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ORIGINAL RESEARCH

MiRNA-128 and MiRNA-142 Regulate Tumorigenesis and EMT in Oral Squamous Cell Carcinoma Through HOXA10

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Background: Oral squamous cell carcinoma (OSCC) accounts for more than 90% of all oral cavity cancers, and the 5-year survival rate for OSCC patients remains unsatisfactory. MiRNA-128/miRNA-142 has been reported to work as a tumor suppressor in diverse tumors. However, the biological function of miR-128/miR-142 in OSCC is still unknown.

Methods: The expression of miR-128/miR-142 and homeobox A10 (HOXA10) in OSCC tissues and cells was measured by quantitative real-time polymerase chain reaction (RT-qPCR). The effects of miR-128/miR-142 or HOXA10 on proliferation, migration, invasion and apoptosis were detected by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT), transwell and flow cytometry assays, respectively. The expression levels of epithelial–mesenchymal transition (EMT)-associated proteins (E-cadherin, N-cadherin and Vimentin), proliferation-associated protein ki-67 and HOXA10 were detected by Western blot assay. The interaction between HOXA10 and miR-128/miR-142 was predicted by TargetScan, and then confirmed by dual-luciferase reporter assay.

Results: MiR-128/miR-142 was downregulated in OSCC tissues and cells. Overexpression of miR-128/miR-142 inhibited proliferation, migration, invasion and EMT and induced apoptosis in OSCC cells. HOXA10 as the target of miR-128/miR-142 was verified in OSCC cells. Knockdown of HOXA10 also repressed proliferation, migration, invasion and EMT and boosted apoptosis in OSCC cells. Upregulation of miR-128/miR-142 hindered the expression level of HOXA10, while introduction of HOXA10 weakened the effect.

Conclusion: MiR-128/miR-142 suppressed OSCC tumorigenesis and metastasis by targeting HOXA10, providing a new promising therapeutic approach for OSCC patient diagnosis and treatment.

Keywords: miR-128, miR-142, oral squamous cell carcinoma, HOXA10, tumorigenesis, EMT

Introduction

Oral squamous cell carcinoma (OSCC), an epithelial neoplasm, originates from the epithelium lining of the oral cavity.[1] OSCC comprises more than 90% of all oral cavity cancers, and approximately 354, 864 new cases and 177, 384 deaths occurred in global in 2018.[2] Numerous pathogenic factors have been considered to be associated with the tumorigenesis of OSCC, such as smoking alcohol abuse, human papillomavirus infection.[3] Despite the great progress in diagnosis, surgery and chemotherapy strategy, the 5-year survival rate for OSCC patients remains unsatisfactory due to local recurrence and metastasis.[4] Hence, it is still essential and urgent to appraise new therapeutic targets for OSCC treatment.

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MicroRNAs (miRNAs) are a type of short, endogenous, non-coding RNAs that participate in regulation of protein-coding genes by binding to 3′UTR of target mRNA.[5] Increasing studies indicated that ectopic expression of miRNA has been frequently found and closely correlated with tumor initiation, progression and epithelial–mesenchymal transition (EMT).[6–8] Also, miRNAs could serve as potential biomarkers for the diagnosis of oral diseases.[9,10] MiRNA-128 (miR-128), a brain-enriched miRNA, has tissue-specific and development-specific expression patterns in neurons.[11] Moreover, studies showed that miR-128 was lower expressed and functioned as a tumor suppressor in diverse tumors.[12–15] MiRNA-142 (miR-142), a hematopoietic miRNA,[16] has been proved to repress tumor progression in multiple cancers.[17,18] Besides, some reports have indicated that miR-142 can be a potential therapeutic agent for patients with gastric cancer[19] and ovarian cancer.[20] However, the biological function of miR-128 and miR-142 in OSCC is still unknown.

Homeobox A10 (HOXA10), a member of the superfamily of homeobox genes, plays an important role in fertility, embryonic viability and regulates hematopoietic lineage commitment.[21] HOXA10 has been verified to serve as an oncogene and promote the development of tumor by interacting with miRNA in head and neck squamous cell carcinoma[22] and oral cancer.[23] Moreover, it was proved that HOXA10 could regulate EMT-relative protein expression to promote EMT process in lung adenocarcinoma,[24] ovarian cancer[25] and head and neck squamous cell carcinoma.[26] Furthermore, a prior report proved that HOXA10 was highly expressed and closely related to proliferation, migration, invasion, apoptosis and expression of EMT markers in OSCC cells,[27] suggesting that HOXA10 is crucial in OSCC progression.

Therefore, in this study, we analyzed the expression levels of miR-128 and miRNA-142, as well as their effects on cell proliferation, migration, invasion, apoptosis and EMT of OSCC cells. Besides, the potential mechanism was investigated in order to offer potential therapeutic strategies for OSCC.

Materials and Methods

Clinical Specimens and OSCC Cell Culture

Samples of histopathologically confirmed OSCC tumor tissues and paired nontumor tissues (over 2 cm from tumor tissues) were collected from 60 patients (33 males, 27 females; aged 43–68 years, mean age 61.7) undergone oral surgical operation at Central Hospital of Taian from 2017 March to 2019 January. All patients did not suffer other treatment before the operation. Patients with complete information of medical records were included, and patients with distant metastasis, clinical disorders or history of serious disease such as heart disease and other malignancies were excluded. This research was approved by the Central Hospital of Taian and every participant signed the written informed consent.

Normal human Oral Keratinocyte cell line (NOK) was collected from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Human OSCC cell lines (SCC-9 and SSC-25) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All OSCC cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA). NOK was incubated in Keratinocyte-SFM medium (Invitrogen). All cells were cultured in a 5% CO_2 incubator at 37°C.

