Am J Transl Res 2023;15(3):1626-1639
www.ajtr.org /ISSN:1943-8141/AJTR0135459

Original Article

miR-379-5p regulates the proliferation, cell cycle, and cisplatin resistance of oral squamous cell carcinoma cells by targeting ROR1

Lei Meng, Yanlin Du, Banglian Deng, Yinzhong Duan

State Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi Clinical Research Center for Oral Diseases, Department of Orthodontics, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, P. R. China

Received May 20, 2021; Accepted October 24, 2022; Epub March 15, 2023; Published March 30, 2023

Abstract: Objective: To analyze the regulatory mechanism microRNA miR-379-5p in oral squamous cell carcinoma (OSCC). Methods: We collected serum samples from patients with OSCC and examined the expression of miR-379-5p and receptor tyrosine kinase-like orphan receptor 1 (ROR1) by real-time polymerase chain reaction and western blot. OSCC cells were purchased for molecular research, cell multiplication was tested using the BrdU assay, cell cycle was tested using flow cytometry, and resistance to cisplatin (DDP) was assessed using the MTT assay. Results: miR-379-5p expression was downregulated and ROR1 expression was upregulated in the serum of OSCC patients, and the area under the curve for OSCC identified by miR-379-5p and ROR1 was not less than 0.800. In the cell function test, overexpression of miR-379-5p could suppress the proliferation, cell cycle, and DDP resistance of OSCC cells. miR-379-5p could negatively regulate ROR1. Inhibition of ROR1 expression had a similar effect after the re-expression of miR-379-5p. Co-overexpression of miR-379-5p and ROR1 counteracted the above inhibitory effects on the proliferation, cell cycle, and DDP resistance of OSCC cells. Conclusion: miR-379-5p in OSCC regulates the proliferation, cell cycle, and DDP resistance of tumor cells by targeting ROR1.

Keywords: Oral squamous cell carcinoma, miR-379-5p, ROR1, DDP resistance

Introduction

Oral squamous cell carcinoma (OSCC) accounts for over 90% of oral carcinomas. To date, its pathogenesis has not been clearly clarified [1, 2]. Currently, patients with advanced OSCC account for a high proportion of OSCC patients, with a high metastasis rate and a 5-year survival rate of < 50% [3]. Although treatment methods (e.g., surgery, radiotherapy, and chemotherapy) for OSCC have progressed, difficulties caused by the advanced disease stage limit the effectiveness of these treatments [4, 5].

The pathogenesis of OSCC needs to be explored further. In this study, we analyzed OSCC pathology based on the proliferation, cell cycle, cisplatin (DDP) resistance, and other aspects of OSCC cells and explored molecular markers that were strongly related to the pathology to investigate their potential diagnostic value. The survival performance of tumor cells can determine the metastatic potential of tumors, and cycle arrest is helpful for suppressing tumor cell propagation [6-8]. However, DDP resistance can determine patients' sensitivity to chemotherapy, which is important for its efficacy [9]. Therefore, our study results can provide guidance for suppressing OSCC tumor growth and improving the curative effects of chemotherapy.

MicroRNAs (miRNAs), which play an important regulatory role in cancer, can regulate the pathologic microenvironment of tumors by affecting the expression of messenger RNA (mRNA) [10, 11]. miRNAs can be used as serum markers for tumor screening with reliability and convenience [12]. The miRNA miR-379-5p has been reported to be abnormally downregulated in nasopharyngeal carcinoma (NPC) and hepa-

tocellular carcinoma (HCC). It can suppress the metastasis of NPC tumors by targeting Y-box binding protein 1 in NPC and can inhibit AKT signal transduction by inhibiting the expression of focal adhesion kinase in HCC, thereby improving the progression of HCC [13, 14].

miR-379-5p may have a universal tumor suppression function, and restoration of its expression can inhibit carcinoma. miR-379-5p also targets DNA methyltransferase 3B expression to inhibit OSCC growth, suggesting that miR-379-5p also possesses anti-OSCC activity [15]. Receptor tyrosine kinase-like orphan receptor 1 (ROR1), a transmembrane protein, is widely upregulated in malignant tumors, and its specific antibody was reported to inhibit the pathologic process in a mouse tumor model [16]. ROR1 has been identified as a downstream target of miR-379-5p by bioinformatics analysis. Thus, we suspected that the miR-379-5p-ROR1 axis can mediate the molecular mechanism of OSCC development and therefore conducted this study to examine this concept.

Materials and methods

Baseline data

From September 2016 to September 2020, we collected the serum samples of 58 patients with OSCC (OSCC group) and 50 healthy individuals (control group) from the School of Stomatology at the Fourth Military Medical University in China.

Inclusion criteria: The patients' tumor foci originated in the oral cavity and were pathologically diagnosed as squamous cell carcinoma; The patients received no other treatment before admission; The patients had undergone systematic and standardized surgery and/or had received radiotherapy and chemotherapy at our hospital; The patients had complete clinicopathologic and follow-up data; and the patients had no history of other malignant tumors or serious systemic diseases.

Exclusion criteria: The patients' tumor lesions originated in the oral cavity and were pathologically diagnosed as sarcoma or other non-squamous cell carcinoma types. The patients had secondary oral malignancies; the patients had undergone surgery or received chemotherapy or radiotherapy before admission to our hospital; the patients had incomplete clinical pathological data; or the patients had a history of malignant tumors in other areas or severe cardiovascular or cerebrovascular diseases.

Ethical considerations

This research was approved by the Ethics Committee of the School of Stomatology, the Fourth Military Medical University (approval number 2019KD251), and strictly complied with the principles of the Declaration of Helsinki. All patients provided written informed consent before participating in the study. Age, sex, and other general data were comparable between the two groups (**Table 1**).

Cell culture and transfection

Human OSCC cell lines (HSC-3, HSC-4, CAL-27, UPCI-SCC-040) and normal human oral keratinocytes (NHOK) were purchased from Otwo Biotech Inc. (Shenzhen, China). The cells were cultured in RPMI-1640 medium (R1145; Shanran Biotechnology Co., Ltd., Shanghai, China) containing 10% heat-inactivated fetal bovine serum (A11-704; Lianshuo Biological Technology Co., Ltd., Shanghai, China) and 100 units of penicillin/ml and 100 mg of streptomycin/ml (07500; Yihui Biological Technology Co., Ltd., Shanghai, China). The medium was placed in an incubator containing 5% CO_2 at 37°C.

Lipofectamine 3000 transfection reagent (L3000-001; Yanhui Biotechnology Co., Ltd., Shanghai, China) was used for cell transfection following the manufacturer's instructions. The main transfectants included miR-379-5p mimetic (miR-379-5p, 5'-UGGUAGACUAUGGAACGUAGG-3') and its negative control (miR-NC, 5'-CAGUACUUUUGUGUAGUACAAA-3') as well as ROR1-targeted inhibitory sequence (si-ROR1, 5'-GAGGAAGACCAAAGC-3') and its negative control (si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3').

Construction of DDP-resistant OSCC cells (HSC-3/DDP)

DDP-resistant OSCC cells were established by exposure to stepwise-increased DDP concentrations. The exposure was terminated when the cells could divide normally in medium containing 10 μM of DDP.

