpression of miR-149-5p impeded cisplatin resistance, measured by the IC50 to cisplatin. Nevertheless, up-regulation of miR-149-5p reversed the suppressive role of miR-149-5p overexpression in both CAL-27 and CAL-27/CDDP cells (Figure 5D and 5E). These data suggested that miR-149-5p regulated cisplatin chemosensitivity by binding to TGFβ2 directly in CAL-27 and CAL-27/CDDP cells.

MiR-149-5p regulates cell proliferation, apoptosis, migration, and invasion in CAL-27 and CAL-27/CDDP cells by targeting TGFβ2

To further define the mechanism of miR-149-5p function in CAL-27 and CAL-27/CDDP cells, cell proliferation, apoptosis, migration, and invasion were detected in two cell lines with different groups. As we saw in Figure 6A and 6B, miR-149-5p mimics + TGFβ2 cotransfection restored the inhibited role of miR-149-5p mimic transfection in CAL-27 and CAL-27/CDDP cells compared with controls. Furthermore, the number of apoptotic cells in the two cell lines transfected with miR-149-5p mimics was rapidly increased compared to miR-NC, while miR-149-5p mimics + TGFβ2 transfection reduced the amount of apoptotic cells (Figure 6C). As for cell migration and invasion, miR-149-5p mimics + TGFβ2 transfection improved the number of migrated and invaded cells that had been reduced by miR-149-5p overexpression (Figure 6D and 6E).

Discussion

The prognosis of OSCC is poor due to the complex metastasis mechanism and chemical resistance. Thus, an understanding of the tumor growth, metastasis, and drug resistance mech-
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Figure 5. Regulatory effect of TGFβ2 overexpression on miR-133b-mediated cisplatin resistance in CAL-27 and CAL-27/CDDP cells. (A–C) The efficiency of TGFβ2 overexpression was measured in miR-149-5p overexpressed CAL-27 and CAL-27/CDDP cells treated with pcDNA-TGFβ2 at the mRNA level (A) and protein level (B and C). (D and E) The IC50 of cisplatin in CAL-27 and CAL-27/CDDP cells treated with miR-149-5p mimics + TGFβ2, miR-149-5p mimics + vector, miR-149-5p mimics, or miR-NC was examined by CCK-8. **P < 0.01.

pathways is indispensable, and a systemic attempt is necessary to improve the outcome of OSCC patients. In the present study, we determined that miR-149-5p acted as a tumor suppressor and sensitized cells to cisplatin in OSCC. Also, the target mRNA TGFβ2 of miR-149-5p was confirmed here, which revealed a novel biological axis of miR-149-5p/TGFβ2 in cisplatin chemosensitivity and cell proliferation, migration, and invasion in OSCC.

It is well-demonstrated that ectopic expression of miR-149-5p plays a crucial role in tumor development and metastasis in human cancers. For example, miR-149-5p level was obviously lower in medullary thyroid carcinoma (MTC) and overexpression of miR-149-5p inhibited cell proliferation and invasion to some extent [30]. The overexpression of miR-149-5p notably slowed melanoma cell proliferation, and colony formation, and promoted cell apoptosis [31]. MiR-149 was markedly down-regulated in human sarcoma samples and functioned as a tumor suppressor in osteosarcoma by inhibition of the TWEAK-Fn14 axis [32]. miR-149 (miR-149-5p) acts as either a tumor suppressor or oncogene [33-35] in different human cancers, suggesting that miR-149 exhibits diverse roles in tumor progression. Unfortunately, a functional study of miR-149-5p in OSCC has hardly been referenced. Our study claims that miR-149-5p was down-regulated in OSCC tissues and cells, and serving as tumor suppressor, inhibited cell proliferation, migration, and invasion, and accelerated apoptosis in OSCC. As for drug resistance, a microRNA profiling of...
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cisplatin-resistant oral squamous cell carcinoma cell lines showed that the expression of miR-149 in cisplatin-resistant cell lines was lower than that in their parental OSCC cell lines [29], which was consistent with the results in our study. Moreover, the research on miR-149-5p regulating chemotherapeutic resistance has been reported in other cancers. For instance, Xu et al. showed that miR-149-5p promoted chemotherapeutic resistance in ovarian cancer through inactivation of the Hippo signaling pathway [36]. Li et al. showed that miR-149 was down-regulated in SGC7901/DDP cells compared with SGC7901 cells and played important roles in determining sensitivity of cisplatin-resistant gastric cancer (GC) cells by targeting FoxM1 [37]. These findings revealed a role of miR-149-5p in tumor growth, metastasis, and drug resistance in human cancers.

TGFβ mainly acts through the classical signaling pathway SMAD, which requires continuous binding of TGFR-II and TGFR-I by TGFβs to form a ternary complex, which triggers phosphorylation of SMAD and regulates various intracellular biologic processes [38, 39]. In this study, we additionally ascertained the protein expression of SMAD pathway-related proteins to determine the expression of TGFβ2. We showed this signal pathway to be mostly associated with OSCC. Lamaroon et al. illustrated that abnormality of the TGF-beta pathway, as indicated by a reduction or absence of Smad4 expression, promotes tumorigenesis in OSCC [40]. Podoplanin is expressed highly in OSCC and has the ability to promote metastasis and invasion, and TGFβ induced podoplanin expression in the majority of human OSCC cell lines [41]. Oshimori et al. showed that TGF-β signaling promoted heterogeneity, tumor characteristics, and drug resistance of squamous cell carcinoma stem cells (SCCSCs) [42]. All findings elucidated the crucial role of TGFβ in carcinogenesis, metastasis, and drug resistance, which provides decisive guidance for our research. We did not address the effects of TGFβ2 alone on tumor cell proliferation, migration, and drug resistance, which is subject to further research.

In sum, we demonstrated that miR-149-5p augmented chemosensitivity to CDDP, inhibited cell proliferation, migration, and invasion, and promoted apoptosis in OSCC cells in vitro. Furthermore, miR-149-5p might increase chemosensitivity to CDDP and suppress growth and metastasis of OSCC cells by targeting the

Figure 6. Regulatory effect of TGFβ2 overexpression on miR-133b-regulated cell proliferation, apoptosis, migration and invasion in CAL-27 and CAL-27/CDDP cells. CAL-27 and CAL-27/CDDP cells were transfected with miR-149-5p mimics + TGFβ2, miR-149-5p mimics + vector, miR-149-5p mimics, or miR-NC for 24 h. (A-E) Cell proliferation (A and B), apoptosis (C), cell migration (D), and invasion (E) were measured by CCK-8, flow cytometry, or transwell, respectively. *P < 0.05, **P < 0.01.
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TGFβ2. Our findings suggested that the miR-149-5p/TGFβ2 axis might be a promising prognostic and therapeutic target in OSCC.

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Disclosure of conflict of interest

None.

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