Cell Transfection

HOXA10 overexpression vector was obtained by cloning the sequences of HOXA10 into pcDNA3.1 (Invitrogen), termed pcDNA3.1-HOXA10 (HOXA10). HOXA10 small interference RNA (si-HOXA10) and its scrambled negative control (si-control), miR-128 mimic (miR-128), miR-142 mimic (miR-142) and their scrambled negative control (miR-control) were collected from RiboBio (RiboBio, Guangzhou, China). All aforementioned plasmids or oligonucleotides were transfected into SCC-9 and SSC-25 cells in accordance with the operation manual of Lipofectamine 2000 reagent (Invitrogen).

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Assay

Total RNA was extracted from OSCC tissues and OSCC cell lines (after 48-h transfection) in accordance with the instructions of TRIzol reagent (Gibco). Reversely transcription assays were carried out to synthesize complementary DNA (cDNA) using PrimeScript™ RT Master Mix Kit (Takara, Tokyo, Japan). The quantitative analysis HOXA10 was carried out with the SYBR® Premix Ex Taq™ (Takara). The cDNA amplification of miR-128/miR-142 was implemented with a mirVana™ real-time RT-PCR microRNA detection kit

(Life Technologies, Carlsbad, CA, USA). The relative expression of HOXA10 and miR-128/miR-142 was calculated using $2^{-\Delta\Delta Ct}$ method, and normalized by house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6 small nuclear RNA, respectively. The primer sequences in RT-qPCR assay were listed as follows: HOXA10: 5′-GGATCGGACAGACAAGTGAAAATCTT-3′ (sense), 5′-GGAAGTGAAAAAACCGCGTCGCCTGG −3′ (antisense); GAPDH: 5′-AGAAGGCTGGGGCTCATTTG-3′ (sense), 5′-AGGGGCCATCCACAGTCTTC-3′ (antisense); miR-128: 5′-ACACTCCAGCTGGGTCACAGTGAACCGGTC −3′ (sense), 5′- TGGTGTCGTGGAGTCG-3′ (antisense); miR-142: 5′-GTATGGATCCTCTTAGGAAGCCACAAGGAG-3′ (sense), 5′- TATCAAGCTTTAAGGTGCTCACCT GTCACA-3′ (antisense); U6, 5′-GCTTCGGCAGCAC ATATACTAAAA-3′ (sense), 5′-CGCTTCACGAATTTG CGTGTCAT −3′ (antisense).

Cell Proliferation Assay

Cell proliferation was detected with 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay according to user's guidebook. Briefly, 6×10^3 transfected SCC-9 and SSC-25 cells were seeded into 96-well plates and cultured for 48 h. Then, at the different time points (0 h, 24 h, 48 h, 72 h), each well was added with 20 μL MTT solution (5 mg/mL; Sigma-aldrich Corp, St. Louis, MO, USA) and then cells incubated for another 4 h at 37°C. Cell supernatants were removed and each well was added with 100 μL dimethyl sulfoxide (DMSO, Sigma-aldrich) to dissolve formed formazan crystals. The optical density (OD) was detected with a microplate reader at 490nm.

Cell Apoptosis Assay

The apoptosis of SCC-9 and SSC-25 cells was monitored by flow cytometry with an Annexin V-FITC/PI Apoptosis Detection Kit (Invitrogen) following the user's guidebook. Generally, after 48-h transfection, SCC-9 and SSC-25 cells were washed and fixed on ice for 1 h. Then, treated SCC-9 and SSC-25 cells were resuspended with Binding buffer and stained with 5 μL Annexin V-FITC/PI referring to the operation manual. At last, apoptotic SCC-9 and SSC-25 cells were detected using the FACSan flow cytometry (BD Bioscience, San Jose, CA, USA) and analyzed using Cell Quest software (BD Bioscience).

Western Blot Assay

Total protein was harvested using pre-cold RIPA buffer reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Extracted proteins were separated by 10% SDS-PAGE followed by electrophoretically transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA). Then the membrane was sealed with 5% skim milk (dissolved in TBST buffer) at room temperature for 1h. Then, the membrane was incubated with primary antibody against HOXA10 (1:500, ab90641, Abcam, Cambridge, UK), ki-67 (1:500, ab16667, Abcam), E-cadherin (1:200, ab1416, Abcam), N-cadherin (1:600, ab76011, Abcam), Vimentin (1:200, ab92547, Abcam) or β-actin (1:1000, ab3280, Abcam) overnight at 4°C, following by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at 37°C. Finally, the blots were detected with the ECL detection kit (Pierce Biotechnology, Rockford, IL, USA).

Cell Migration and Invasion Assay

Cell migration and invasion were detected using Transwell chambers (24-well, Sigma-Aldrich) based on the operation manual. In briefly, transfected SCC-9 and SSC-25 cells were resuspended in serum-free DMEM (Gibco). Then, SCC-9 and SSC-25 cells were seeded into the upper chamber without coated-matrigel (BD Biosciences, Franklin Lakes, NJ, USA) at a density of 8×10^4 cells/well for the migration assay, whereas SCC-9 and SSC-25 cells were introduced into the upper chamber coated with matrigel (BD Biosciences) at a density of 1×10^5 cells/well for invasion assay. Meanwhile, the lower chamber was added with the DMEM (Gibco) containing 10% FBS (Invitrogen) as chemoattractant. After 24-h incubation, the non-migrated and non-invasive cells were scraped with cotton swabs on the upper chamber, while cells transferred to membrane were fixed with methanol, and stained with crystal violet solution. Finally, migrated or invaded cells were observed with the inverted microscope (magnification × 400, Olympus).

Dual-Luciferase Reporter Assay

The target gene of miR-128/miR-142 was predicted by TargetScan online prediction software. Based on the bioinformatics prediction results, partial HOXA10 3′UTR sequence containing wild-type or mutant-type miR-128/miR-142 targeting sites were amplified and cloned into pmirGLO vector (Promega, Madison, Wisconsin, USA), termed WT-HOXA10-3′UTR and MUT-HOXA10 reporter plasmids. Then, these constructed plasmids were cotransfected with miR-control, miR-128 or miR-142 into SCC-9 or SSC-25 cells and incubated for 48 h. Finally, dual-luciferase reporter

assays kit (Promega) was used to detect the luciferase activity in cell lysates.