Table 1. Baseline characteristics of patients

Characteristic	OSCC group (n = 58)	Control group (n = 50)	P
Age	55.34 ± 9.27	54.78 ± 10.41	0.512
Sex (%)			
Male	49 (84.4)	42 (84.0)	0.665
Female	9 (15.6)	8 (16.9)	
Smoking history			
Yes	14 (24.1)	11 (22.0)	0.572
No	35 (75.9)	39 (78.0)	
Drinking history			
Yes	6 (10.3)	7 (14.0)	0.811
No	52 (89.7)	43 (86.0)	

OSSC, Oral Squamous Cell Carcinoma.

Table 2. Primer sequences for qRT-PCR

Gene	Sequence
miR-379	F: 5'-GTGGTAGACTATGGAACGTAGG-3'
	R: 5'-TACGTTCCATAGTCTACCA-3'
ROR1	F: 5'-CTGACGAAACACTGGAACTC-3'
	R: 5'-GTCTGATTTGGTAGCTTGGATG-3'
U6	F: 5'-CTCGCTTCGGCAGCACA-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'
β-actin	F: 5'-AGGGGCCGGACTCGTCATACT-3'
	R: 5'-GGCGGCACCACCATGTACCCT-3'

RT-PCR

TRIzol reagent (Invitrogen; Wenren Biotechnology Co., Ltd., Shanghai, China) was used to extract total RNA from the serum and cell samples. Superscript II reverse transcriptase (B1259; Westang Bio-Tech Co., Ltd., Shanghai, China) was used to reverse transcribe 2 μg of total RNA into complementary DNA. The levels of miR-379-5p and ROR1 were quantitatively assessed using a real-time polymerase chain reaction (RT-PCR) system (100089; Image Trading Co., Ltd., Beijing, China).

A Primescript™ RT Reagent Kit (TaKaRa Biotechnology Ltd., Dalian, China) was used for reverse transcription, and a SYBR® Premix Ex Taq™ Quantitative Real-Time PCR Kit (TaKaRa Biotechnology) was used for PCR amplification. Opticon Monitor 3 software (BioRad Laboratories, Inc., CA, USA) was used to analyze the PCR results. The quantitative evaluation formula was $2^{-\Delta\Delta Ct}$. Primer sequences are listed in **Table 2**.

Western blot analysis

RIPA lysis buffer (N653-100 ml; Runwell Technology Co., Ltd., Shanghai, China) was used to extract total protein. A BCA protein determination kit (FT-P91144T; Fantai Biotechnology Co., Ltd., Shanghai, China) was used to quantify the protein concentration. 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (AT1210-100 ml; Acmec Biochemical Co., Ltd., Shanghai, China) was used to isolate 30 μg of protein. The protein was transferred to a polyvinylidene fluoride membrane (BSP0161; Zeping Bioscience & Technologies Co., Ltd., Beijing, China). The membrane was sealed with 5% bovine serum albumin sealing solution (QN1119-ANF; Biolab Technology Co., Ltd., Beijing, China) at ambient temperature for 1 h and then incubated overnight with ROR1 (Abcam, ab91187, 1:1000), GADPH (Abcam, ab9485, 1:1000), and other primary antibodies (CABT-34798-MH, DPAB2596RH, 1:1000; Abace Biotechnology Co., Ltd., Beijing, China) at 4°C. The washed membrane was incubated with a horseradish peroxidase-conjugated secondary antibody (434323, 1:5000; Tideradar Technology Development Co., Ltd., Beijing, China) for 2 h. Finally, an enhanced chemiluminescence reagent (ZY-252FP; Zeye Biotechnology Co., Ltd., Shanghai, China) was used to determine the protein content.

The gray values were analyzed using Image software. The relative expression of the proteins was expressed as the gray value of the target protein and in proportion to that of the reference protein. The experiment was repeated three times for each group.

Cell proliferation assay

The transfected cells were collected, digested with trypsinase (E0022; Baomanbio Biotechnology Co., Ltd., Shanghai, China), resuspended, and inoculated in a 96-well plate (5×10^3 cells/well). Then, 5 mg/mL CCK-8 reagent (10 μL) (CA1210-5000T; Acmec Biochemical Co., Ltd., Shanghai, China) was added to each well (at 0, 24, 48, and 72 h), and the cells were cultured at 37°C for 4 h. Next, 200 μL of dimethyl sulfoxide (SD8580; Kairuiji Biotechnology Co., Ltd., Beijing, China) was added to each well, and the absorbance at 595 nm was measured using an enzyme-labeling instrument

(257158657; Image Trading Co., Ltd., Beijing, China).

The DDP resistance experiment was also conducted using the CCK-8 assay as described above, except that the culture medium was freshly prepared and contained various DDP concentrations. The DDP resistance was assessed using the IC50 value, which was directly proportional to the DDP resistance. The IC50 value was the DDP concentration that resulted in a 50% reduction in cell growth compared with that in the control group.

Cell cycle assay

The transfected cells were inoculated into a 6-cm Petri dish (2×10^5 cells/dish) until a confluency of approximately 80% was attained. The cells were digested with trypsinase, washed with phosphate-buffered saline, fixed with 75% ethanol, and stained with RNase A (R1030-1; Solarbio Science & Technology Co., Ltd., Beijing, China) and 500 μL of propidium iodide (C0123; Baomanbio Biotechnology Co., Ltd., Shanghai, China). The distribution of the cell cycle (percentage of cells in the G0/G1, S, and G2/M phases) was analyzed by flow cytometry (Ranger Apparatus Co., Ltd., Shanghai, China).

Cell apoptosis

Cell apoptosis was assessed by flow cytometry, using the Annexin V-Fluorescein isothiocyanate (FITC)/Propidium iodide (PI) apoptosis detection kit (556547, BD Pharmingen). Cells were suspended in binding buffer (20 μL) and treated with annexin V-FITC (10 μL) and PI (5 μL). The cell apoptosis rate was determined using a flow cytometer (Ranger Apparatus Co., Ltd., Shanghai, China).

Detection of dual-luciferase gene

The ROR1 3'-UTR fragment was amplified and cloned into the psiCHECK-2 vector. For the luciferase assay, the control psiCHECK-2 vector, ROR1 mutant (ROR1-Mut), or ROR1 wild type (ROR1-WT) was co-transfected into the cells with miR-NC and miR-379-5p. The PCR products were cloned into multiple cloning sites of pmirGLO luciferase (Promega Corp., Madison, WI, USA). The number of cells and transfection efficiency were normalized using promoter-*Renilla* luciferase reporter plasmid (TaKaRa Biotechnology) as an internal reference, which expressed R. luciferase. After transfection for 48 h, the luciferase activity was examined using a dual-luciferase reporter gene detection system (GB3001; Giantagen Biosciences, Inc., Beijing, China) following the manufacturer's specifications.

Statistical analysis

The data were expressed as mean ± standard deviation, and GraphPad Prism 7.0 (La Jolla, CA, USA) was used for analysis. We used Student's *t*-test and one-way analysis of variance to analyze the differences between groups. ANOVA with post hoc Bonferroni test was performed for comparisons among multiple groups. We analyzed the diagnostic value of serum miR-379-5p and ROR1 in OSCC using receiver operating characteristic (ROC) curves. $P < 0.05$ was considered significant.

Results

Expression of miR-379-5p and ROR1 in serum samples of patients with OSCC

To investigate the biological effects of miR-379-5p and ROR1 in OSCC, we determined their expression in the serum of patients in the OSCC group (n = 58) and the control group (n = 50) using RT-PCR. The expression of miR-379-5p in the OSCC group was lower than that of the control group (**Figure 1A**), whereas the expression of ROR1 in the OSCC group was higher than that of the control group (**Figure 1B**). Correlation analysis revealed a significant negative correlation between their expressions ($r = -0.692$, $P < 0.001$; **Figure 1C**). ROC curve analysis revealed that the area under the curve (AUC) identified by miR-379-5p for OSCC was 0.898 and that identified by ROR1 was 0.810 (**Figure 1D**). Thus miR-379-5p and ROR1 have strong potential to discriminate OSCC, and might mediate its pathological process.

