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Mechanism of lncRNA FEZF1-AS1 in promoting the occurrence and development of oral squamous cell carcinoma through targeting miR-196a

L. XU, T.-J. HOU, P. YANG

Department of Stomatology, Liaocheng People's Hospital, Liaocheng, China

Lin Xu and Tiejun Hou contributed equally to this work

Abstract. – OBJECTIVE: Previous studies have demonstrated that long non-coding ribonucleic acid (lncRNA) FEZF1-AS1 acts as a cancer-promoting gene. However, no reports have investigated the role of FEZF1-AS1 in oral squamous cell carcinoma (OSCC) yet. Therefore, the aim of this study was to explore whether FEZF1-AS1 promoted the expression characteristics of OSCC by targeting miR-196a and to further elucidate the underlying mechanism of FEZF1-AS1 in promoting the metastasis of OSCC.

PATIENTS AND METHODS: The expression levels of FEZF1-AS1 and miR-196a in 42 pairs of OSCC tissues and para-carcinoma tissues were detected via quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The correlation of FEZF1-AS1 expression with clinical indexes and prognosis of OSCC patients was analyzed. Moreover, the expression levels of FEZF1-AS1 and miR-196a in OSCC cells were detected via qRT-PCR. FEZF1-AS1 knockdown and miR-196a over-expression models were established using lentivirus transfection in OSCC cell lines (CAL-27 and Tca8113). Subsequently, the influences of FEZF1-AS1 and miR-196a on the biological functions of OSCC cells were analyzed via Cell Counting Kit-8 (CCK-8) assay, colony formation assay and 5-Ethynyl-2'-deoxyuridine (EdU) assay, respectively. Furthermore, the potential mechanism was explored using the Luciferase reporter gene and recovery assays.

RESULTS: The results of qRT-PCR proved that the expression level of FEZF1-AS1 in OSCC tissues was significantly higher than that of para-carcinoma tissues, and the difference was statistically significant. The pathological stage was significantly higher in patients with high-expression FEZF1-AS1 than those with low-expression FEZF1-AS1, while the overall survival rate was remarkably lower. The proliferation ability of cells in FEZF1-AS1 silencing group declined significantly when compared with the NC group. Similarly, qRT-PCR results verified that the expression of miR-196a in OSCC cell lines and tissues was significantly reduced as well. Meanwhile, the miR-196a expression was negatively correlated with FEZF1-AS1. Subsequent Luciferase reporter gene assay confirmed that overexpression of miR-196a could markedly reduce the activity of Luciferase containing wild-type FEZF1-AS1 vector rather than decrease the activity of Luciferase containing mutant-type vector or empty vector. These findings further indicated that FEZF1-AS1 could be targeted by miR-196a through this binding site. In addition, recovery assay demonstrates that there was a mutual regulatory effect between FEZF1-AS1 and miR-196a, jointly affecting the malignant progression of OSCC.

CONCLUSIONS: The expression of lncRNA FEZF1-AS1 was markedly up-regulated in OSCC, which was significantly correlated with pathological stage and poor prognosis of OSCC patients. Therefore, it was believed that FEZF1-AS1 might promote the malignant progression of OSCC by regulating miR-196a.

Key Words:
LncRNA FEZF1-AS1, MiR-196a, Oral squamous cell carcinoma (OSCC), Proliferation.

Introduction

The oral cavity is located at the initial position of the digestive and respiratory tract, which possesses important physiological functions[1,2]. Oral cancer is considered one of the top ten frequently-occurring cancers in the body. It can cause dysfunctions of eating, speech and respiratory in severe cases, seriously affecting the quality of life of patients[1,2]. Squamous cell carcinoma is the most common type of oral cancer, accounting for more than 90% of oral cancer patients. The prognosis of squamous cell carcinoma is relatively poor, with a 5-year survival rate of 50-60% only[3-5]. Oral squamous cell carcinoma (OSCC) is generally derived

Corresponding Author: Tiejun Hou, MM; e-mail: htjdentist@163.com

from oral mucosal epithelium. Due to the reasons that the oral cavity is located at the key position of the digestive and respiratory tract and there are many important organs, postoperative recurrence and cervical lymph node metastasis of OSCC occur easily[6,7]. In recent years, synthetic serial therapy based on surgery has been widely applied in the diagnosis and treatment of OSCC. Meanwhile, the importance of radical and complete treatment in the prognosis of patients has been emphasized. However, the 5-year survival rate of patients with advanced tumor and recurrence remains far from satisfactory despite various treatment methods[8-10]. Therefore, the study of the pathogenesis of OSCC, especially the detection and diagnosis of specific early tumor markers, has been increasingly more important[8,10].