Statistical Analysis

Data were analyzed with the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Experimental data were presented as mean ± standard deviation (SD). Every assay was repeated independently at least three times. For statistical comparisons, two groups or numerous groups were treated with Student's *t*-test or one way analysis of variance (ANOVA). *P*-value<0.05 was considered to suggest a statistically significant difference.

Results

MiR-128 and MiR-142 Expression are Downregulated in OSCC Tissues and Cells

To ascertain the roles of miR-128 and miR-142 in OSCC, their expression patterns were firstly measured by RT-qPCR assay. According to the results shown in Figure 1A and B, miR-128 and miR-142 expression were downregulated in cancerous tissues in comparison with adjacent normal tissues collected from 60 patients with OSCC. Similarly, the obvious low levels of miR-128 and miR-142 in OSCC cell lines (SCC-9 and SSC-25) were determined (Figure 1C and D), indicating the involvement of miR-128 and miR-142 in OSCC progression.

MiR-128 and MiR-142 Suppress Proliferation, Migration and Invasion, While Induce Apoptosis in OSCC Cells

Next, to identify the functional roles of miR-128 and miR-142, the gain-of-function studies using miRNA mimics transfected SCC-9 and SSC-25 cells were performed. As exhibited in Figure 2A and B, the expression levels of miR-128 and miR-142 were remarkably upregulated in SCC-9 and SSC-25 cells transfected with miRNA mimics relative to cells transfected with control. Thus, we adopted the gain-of-function experiments to further probe the roles of miR-128 and miR-142 in OSCC development, including in proliferation, apoptosis, migration and invasion. Firstly, the results of MMT assay and proliferation index ki-67 expression showed that cell proliferation was significantly decreased after transfection of miR-128 or miR-142, while flow cytometry assays displayed that transfection of miR-128 or miR-142

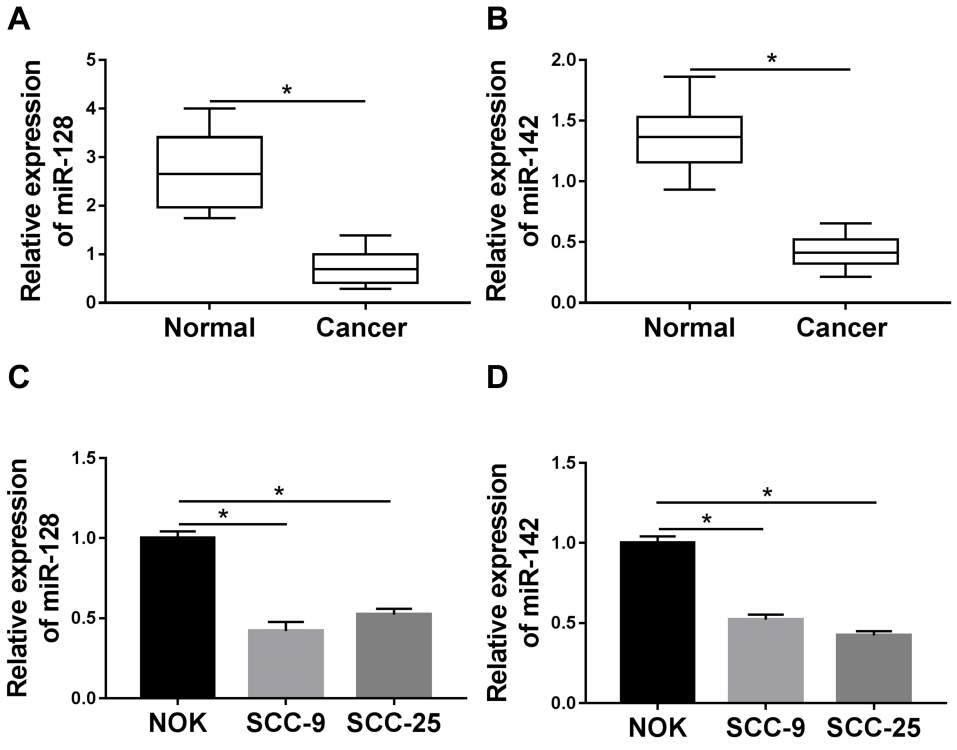

Figure 1 MiR-128 and miR-142 are downregulated in OSCC tissues and cells. (**A** and **B**) RT-qPCR assay was implemented to detect the expression profiles of miR-128 and miR-142 in 60 pairs of OSCC tissues and adjacent nontumor tissues. (**C** and **D**) Expression levels of miR-128 and miR-142 in OSCC cell lines (SCC-9 and SSC-25) and normal Human Oral Keratinocyte cell line (NOK) were measured by RT-qPCR. **P* <0.05.