Effect of miR-379-5p expression on OSCC cell proliferation and cycle

We evaluated the expression of miR-379-5p in four OSCC cell lines, showing that miR-379-5p expression was generally low (**Figure 2A**). We selected the HSC-3 and CAL-27 cell lines, which had the lowest expression levels, to study cell

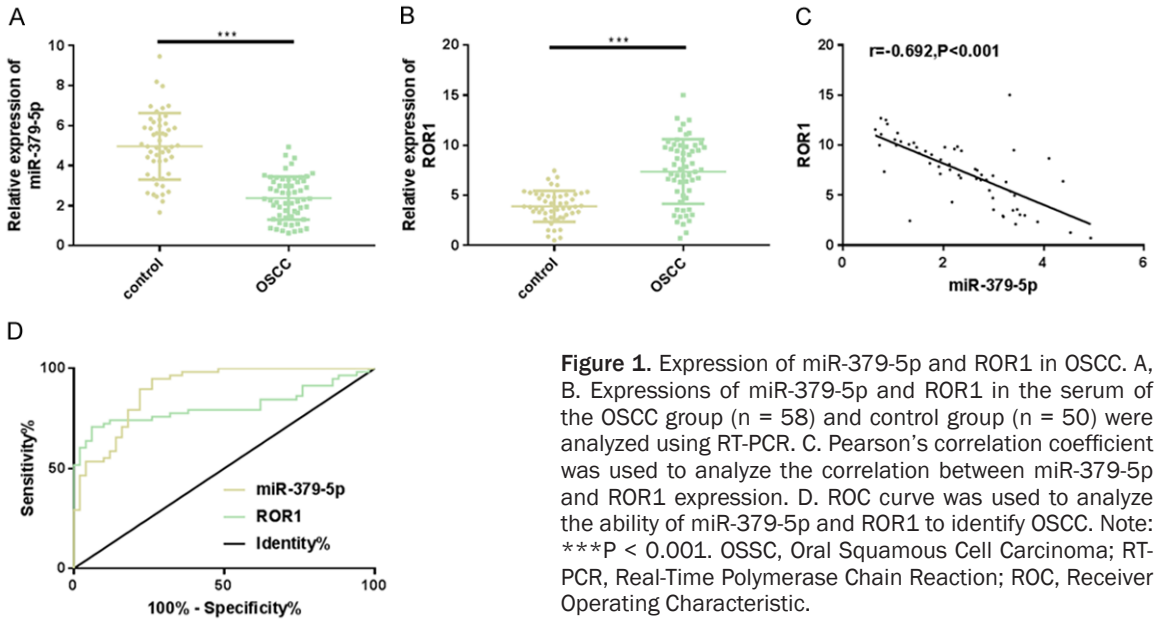

Figure 1. Expression of miR-379-5p and ROR1 in OSCC. A, B. Expressions of miR-379-5p and ROR1 in the serum of the OSCC group (n = 58) and control group (n = 50) were analyzed using RT-PCR. C. Pearson's correlation coefficient was used to analyze the correlation between miR-379-5p and ROR1 expression. D. ROC curve was used to analyze the ability of miR-379-5p and ROR1 to identify OSCC. Note: ***P < 0.001. OSSC, Oral Squamous Cell Carcinoma; RT-PCR, Real-Time Polymerase Chain Reaction; ROC, Receiver Operating Characteristic.

function. miR-379-5p mimics were transfected into HSC-3 and CAL-27 cells to achieve a logarithmically upregulated expression level (**Figure 2B**). Compared to miR-NC transfection, the number of proliferating HSC-3 and CAL-27 cells decreased after upregulation of miR-379-5p expression (**Figure 2C**, **2D**). The percentage of cells in the G0/G1 phase decreased and those in the G2/M phase increased (**Figure 2E**, **2F**). All these results were significant (P < 0.05), indicating that the upregulation of miR-379-5p blocked the OSCC cells in the G2/M phase and partially suppressed their proliferation, thereby inhibiting the OSCC progression.

Targeted relationship between miR-379-5p and ROR1

The above results indicate that miR-379-5p may inhibit OSCC. We studied the downstream target genes through biologic analysis to explore the regulatory mechanisms. The target genes were predicted using the TargetScan database. Because ROR1 plays a regulatory role in the cell cycle to varying degrees in lung adenocarcinoma, chronic lymphocytic leukemia, HCC, and other tumors, and its high expression is closely associated with concealed OSCC metastasis, it has attracted much attention from researchers [17-20]. We found that ROR1 and miR-379-5p had possible binding sites (**Figure 3A**). Based on the dual-luciferase gene reporter assay results, we concluded that ROR1-WT expression was decreased by miR-379-5p, whereas ROR1-Mut expression was not affected (**Figure 3B**, **3C**).

Western blot analysis revealed that the ROR1 protein level was negatively regulated by miR-379-5p; that is, when miR-379-5p expression was upregulated, the ROR1 protein level was inhibited (P < 0.05) (**Figure 3D**, **3E**). These results , suggest that ROR1 is a target gene of miR-379-5p, which can be overexpressed to inhibit ROR1 protein.

Effects of inhibiting ROR1 expression on OSCC cell proliferation and cell cycle

We evaluated the ROR1 protein level in four OSCC cell lines using western blot analysis. Compared to NHOK cells, the ROR1 protein levels in the OSCC cells were high (**Figure 4A**), a finding contrary to the expression level of miR-379-5p that was previously observed. We selected two OSCC cell lines (HSC-3 and CAL-27) with the highest expression for further study.

We transfected si-ROR1 into HSC-3 and CAL-27 cells to significantly reduce the ROR1 protein level (**Figure 4B**). In the CCK-8 analysis, compared to the si-NC transfection, the proliferation of HSC-3 and CAL-27 cells was suppressed under si-ROR1 (**Figure 4C**, **4D**). By flow cytometry analysis, downregulation of ROR1