Previous molecular biological studies[11,12] on tumors have mainly focused on genes that can encode proteins. However, non-coding genes are ignored. Non-coding genes are a general term for genes with no protein-encoding functions, and their transcription products are non-coding ribonucleic acid (ncRNA)[13,14]. There are many members of the ncRNA family, of which micro RNA (miRNA) and long ncRNA (lncRNA) are the most notable[15,16]. Currently, molecular biological mechanism of miRNA has been studied. It has been found that miRNA can silence mRNA at the post-transcriptional level through complementary base pairing. The correlations of miRNA with the pathogenesis of malignancies and diagnostic values have been confirmed as well[17,18]. LncRNAs are a kind of RNA molecules with more than 200 nt in length, with no protein-encoding ability. Previous studies[19-21] have indicated that they exert biological functions in the form of RNA. LncRNA was regarded as "noise" produced in the gene transcription process in the past. However, increasingly more evidence has suggested that lncRNAs are involved in regulating gene expression at epigenetic, transcriptional and post-transcriptional levels. Moreover, lncRNAs play extensive and important roles in life processes, such as cell differentiation, cell fate determination, proliferation and migration[12,21].

In the present work, the expressions of FEZF1-AS1 and miR-196a in 42 pairs of OSCC tissues and para-carcinoma tissues were analyzed. The influences of FEZF1-AS1 and miR-196a on the biological functions of OSCC cells were explored as well. Previous studies have pointed out that FEZF1-AS1 and miR-196a play important roles in the occurrence and development of tumors. Therefore, the aim of this study was to investigate the mechanism of lncRNA FEZF1-AS1 in affecting clinical parameters, prognosis and malignant progression of OSCC by absorbing miR-196a.

Patients and Methods

Patients and OSCC Specimens

A total of 42 paired OSCC tissues and para-carcinoma tissues were collected. All specimens were obtained from tissues subjected to operation and needle biopsy or bronchoscopic biopsy in the Oncology Department and Stomatology Department. In addition, no patient underwent anti-tumor therapies such as radiotherapy and chemotherapy before the operation. This study was approved by the Ethics Committee of Liaocheng People's Hospital. Informed consent was obtained from each subject before the study. All patients were followed up *via* telephone and clinic after discharge. Follow-up information was collected, including general conditions, clinical symptoms and imaging examinations.

Cell Lines and Reagents

Four human OSCC cell lines (Fadu, SCC-25, CAL-27, and Tca8113) and one normal human oral cell line (Hs 680.Tg) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). All cells were cultured in DMEM containing 10% FBS in an incubator with 5% CO_2 at 37°C.

Cell Transfection

Control group (NC) and FEZF1-AS1 silencing group containing FEZF1-AS1 lentiviral sequence (sh-FEZF1-AS1), and control group (miR-NC) and overexpression group containing miRNA-196a lentiviral sequence (miRNA-196a mimics) were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The cells were first seeded into 6-well plates, followed by culture until cell density reached 70%. Subsequently, the cells were transfected with lentivirus according to the manufacturer's instructions. 48 h after transfection, the cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and functional cell assays.

Cell Proliferation Assay

48 h after transfection, the cells were collected and seeded into 96-well plates (2000 cells/well). After culturing for 6 h, 24 h, 48 h, and 72 h, the cells were added with Cell Counting Kit-8 (CCK-8) reagent (Dojindo Laboratories, Kumamoto, Japan), respectively. After incubation for another 2 h in the dark, the optical density (OD) value of each well at the wavelength of 490 nm was measured using a microplate reader. Finally, experimental data were analyzed.

Colony Formation Assay

48 h after transfection the cells were collected, and 200 cells were inoculated into each well of 6-well plates. Then, the cells were cultured in complete medium for 2 weeks. The culture medium was replaced after 1 week, and then replaced twice a week. The medium should not be replaced as far as possible in the first week to prevent non-adherent cells from being washed away. After 2 weeks, the medium was sucked dry when colonies formed. Subsequently, the cells were washed with Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA) twice and fixed with 2 mL methanol for 20 min. After washing again with PBS, the cells were stained with 0.1% crystal violet dye for 20 min. The cells were washed with PBS 3 times. Finally, the cells were photographed in a bright environment, and the number of formed colonies was calculated.