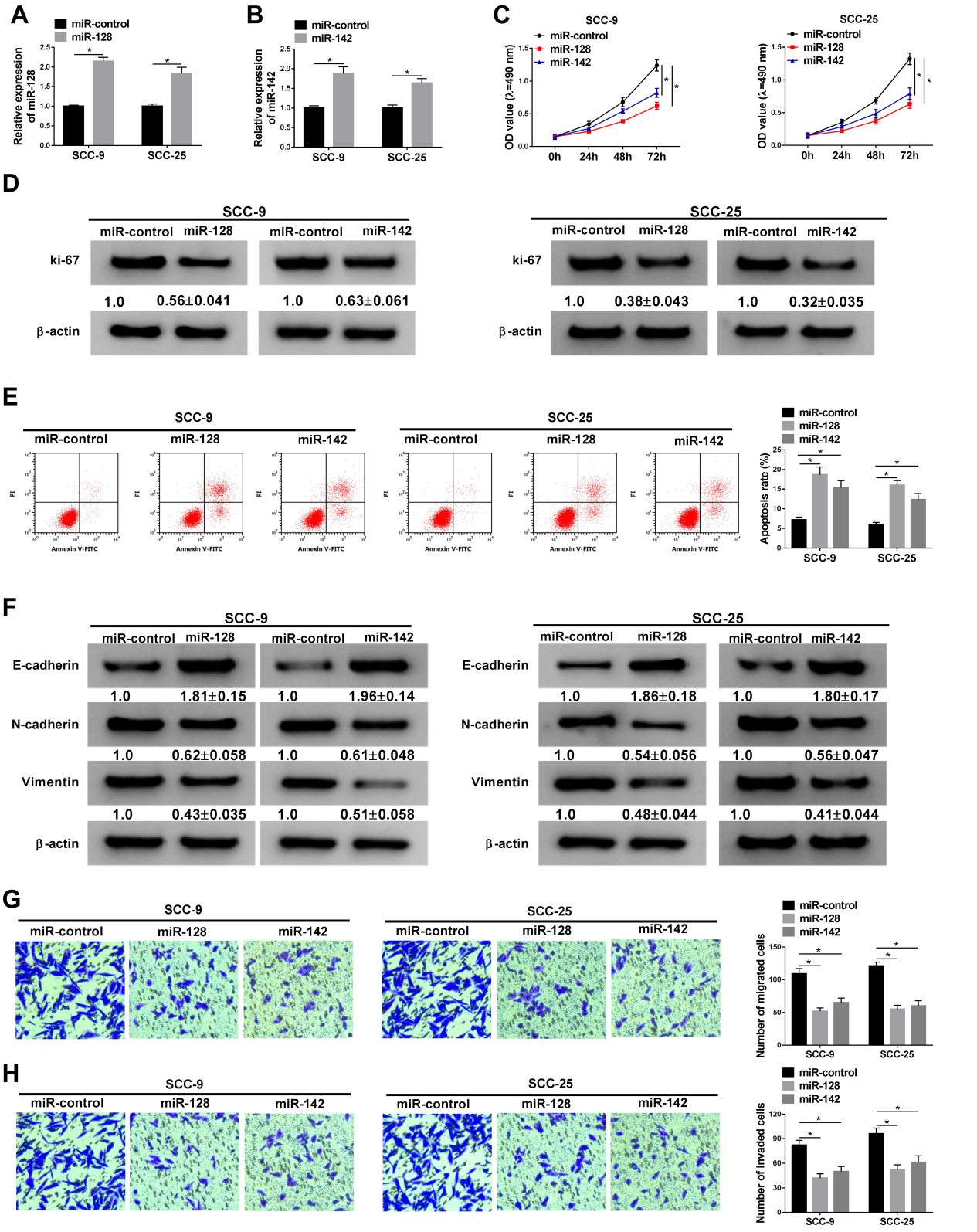

Figure 2 MiR-128 and miR-142 suppress proliferation, migration and invasion, while induce apoptosis in OSCC cells. SCC-9 and SSC-25 cells were transfected with miR-128, miR-142 or miR-control. (**A** and **B**) Transfection efficiency of mature miR-128 or miR-142 in SCC-9 and SSC-25 cells was determined by RT-qPCR. (**C**) Proliferation of transfected SCC-9 and SSC-25 cells was tested by MTT assay. (**D**) The expression level of proliferation-related protein ki-67 was detected by Western blot assays. (**E**) Apoptosis analysis in mature miR-128 or miR-142-transfected SCC-9 and SSC-25 cells was conducted by flow cytometry. (**F**) Levels of EMT-related proteins (E-cadherin, N-cadherin and Vimentin) were detected by Western blot assay in transfected SCC-9 and SSC-25 cells. (**G** and **H**) Cell migration and invasion in mature miR-128 or miR-142-transfected SCC-9 and SSC-25 cells were analyzed via Transwell assay. *P <0.05.

elevated the apoptotic rate of SCC-9 and SSC-25 cells (Figure 2C–E). Then, we further explored the impact of miR-128 and miR-142 on the migratory and invasive abilities of OSCC cells. Since EMT is a key step in tumor metastasis,[28] EMT-associated markers or transcription factors were detected by Western blot assays. The protein levels of N-cadherin and Vimentin were decreased, but E-cadherin expression was increased in SCC-9 and SSC-25 cells transfected with miR-128 or miR-142 (Figure 2F), suggesting the suppressed roles of miR-128 and miR-142 in the EMT of OSCC cells. As presented in Figure 2G and H, the overexpression of miR-128 and miR-142 obviously constrained the capabilities of migratory and invasive abilities in SCC-9 and SSC-25 cells. Collectively, miR-128/miR-142 inhibited the OSCC tumorigenesis in vitro.

HOXA10 is the Target Gene of MiR-128/MiR-142

It is well known that miRNA can exert the function by interacting with the expression of target mRNAs. Therefore, an online predicted website TargetScan was used to search the potential target mRNA of miR-128/miR-142. As a result, HOXA10 possessed complementary sites with miR-128/miR-142 (Figure 3A and B). To further validate the prediction, WT-HOXA10 and MUT-HOXA10 reporters were constructed. Subsequently, dual-luciferase assay manifested that miR-128/miR-142 prompted an overt reduction of the luciferase activity of WT-HOXA10 reporter, but without an evident effect on the luciferase activity of MUT-HOXA10 reporter in SCC-9 and SSC-25 cells (Figure 3C and D). Then, RT-qPCR analysis indicated that HOXA10 expressed at high level in OSCC tissues (Figure 3E and F), and was inversely associated with miR-128/miR-142 expression in OSCC tissues (Figure 3G and H). Taken together, all these data implied that miR-128/miR-142 could interact with HOXA10 to block its expression.