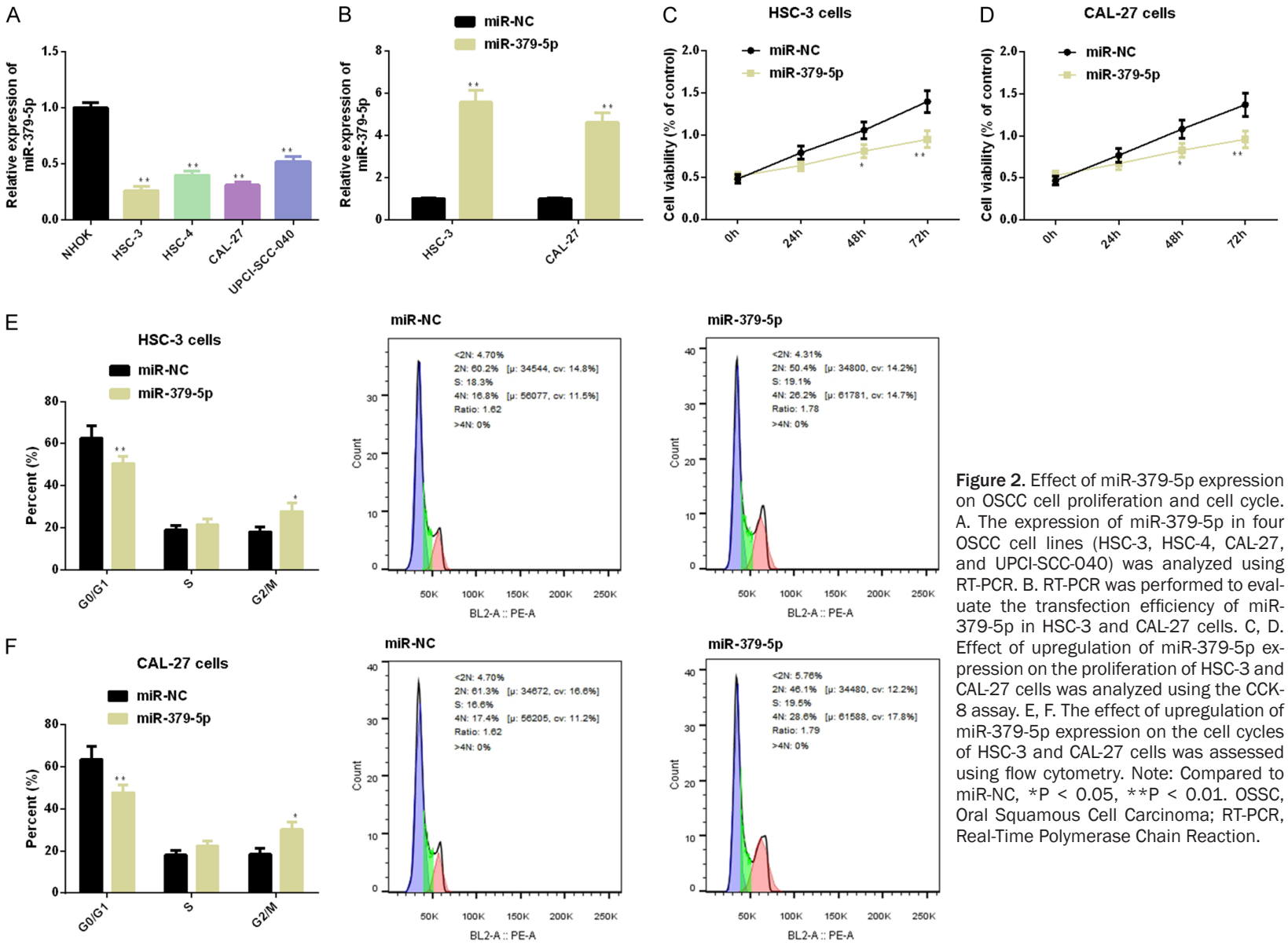

Figure 2. Effect of miR-379-5p expression on OSCC cell proliferation and cell cycle. A. The expression of miR-379-5p in four OSCC cell lines (HSC-3, HSC-4, CAL-27, and UPCI-SCC-040) was analyzed using RT-PCR. B. RT-PCR was performed to evaluate the transfection efficiency of miR-379-5p in HSC-3 and CAL-27 cells. C, D. Effect of upregulation of miR-379-5p expression on the proliferation of HSC-3 and CAL-27 cells was analyzed using the CCK-8 assay. E, F. The effect of upregulation of miR-379-5p expression on the cell cycles of HSC-3 and CAL-27 cells was assessed using flow cytometry. Note: Compared to miR-NC, *P < 0.05, **P < 0.01. OSSC, Oral Squamous Cell Carcinoma; RT-PCR, Real-Time Polymerase Chain Reaction.

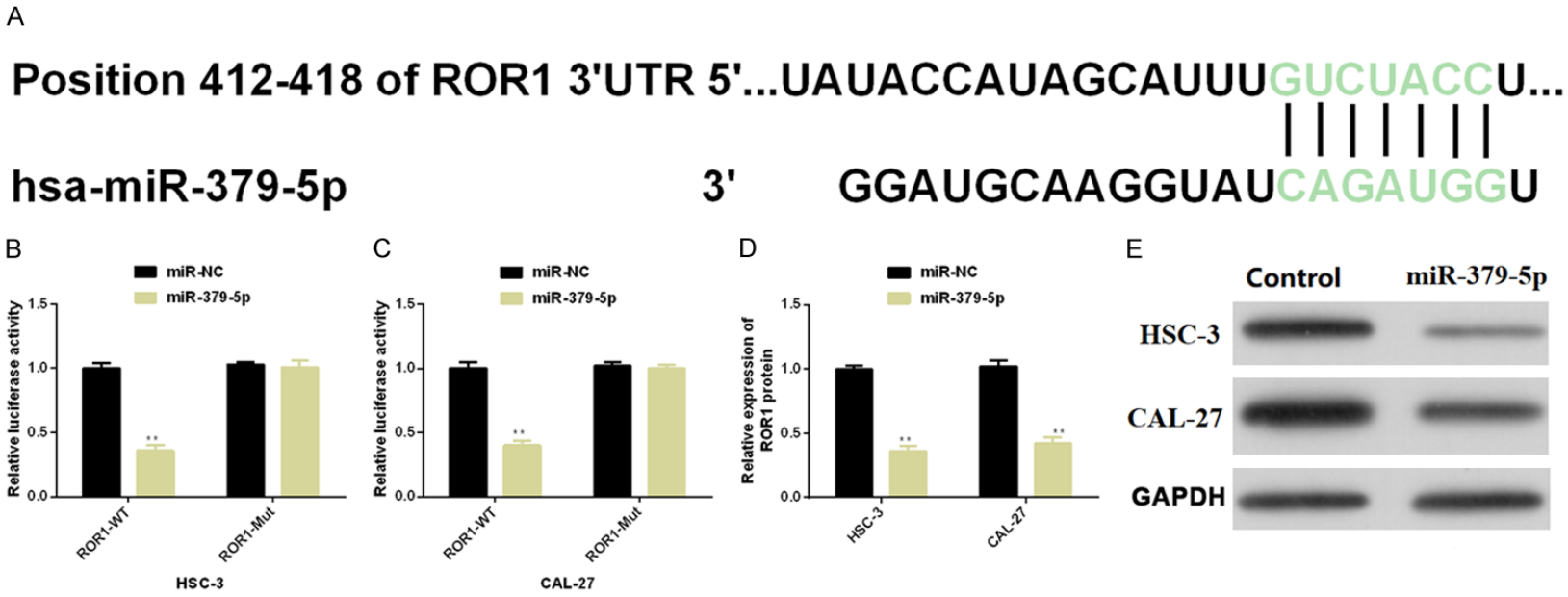

Figure 3. Targeted relationship between miR-379-5p and ROR1. A. Biological analysis was used to analyze the targeted binding sites of miR-379-5P with ROR1. B, C. The effects of miR-379-5p on the luciferase activities of ROR1-WT and ROR1-Mut were analyzed using the dual-luciferase gene reporter assay. D. Western blot was performed to analyze the effect of miR-379-5p on ROR1 protein level. E. Western blot images of ROR1 protein level. Note: Compared to miR-NC, **P < 0.01.