5-Ethynyl-2'-Deoxyuridine (EdU) Proliferation Assay

To reveal the effects of FEZF1-AS1 and miR-196a on cell proliferation, EdU proliferation assay was performed according to the manufacturer's instructions (RiboBio, Guangzhou, China). Briefly, 24 h after transfection, the cells were incubated with 50 μm EdU for 2 h and stained with AdoLo and 4',6-diamidino-2-phenylindole (DAPI; Solarbio, Beijing, China). The number of EdU-positive cells was detected under a fluorescence microscope. EdU-positive rate = the number of EdU-positive cells/the total number of DAPI-colored cells (blue cells).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from tissue specimens using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). 2 μg of total RNA in 20 μL system was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) according to the instructions of AMV reverse transcription kit. A 15 μL system was used for amplification, including 2×SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), an appropriate amount of cDNA as a template and primers (0.4 mol/L). Three replicates were set for each sample. The corresponding forward and reverse primers were designed and synthesized according to target genes. PCR amplification was conducted on a qPCR instrument, with β-actin as an internal reference. The expression level of the genes was calculated by the $RQ=2^{-\Delta\Delta Ct}$ method. This experiment was repeated three times

Primers used in this study were as follows: FEZF1-AS1: forward: 5'-TCGCTCAATGGAGTGACGGCA-3', reverse: 5'-CGGCTGAGACCGCTGAGAACTT-3'. β-actin: forward: 5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-TGCCGTAGGTGTCCCTTTG-3'. MiR-196a: forward: 5'-UAGGUAGUUUCAUGUUGUUGGG-3', reverse: 5'-UAGGUAGUUUCAUGUUGUUGGG-3'. U6: forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'. Experimental data were analyzed using ABI Step One software (Applied Biosystems, Foster City, CA, USA), and the relative expression level of mRNA was calculated using $2^{-\Delta\Delta Ct}$.

Dual-Luciferase Reporter Gene Assay

A reporter gene plasmid with a specific fragment of target promoter inserted in front of the Luciferase expression sequence was first constructed. Transcription factor expression plasmid and reporter gene plasmid were co-transfected into CAL-27 and SPC-A1 cells or other related cell lines. If the transcription factor could activate the target promoter, the Luciferase gene would be expressed. Meanwhile, the expression level of Luciferase was directly proportional to the effect intensity of the transcription factor. Specific Luciferase substrate was added, and Luciferase reacted with the substrate to produce fluorescence. The intensity of fluorescence was measured to indicate the activity of Luciferase, to determine whether the transcription factor could exert an effect on target promoter fragment.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. The difference in the expression of lncRNA FEZF1-AS1 between OSCC tissues and para-carcinoma tissues was analyzed *via* analysis of variance, followed by Post-Hoc Test (Least Significant Difference). The expression of lncRNA FEZF1-AS1 in OSCC tissues and para-carcinoma tissues and its corre-

lations with clinicopathological parameters were detected *via* the chi-square test. The correlations of lncRNA FEZF1-AS1 expression with survival time and prognosis of patients were analyzed using the Kaplan-Meier method. Cox proportional hazard model was used to analyze possible influencing factors for the prognosis of OSCC patients. Experimental data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

LncRNA FEZF1-AS1 Was Upregulated in Human OSCC Tissues and Cell Lines

The expression of FEZF1-AS1 in 42 paired OSCC tissues and the corresponding para-carcinoma tissues, as well as OSCC cell lines, was detected *via* qRT-PCR. The results revealed that the expression level of FEZF1-AS1 in OSCC tissues increased significantly when compared with that in para-carcinoma tissues, and the difference was statistically significant (Figure 1A and 1C). The expression of FEZF1-AS1 in OSCC cell lines was significantly higher than that of Hs 680.Tg cells as well (Figure 1C). CAL-27 and Tca8113 cell lines expressed the highest level of FEZF1-AS1, which were selected for transfection and subsequent experiments.

FEZF1-AS1 Expression Was Correlated With Pathological Stage and Overall Survival of OSCC Patients

According to the expression of lncRNA FEZF1-AS1 in 42 pairs of OSCC and para-carcinoma tissues, patients were divided into two groups, including the high-expression group and