Silenced HOXA10 Curbs Proliferation, Migration and Invasion, Whereas Boosts Apoptosis in OSCC Cells

Then, we further confirmed that HOXA10 expression level was upregulated in SCC-9 and SSC-25 cells in contrast to

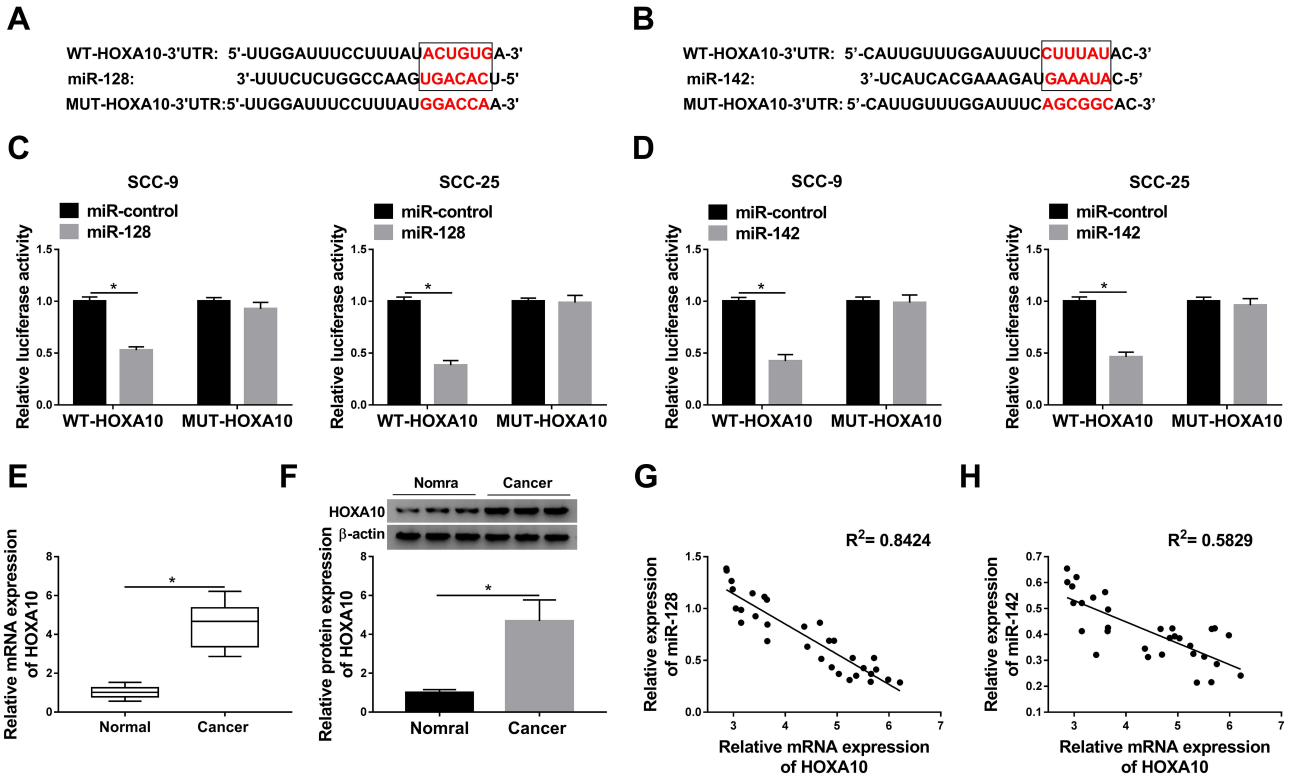

Figure 3 HOXA10 is the target gene of miR-128 and miR-142. (**A** and **B**) The binding sites between miR-128 or miR-142 and HOXA10, as well as the sequences of MUT-HOXA10 were exhibited. (**C** and **D**) The interaction between miR-128 or miR-142 and HOXA10 was confirmed by luciferase activity analysis. (**E** and **F**) The expression level of HOXA10 in OSCC tissues was detected by RT-qPCR and Western blot assays. (**G** and **H**) The expression correlation of HOXA10 with miR-128 or miR-142 in OSCC tissues was analyzed by Pearson correlation analysis. *P <0.05.

that in normal Human Oral Keratinocyte cell line (NOK) (Figure 4A and B). In order to investigate the effect of HOXA10 expression on tumor progression, the small interference RNA (siRNA) of HOXA10 and its scramble control (si-control) were synthesized and transfected into SCC-9 and SSC-25 cells. According to the data exhibited in Figure 4C and D, compared with SCC-9 and SSC-25 cells transfected with si-control, HOXA10 expression was effectively decreased in si-HOXA10-transfected SCC-9 and SSC-25 cells. Whereafter, we further explored the effect of HOXA10 knockdown on OSCC cell development. Functional assays revealed that the depletion of HOXA10 notably curbed proliferation (Figure 4E and F) and facilitated apoptosis in SCC-9 and SSC-25 cells (Figure 4G). Moreover, we also proved that deficiency of HOXA10 retarded the EMT of OSCC cell, presented as the decline of N-cadherin and Vimentin expression, and the increase of E-cadherin expression in si-HOXA10-transfected SCC-9 and SSC-25 cells (Figure 4H and I). Furthermore, the data of transwell assay showed that knockdown of HOXA10 inhibited the cell migration (Figure 4J) and invasion (Figure 4K) in OSCC cells. In a word, HOXA10 downregulation suppressed tumorigenesis and EMT of OSCC cells in vitro.

Validation of MiR-128/MiR-142-HOXA10 Regulatory Axis in OSCC Cells

Based on the above findings, we speculated that miR-128/miR-142 exerted tumor-suppressive roles in OSCC cells partly by regulating HOXA10. To verify the hypothesis, we further probed whether miR-128/miR-142 affecting the expression of HOXA10. First of all, we discovered that transfection of miR-128 induced an evident reduction of HOXA10 mRNA and protein expression in SCC-9 and SSC-25 cells (Figure 5A and B), manifesting that miR-128 could negatively regulated HOXA10 expression. Conversely, gain of HOXA10 reversed the inhibitory effect. Additionally, similar results were observed for miR-142 that transfection with HOXA10 could partially restore the inhibitory effect of HOXA10 expression by miR-142 overexpression (Figure 5C and D).