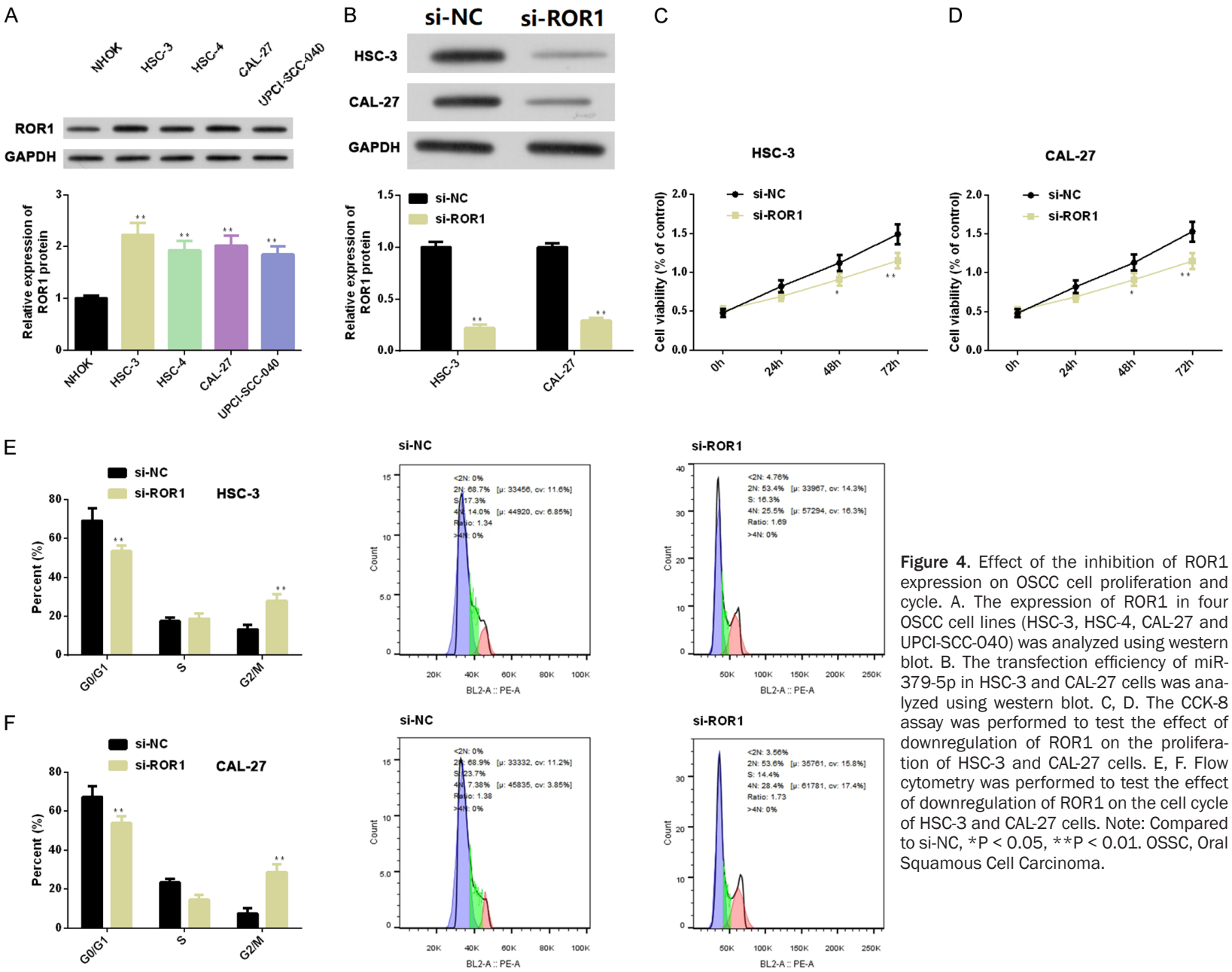

Figure 4. Effect of the inhibition of ROR1 expression on OSCC cell proliferation and cycle. A. The expression of ROR1 in four OSCC cell lines (HSC-3, HSC-4, CAL-27 and UPCI-SCC-040) was analyzed using western blot. B. The transfection efficiency of miR-379-5p in HSC-3 and CAL-27 cells was analyzed using western blot. C, D. The CCK-8 assay was performed to test the effect of downregulation of ROR1 on the proliferation of HSC-3 and CAL-27 cells. E, F. Flow cytometry was performed to test the effect of downregulation of ROR1 on the cell cycle of HSC-3 and CAL-27 cells. Note: Compared to si-NC, *P < 0.05, **P < 0.01. OSSC, Oral Squamous Cell Carcinoma.

expression could significantly decrease the percentage of cells in the G0/G1 phase and increase the percentage of cells in the G2/M phase ($P < 0.05$, **Figure 4E**, **4F**). This indicates that downregulation of ROR1 expression had an effect similar to that of the upregulation of miR-379-5p expression; that is, downregulation of ROR1 could further suppress cell proliferation by blocking the OSCC cells in the G2/M phase, thereby inhibiting OSCC progression.

Effects of miR-379-5p targeting ROR1 on DDP resistance of OSCC cells

We established DDP-resistant OSCC cells (HSC-3/DDP) to further analyze the influence of the miR-379-5p-ROR1 axis on the DDP resistance of OSCC cells. miR-379-5p expression in HSC-3/DDP cells was lower than that of HSC-3 cells (**Figure 5A**). Under DDP stimulation, the proliferation ability of HSC-3/DDP cells was higher than that of HSC-3 cells (**Figure 5B**), while the apoptosis level of HSC-3/DDP cells was lower than that of HSC-3 cells (**Figure 5C**). In the analysis of DDP resistance, the upregulation of miR-379-5p expression or the downregulation of ROR1 expression could suppress the proliferation of HSC-3 cells and reduce DDP resistance, whereas co-overexpression of miR-379-5p and ROR1 could offset these effects ($P < 0.05$) (**Figure 5D**, **5E**). Thus, miR-379-5p can target ROR1 to regulate the DDP resistance of OSCC cells.

Effects of miR-379-5p and ROR1 co-overexpression on proliferation and cell cycle of OSCC cells

miR-379-5p and ROR1 were overexpressed simultaneously. The results revealed that the proliferation (**Figure 6A**, **6B**) and cycle (**Figure 6C**, **6D**) of OSCC cells transfected with miR-379-5p+AAV-NC were not significantly different from those of OSCC cells transfected with miR-379-5p mimetic ($P > 0.05$). Compared to the transfection of miR-379-5p+AAV-NC, the co-overexpression of miR-379-5p and ROR1 could offset the inhibitory effect of miR-379-5p mimetic on OSCC cell proliferation ability and the blocking effect on the G2/M phase ($P < 0.05$), which was not significantly different from miR-NC ($P > 0.05$; **Figure 6A-D**). The above results reveal that the overexpression of ROR1 can counteract the antitumor effect of the upregulation of miR-379-5p expression on OSCC cells.

Discussion

Oral squamous cell carcinoma (OSCC) is one of the most common cancers worldwide [21]. The regulatory mechanism of miRNAs in the carcinoma process has been confirmed [22]. However, the clinical application value of miRNAs in OSCC has not been fully determined [23].

The regulation of miR-379-5p can have therapeutic effects [24]. miR-379-5p is closely associated with patients' epithelial-mesenchymal transformation phenotype [25]. In contrast, ROR1 is overexpressed in breast cancer [26] and is upregulated in pancreatic carcinoma tumor cells; knocking down its expression inhibits metastasis [27]. The AUCs for miR-379-5p and ROR1 for identifying OSCC were above 0.800, with the former at 0.898 and the latter at 0.810, indicating that miR-379-5p and ROR1 have strong diagnostic potential for OSCC.

Researchers have analyzed the regulatory mechanism of the miRNA-mRNA axis in OSCC. Lu et al. [28] reported that miR-654-5p can promote the survival and metastasis of OSCC cells by targeting Grb-2 related adapter protein, and it can also serve as a marker for the diagnosis and prognosis of OSCC. miR-127-3p has been reported to target the driver protein family member 3B, which can suppress the proliferation, migration, invasiveness, and other malignant behaviors of OSCC cells [29]. Maruyama et al. [30] revealed that miR-935 can suppress the development of OSCC tumors by downregulating the expression of type IA inositol polyphosphate-4-phosphatase, possibly exerting a therapeutic effect.

In our study, upregulation of miR-379-5p expression could suppress the proliferation, cell cycle, and DDP resistance of OSCC cells, which suggests that miR-379-5p inhibits OSCC. By exploring the underlying mechanism, we concluded that miR-379-5p could negatively regulate ROR1 and that downregulation of ROR1 expression could suppress the above-mentioned biologic functions of OSCC cells and improve sensitivity to DDP (**Figure 7**).

These findings suggest that knocking down ROR1 or upregulating miR-379-5p expression can exert therapeutic effects on OSCC in vitro. We also performed a miR-379-5p and ROR1 co-transfection experiment and found that their

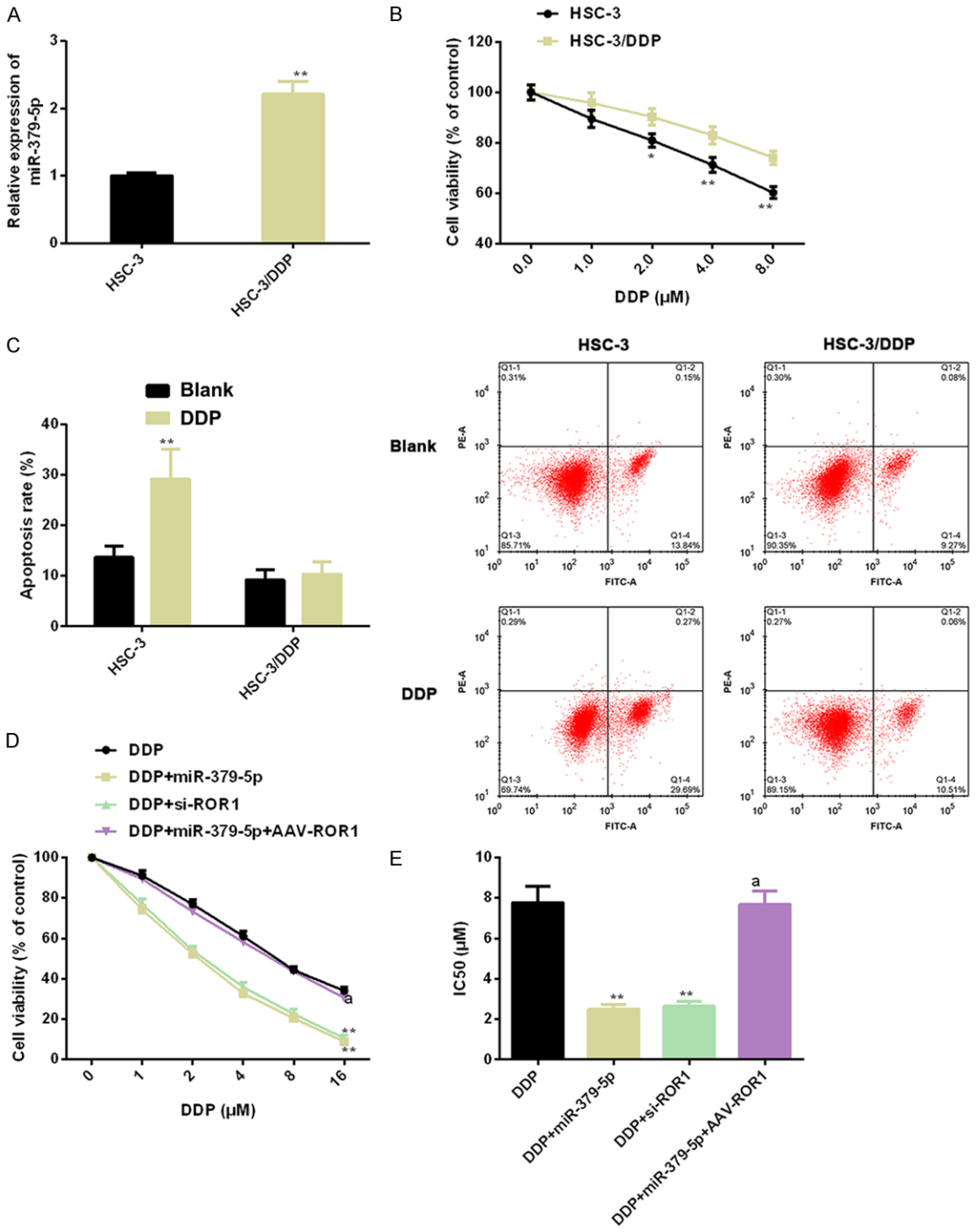

Figure 5. Effect of miR-379-5p targeting ROR1 on the DDP resistance of OSCC cells. A. RT-PCR was performed to determine the expression of miR-379-5p in HSC-3/DDP and HSC-3 cells. B. The CCK-8 assay was performed to determine the effect of DDP stimulation on the proliferative ability of HSC-3/DDP and HSC-3 cells. C. Flow cytometry was performed to determine the effect of DDP stimulation on the apoptosis of HSC-3 cells. D, E. The effects of miR-379-5p targeting ROR1 on the DDP resistance of HSC-3 cells was analyzed using the CCK-8 assay. Note: Compared to HSC-3/DDP, *P < 0.05, **P < 0.01. Compared to DDP+miR-379-5p, [a]P < 0.01. OSSC, Oral Squamous Cell Carcinoma; RT-PCR, Real-Time Polymerase Chain Reaction.