Further, the interaction between miR-128/miR-142 and HOXA10 in OSCC cells was explored. Cell proliferation was significantly decreased after overexpression of miR-128/miR-142, while addition of HOXA10 rescued the effects of miR-128/miR-142 in OSCC cells (Figure 6A and B). And then the expression of proliferation index ki-67 protein reduced after transfection of miR-128/miR-142,

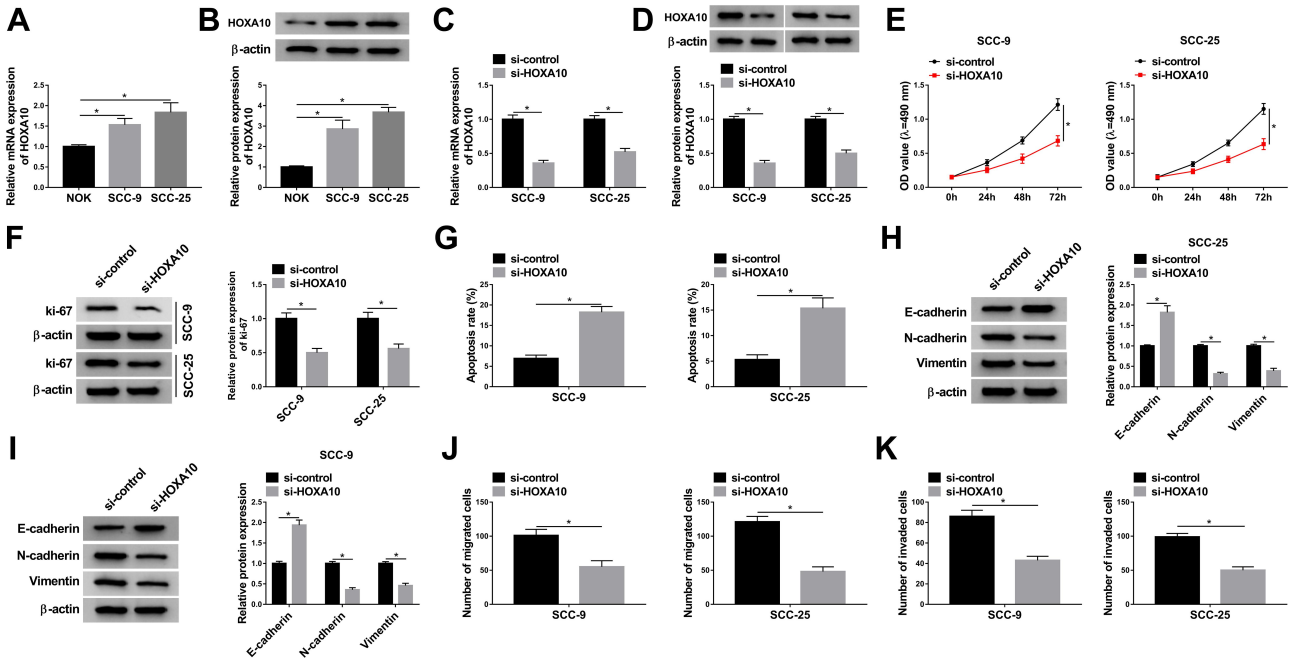

Figure 4 Silenced HOXA10 curbs proliferation, migration and invasion, whereas boosts apoptosis in OSCC cells. (**A** and **B**) HOXA10 expression level was evaluated by RT-qPCR and Western blot assays. (**C–K**) SCC-9 and SSC-25 cells were transfected with si-HOXA10 or si-control. (**C** and **D**) Knockdown efficiency of si-HOXA10 in SCC-9 and SSC-25 cells was determined by RT-qPCR and Western blot assays. (**E**) Cell proliferation of transfected SCC-9 and SSC-25 cells was examined by MTT assay. (**F**) The protein level of ki-67 was detected by Western blot assays. (**G**) Cell apoptosis of transfected SCC-9 and SSC-25 cells was evaluated through flow cytometry. (**H** and **I**) Expression of EMT-related proteins (E-cadherin, N-cadherin and Vimentin) in transfected SCC-9 and SSC-25 cells was measured by Western blot assay. (**J** and **K**) Migration and invasion of transfected SCC-9 and SSC-25 cells were measured through Transwell assay. *P <0.05.

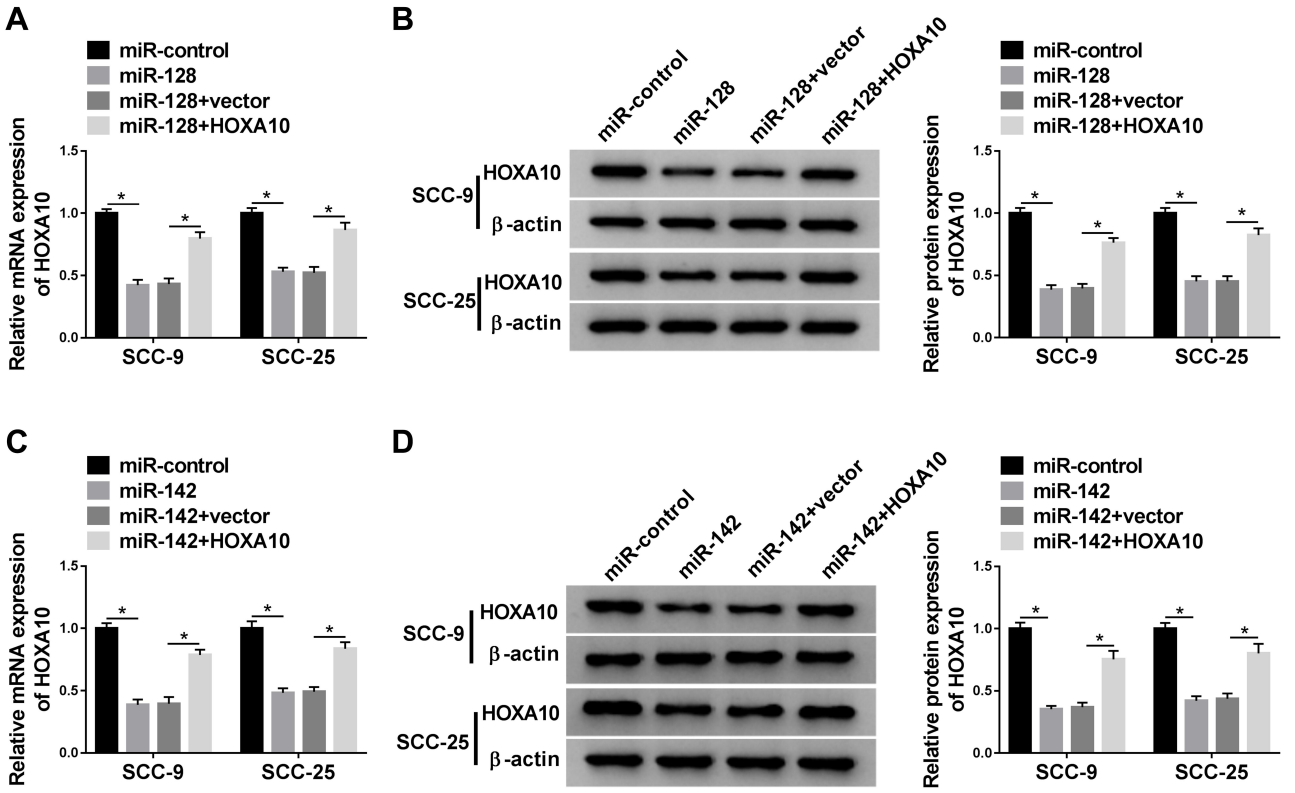

Figure 5 Overexpression of miR-128/miR-142 reduced the expression of HOXA10. (**A** and **B**) The mRNA and protein expression of HOXA10 in SCC-9 and SSC-25 cells transfected with miR-control, miR-128, miR-128 + vector or miR-128 + HOXA10 were detected by RT-qPCR and Western blot assay, respectively. (**C** and **D**) The mRNA and protein expression of HOXA10 in SCC-9 and SSC-25 cells transfected with miR-control, miR-142, miR-142 + vector or miR-142 + HOXA10 were determined through RT-qPCR and Western blot assay, respectively. *P <0.05.

however, this change would be reversed by HOXA10 overexpression (Figure 6C). In addition, cell apoptosis was upregulated after transfection of miR-128/miR-142, whereas HOXA10 could recover the effect of miR-128/miR-142 (Figure 6D). We subsequently studied the EMT process, and the expression of EMT-related proteins was remarkably downregulated after transfection of miR-128/miR-142 in SCC-9 and SCC-25 cell lines, while transfection of HOXA10 rescued the effect of EMT process (Figure 6E and F). The results of transwell assay showed that miR-128/miR-142 significantly decreased the cell migration and invasion, while addition of HOXA10 rescued the function of miR-128/miR-142 in EC cells (Figure 6G and H). Together, these data indicated that miR-128/miR-142 might exert the tumor suppressive function through targeting HOXA10 in OSCC cells.

Discussion

Accumulative evidence has manifested that miRNAs are frequently dysregulated in various tumors and act as important regulatory molecules of pathological processes correlated with oncogenesis and metastasis.[29] Previous reports have disclosed that miR-128/miR-142 could exert the function as tumor suppressors in multiform types of tumors. For example, Li et al reported that overexpression of miR-128 impeded cell proliferation by binding to HOXB8 in ovarian cancer.[30] Su et al found that miR-142 was low-expressed in endometrial cancer and miR-142 upregulation suppressed endometrial cancer proliferation via targeting cell cycle regulatory protein cyclin D1 (CCND1) in vitro and in vivo.[31] However, the roles of miR-128/miR-142 in OSCC have not been completely studied.

In this study, we firstly determined that miR-128/miR-142 was low expression in both OSCC tissues and cells relative to control group. Then, the influence of miR-128/miR-142 on proliferation, apoptosis, migration, invasion and EMT in SCC-9 and SSC-25 cells was evaluated. Results suggested that miR-128/miR-142 restrained proliferation, migration, invasion, EMT and expedited apoptosis in SCC-9 or SSC-25 cells. That is to say, miR-128/miR-142 works as a suppressive factor in OSCC development.