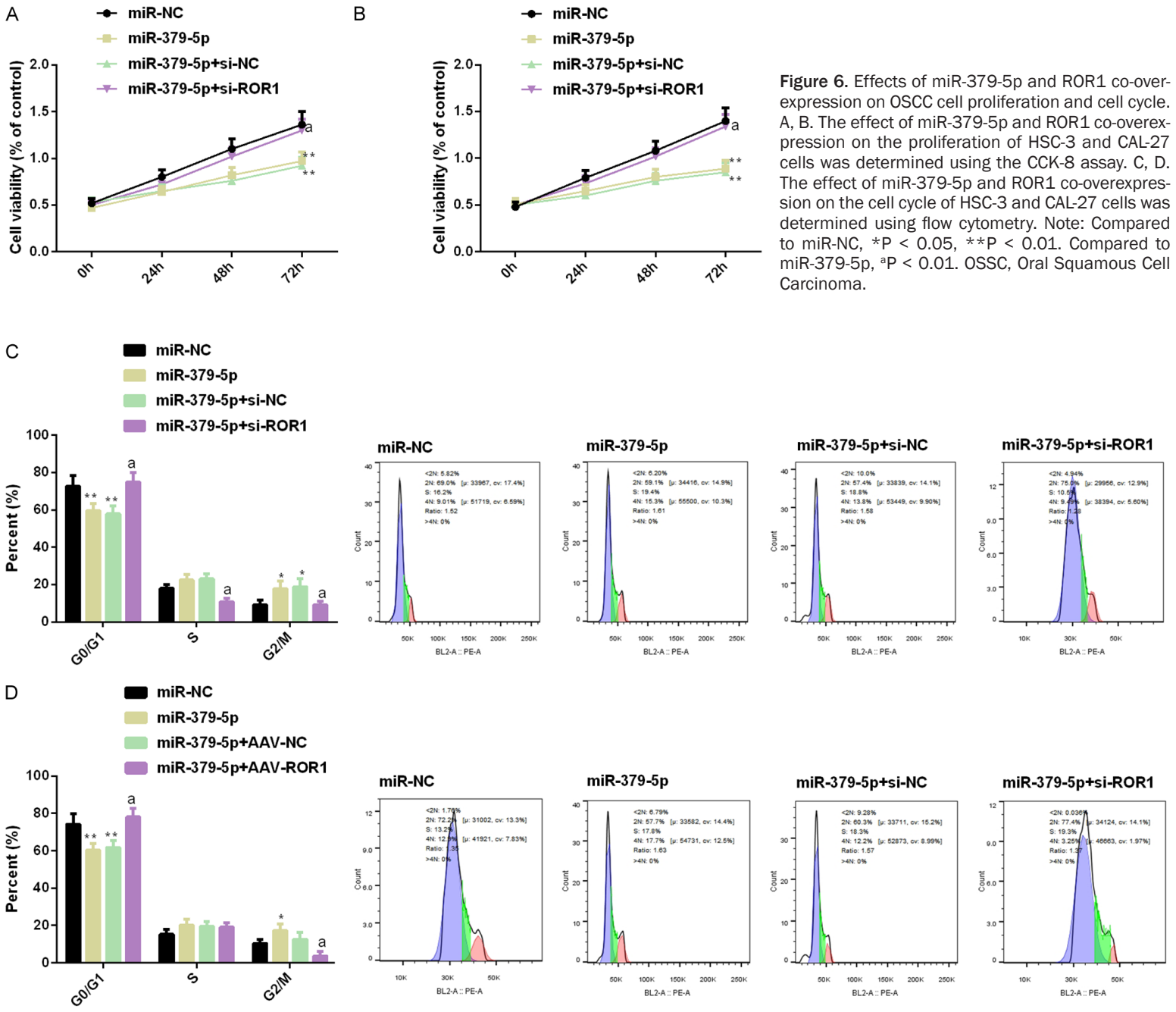

Figure 6. Effects of miR-379-5p and ROR1 co-overexpression on OSCC cell proliferation and cell cycle. A, B. The effect of miR-379-5p and ROR1 co-overexpression on the proliferation of HSC-3 and CAL-27 cells was determined using the CCK-8 assay. C, D. The effect of miR-379-5p and ROR1 co-overexpression on the cell cycle of HSC-3 and CAL-27 cells was determined using flow cytometry. Note: Compared to miR-NC, *P < 0.05, **P < 0.01. Compared to miR-379-5p, [a]P < 0.01. OSSC, Oral Squamous Cell Carcinoma.

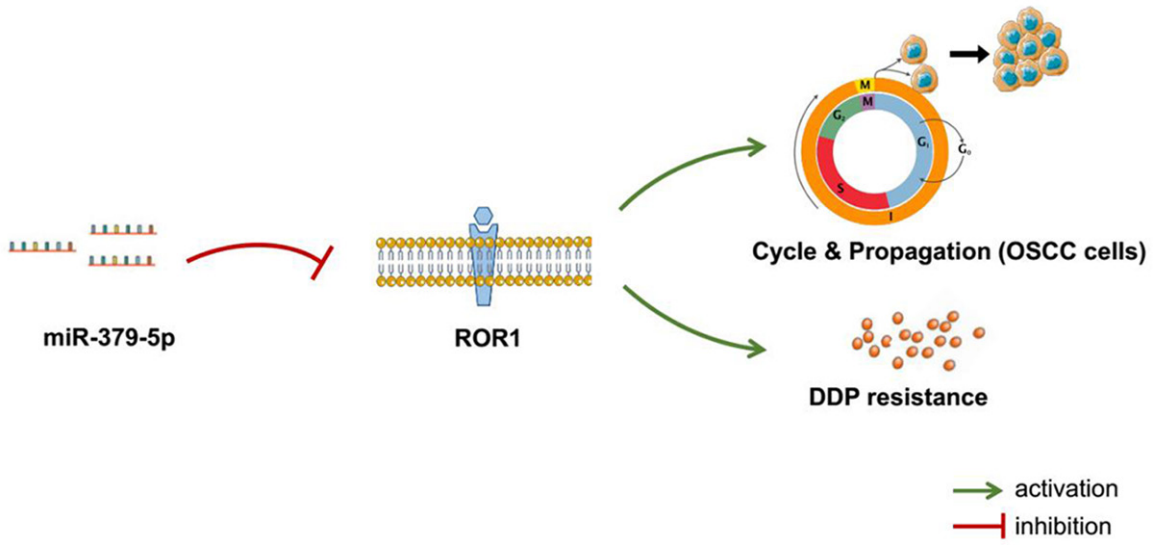

Figure 7. Schematic diagram of the regulatory mechanism of miR-379-5p in OSCC. OSSC, Oral Squamous Cell Carcinoma.

co-overexpression could offset the above anti-OSCC effects, further confirming their targeted regulatory relationship in OSCC.

Although this study demonstrated that restoring miR-379-5p expression and knocking down ROR1 expression can inhibit the progression of OSCC, there is still room for further research. First, we can supplement the analysis of upstream target candidates of miR-379-5p, which is of great significance for expanding the OSCC molecular regulatory network. Second, we can further analyze the molecular regulatory pathways that are highly related to the pathologic progression of OSCC, to clarify the mechanism of the miR-379-5p-ROR1 axis. Finally, we can perform animal studies to investigate the therapeutic effects of this axis in vivo.

In conclusion, we have proven for the first time that miR-379-5p regulates the proliferation, cell cycle, and DDP resistance of tumor cells in OSCC by targeting ROR1 expression and that miR-379-5p can serve as an early biological indicator for OSCC. This research may provide new insight and strategies for the diagnosis and treatment of OSCC.

Disclosure of conflict of interest

None.

Address correspondence to: Yinzhong Duan, State Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi Clinical Research Center for Oral Diseases, Department of Orthodontics, School of Stomatology, The Fourth Military Medical University, No. 145 West Changle Road, Xi'an 710032, Shaanxi, P. R. China. Tel: +86-29-84776137; E-mail: duanyinzhong0311@hotmail.com

References

[1] Li Q, Sun Q and Zhu B. LncRNA XIST inhibits the progression of oral squamous cell carcinoma via sponging miR-455-3p/BTG2 axis. Onco Targets Ther 2020; 13: 11211-11220.

[2] Weng X, Li JS and Fan S. Mechanism of long-chain noncoding RNA PCGEM1 in the regulation of the invasion and metastasis of oral squamous carcinoma cells via transforming growth factor β2/Smad2 signaling pathway. Hua Xi Kou Qiang Yi Xue Za Zhi 2020; 38: 550-557.

[3] Sun M and Shen Z. Knockdown of long noncoding RNA (lncRNA) colon cancer-associated transcript-1 (CCAT1) suppresses oral squamous cell carcinoma proliferation, invasion, and migration by inhibiting the discoidin domain receptor 2 (DDR2)/ERK/AKT axis. Med Sci Monit 2020; 26: e920020.

[4] Manikandan M, Deva Magendhra Rao AK, Arunkumar G, Manickavasagam M, Rajkumar KS, Rajaraman R and Munirajan AK. Oral squamous cell carcinoma: microRNA expression profiling and integrative analyses for elucidation of tumourigenesis mechanism. Mol Cancer 2016; 15: 28.