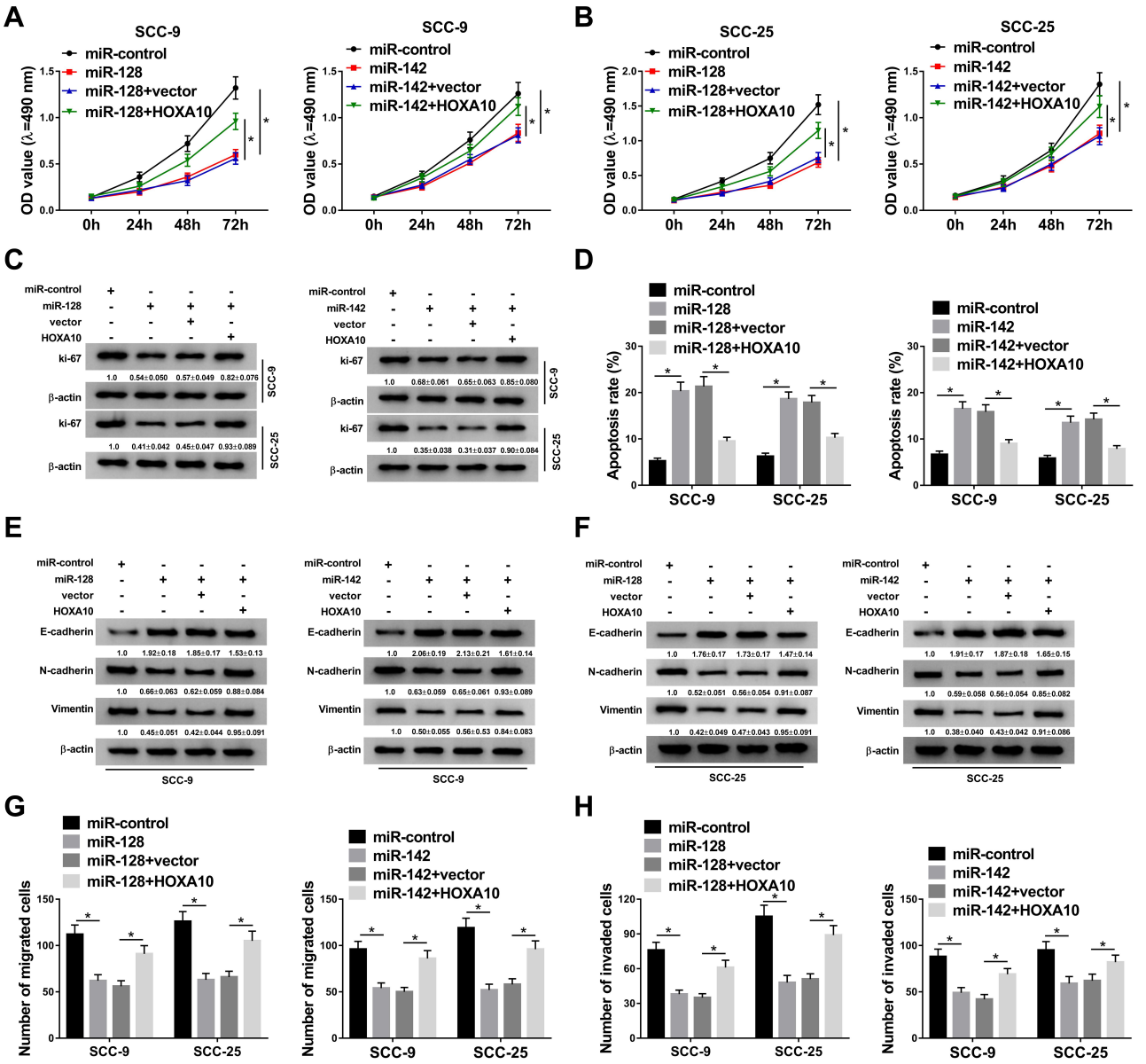

Figure 6 HOXA10 partially rescued the effects of miR-128 and miR-142 in OSCC cells. SCC-9 and SCC-25 cells were transfected with miR-control, miR-128, miR-128 and vector, miR-128 and HOXA10, miR-142, miR-142 and vector or miR-142 and HOXA10. (**A** and **B**) The cell proliferation was detected by MTT assay and Western blot assay in SCC-9 and SCC-25 cell lines. (**C**) The expression of proliferation-related protein of transfected SCC-9 and SCC-25 cells was assessed by Western blot assay. (**D**) Cell apoptosis of transfected SCC-9 and SSC-25 cells was evaluated through flow cytometry. (**E** and **F**) The protein expression of EMT-related protein E-cadherin, N-cadherin and Vimentin in transfected SCC-9 and SSC-25 cells was measured by Western blot assay. (**G** and **H**) The ability of cell migration and invasion were measured by transwell assay in OSCC cells. *P <0.05.

Additionally, we further probed the underlying molecular mechanisms of miR-128/miR-142 in blocking OSCC tumorigenesis. It is widely acknowledged that miRNA could exert its function by interacting with the target mRNAs.[32] For example, miR-128 directly targeted vascular endothelial growth factor (VEGF)-C and impeded cell proliferation in non-small cell lung cancer (NSCLC).[33] MiR-142 suppressed cervical cancer progression by targeting high-mobility group box 1 protein (HMGB1).[34] In the present study, the bioinformatics analysis predicted that HOXA10 acted as the target gene of both miR-128 and miR-142, and these relationships were further confirmed by the dual-luciferase reporter assay. What's more, our study displayed that HOXA10 expression level was upregulated in OSCC tissues and cells, and was inversely associated with miR-128/miR-142 expression level in OSCC tissues. HOXA10 has been pointed out to be tightly correlated with EMT, cell proliferation, migration and invasion in oral cancer.[25,35] Also, HOXA10 suppressed gastric cancer cell apoptosis through inducing BCL2 expression.[36] Therefore,

in the present study, we further investigated the effect of HOXA10 on proliferation, apoptosis, migration, invasion and EMT in OSCC. The function analysis revealed that downregulation of HOXA10 resulted in a marked decrease in proliferation, migration and invasion, and an obvious increase in apoptotic rate in OSCC cells. Moreover, silence of HOXA10 augmented of E-cadherin protein level and curbed N-cadherin and Vimentin protein levels, implicating that HOXA10 knockdown inhibited the EMT of OSCC cells. These data implied that HOXA10 induced tumorigenesis of OSCC in vitro.

To further verify miR-128/miR-142 served as suppressive factors in OSCC cells partially by hindering the expression of HOXA10, the effect of miR-128/miR-142 on the expression of HOXA10 was also explored. Not surprisingly, exogenetic expression of miR-128/miR-142 retarded HOXA10 expression, while this effect was counteracted following introduction of HOXA10. In addition, HOXA10 restored the effects of miR-128/miR-142 on cell proliferation, apoptosis, EMT process, migration and invasion in OSCC cells. These findings indicated that miR-128/miR-142 affected cell proliferation, migration, invasion, apoptosis and EMT process by downregulation of HOXA10 in OSCC cells. However, whether miR-128/miR-142/HOXA10 axis regulates OSCC progression in vivo has not yet been verified, which will be the focus of our further studies.

In conclusion, our study firstly verified that miR-128/miR-142 was downregulated in OSCC tissues and cells. In addition, miR-128/miR-142 acted as a tumor suppresser to markedly inhibited proliferation, migration and invasion and induced apoptosis of OSCC cells targeting HOXA10. The miR-128/miR-142-HOXA10 axis in the regulation of OSCC progression might provide the novel potential therapeutic targets for OSCC.

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Disclosure

The authors declare that they have no financial conflicts of interest.

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