[5] Yang L, Li G, Gao Y, Ou N, Yu T and Ren S. lncRNA NR4A1AS upregulates miR-221 through demethylation to promote cell proliferation in oral squamous cell carcinoma. Cancer Manag Res 2020; 12: 5285-5292.
[6] Hou PF, Jiang T, Chen F, Shi PC, Li HQ, Bai J and Song J. KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. Cell Death Dis 2018; 9: 477.
[7] Lu Q, Ma X, Li Y, Song W, Zhang L, Shu Y and Wan B. Overexpression of FOXS1 in gastric cancer cell lines inhibits proliferation, metastasis, and epithelial-mesenchymal transition of tumor through downregulating wnt/β-catenin pathway. J Cell Biochem 2019; 120: 2897-2907.
[8] Li N, Zeng J, Sun F, Tong X, Meng G, Wu C, Ding X, Liu L, Han M, Lu C and Dai F. P27 inhibits CDK6/CCND1 complex formation resulting in cell cycle arrest and inhibition of cell proliferation. Cell Cycle 2018; 17: 2335-2348.
[9] Feng F, Cheng P, Wang C, Wang Y and Wang W. Polyphyllin I and VII potentiate the chemosensitivity of A549/DDP cells to cisplatin by enhancing apoptosis, reversing EMT and suppressing the CIP2A/AKT/mTOR signaling axis. Oncol Lett 2019; 18: 5428-5436.
[10] Chen L, Sun H, Wang C, Yang Y, Zhang M and Wong G. MiRNA arm switching identifies novel tumour biomarkers. EBioMedicine 2018; 38: 37-46.
[11] Lin X and Chen Y. Identification of potentially functional CircRNA-miRNA-mRNA regulatory network in hepatocellular carcinoma by integrated microarray analysis. Med Sci Monit Basic Res 2018; 24: 70-78.
[12] Mjelle R, Dima SO, Bacalbasa N, Chawla K, Sorop A, Cucu D, Herlea V, Sætrom P and Popescu I. Comprehensive transcriptomic analyses of tissue, serum, and serum exosomes from hepatocellular carcinoma patients. BMC Cancer 2019; 19: 1007.
[13] Zhang F, Duan C, Yin S and Tian Y. MicroRNA-379-5p/YBX1 axis regulates cellular EMT to suppress migration and invasion of nasopharyngeal carcinoma cells. Cancer Manag Res 2020; 12: 4335-4346.
[14] Chen JS, Li HS, Huang JQ, Dong SH, Huang ZJ, Yi W, Zhan GF, Feng JT, Sun JC and Huang XH. MicroRNA-379-5p inhibits tumor invasion and metastasis by targeting FAK/AKT signaling in hepatocellular carcinoma. Cancer Lett 2016; 375: 73-83.
[15] Shiah SG, Hsiao JR, Chang HJ, Hsu YM, Wu GH, Peng HY, Chou ST, Kuo CC and Chang JY. MiR-30a and miR-379 modulate retinoic acid pathway by targeting DNA methyltransferase 3B in oral cancer. J Biomed Sci 2020; 27: 46.
[16] Hassannia H, Amiri MM, Jadidi-Niaragh F, Hosseini-Ghatar R, Khoshnoodi J, Sharifian RA, Golsaz-Shirazi F, Jeddi-Tehrani M and Shokri F. Inhibition of tumor growth by mouse ROR1 specific antibody in a syngeneic mouse tumor model. Immunol Lett 2018; 193: 35-41.
[17] Zhou Q, Zhou S, Wang H, Li Y, Xiao X and Yang J. Stable silencing of ROR1 regulates cell cycle, apoptosis, and autophagy in a lung adenocarcinoma cell line. Int J Clin Exp Pathol 2020; 13: 1108-1120.
[18] Chiang CL, Goswami S, Frissora FW, Xie Z, Yan PS, Bundschuh R, Walker LA, Huang X, Mani R, Mo XM, Baskar S, Rader C, Phelps MA, Marcucci G, Byrd JC, Lee LJ and Muthusamy N. ROR1-targeted delivery of miR-29b induces cell cycle arrest and therapeutic benefit in vivo in a CLL mouse model. Blood 2019; 134: 432-444.
[19] Cetin M, Odabas G, Douglas LR, Duriez PJ, Balcik-Ercin P, Yalim-Camci I, Sayan AE and Yagci T. ROR1 expression and its functional significance in hepatocellular carcinoma cells. Cells 2019; 8: 210.
[20] Shin JH, Yoon HJ, Kim SM, Lee JH and Myoung H. Analyzing the factors that influence occult metastasis in oral tongue cancer. J Korean Assoc Oral Maxillofac Surg 2020; 46: 99-107.
[21] Chen R, Zhang Y and Zhang X. MiR-1254 functions as a tumor suppressor in oral squamous cell carcinoma by targeting CD36. Technol Cancer Res Treat 2019; 18: 1533033819859447.
[22] Aali M, Mesgarzadeh AH, Najjary S, Abdolahi HM, Kojabad AB and Baradaran B. Evaluating the role of microRNAs alterations in oral squamous cell carcinoma. Gene 2020; 757: 144936.
[23] Nakashima H, Yoshida R, Hirosue A, Kawahara K, Sakata J, Arita H, Yamamoto T, Toya R, Murakami R, Hiraki A, Shinohara M, Ito T, Kuwahara Y and Nakayama H. Circulating miRNA-1290 as a potential biomarker for response to chemoradiotherapy and prognosis of patients with advanced oral squamous cell carcinoma: a single-center retrospective study. Tumour Biol 2019; 41: 1010428319826853.
[24] Wu D, Niu X, Tao J, Li P, Lu Q, Xu A, Chen W and Wang Z. MicroRNA-379-5p plays a tumor-suppressive role in human bladder cancer growth and metastasis by directly targeting MDM2. Oncol Rep 2017; 37: 3502-3508.
[25] Ji W, Diao YL, Qiu YR, Ge J, Cao XC and Yu Y. LINC00665 promotes breast cancer progression through regulation of the miR-379-5p/LIN28B axis. Cell Death Dis 2020; 11: 16.
[26] Cao J, Wang X, Dai T, Wu Y, Zhang M, Cao R, Zhang R, Wang G, Jiang R, Zhou BP, Shi J and Kang T. Twist promotes tumor metastasis in

basal-like breast cancer by transcriptionally upregulating ROR1. Theranostics 2018; 8: 2739-2751.

[27] Xu GL, Shen J, Xu YH, Wang WS and Ni CF. ROR1 is highly expressed in circulating tumor cells and promotes invasion of pancreatic cancer. Mol Med Rep 2018; 18: 5087-5094.

[28] Lu M, Wang C, Chen W, Mao C and Wang J. MiR-654-5p targets GRAP to promote proliferation, metastasis, and chemoresistance of oral squamous cell carcinoma through Ras/MAPK signaling. DNA Cell Biol 2018; 37: 381-388.

[29] Ji L, Zhu ZN, He CJ and Shen X. MiR-127-3p targets KIF3B to inhibit the development of oral squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2019; 23: 630-640.

[30] Maruyama N, Umikawa M, Matsumoto H, Maruyama T, Nishihara K, Nakasone T, Matayoshi A, Goto T, Hirano F, Arasaki A, Nakamura H, Matsuzaki G and Takaesu G. MiR-935 inhibits oral squamous cell carcinoma and targets inositol polyphosphate-4-phosphatase type IA (INPP4A). Anticancer Res 2020; 40: 6101-6113.