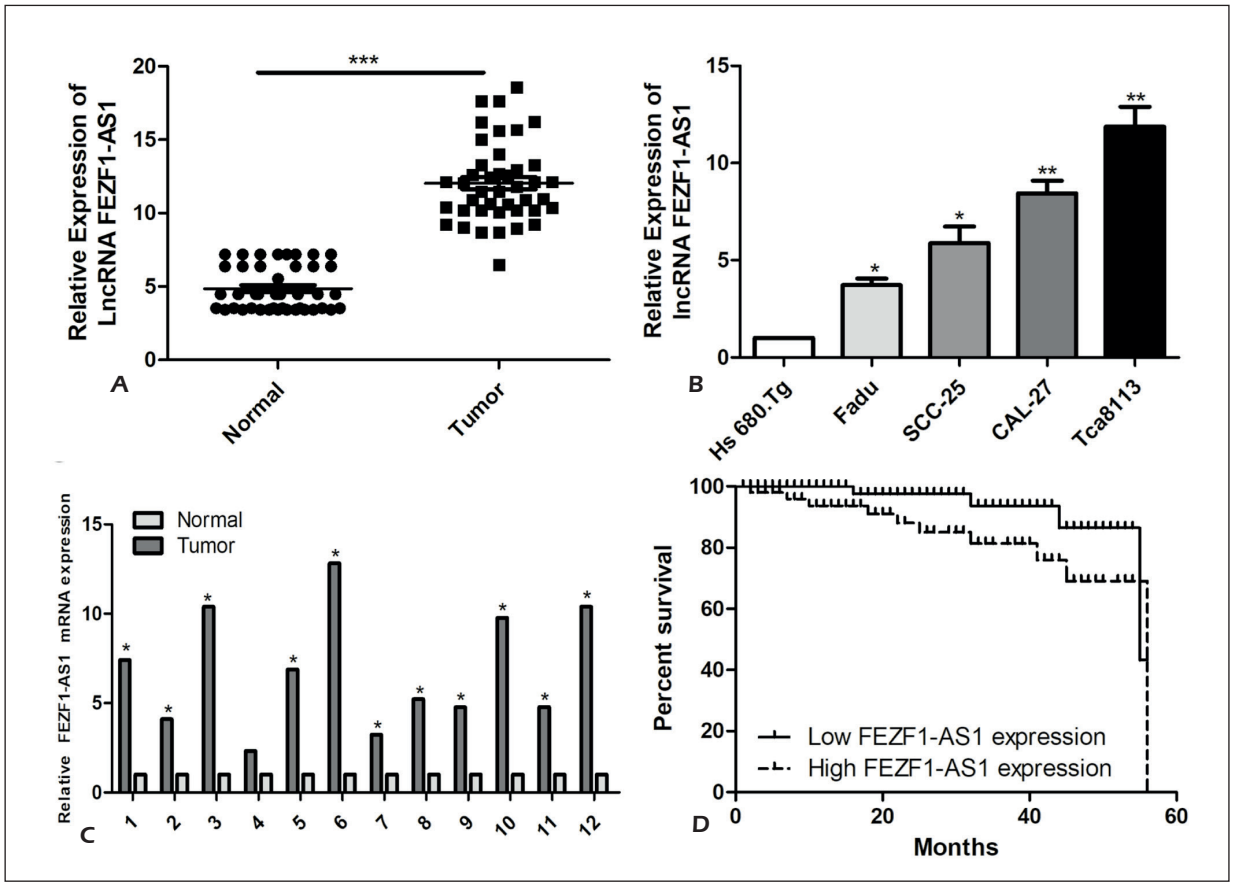

Figure 1. FEZF1-AS1 is highly expressed in OSCC tissues and cell lines. ***A***, Difference in FEZF1-AS1 expression between OSCC tissues and para-carcinoma tissues detected *via* qRT-PCR. ***B***, Expression level of FEZF1-AS1 in OSCC cell lines detected *via* qRT-PCR. ***C***, Difference in FEZF1-AS1 expression in 12 pairs of OSCC tissues and para-carcinoma tissues detected *via* qRT-PCR. ***D***, The Kaplan-Meier survival curve indicated that the prognosis of patients in FEZF1-AS1 high-expression group is significantly worse than that of patients in low-expression group. The data are expressed as mean ± standard deviation, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table I. Association of lncRNA FEZF1-AS1 and miR-196a expression with clinicopathologic characteristics of oral squamous cell carcinoma.

Parameters	Number of cases	FEZF1-AS1 expression		*p*-value	miR-196a expression		*p*-value
		Low (%)	High (%)		Low (%)	High (%)	
Age (years)				0.204			0.474
<60	16	10	6		5	11	
≥60	26	11	15		11	15	
Gender				0.355			0.525
Male	21	12	9		7	14	
Female	21	9	12		9	12	
T stage				0.011			0.011
T1-T2	26	17	9		6	20	
T3-T4	16	4	12		10	6	
Lymph node metastasis				0.095			0.159
No	29	17	12		9	20	
Yes	13	4	9		7	6	
Distance metastasis				0.204			0.057
No	26	15	11		7	19	
Yes	16	6	10		9	7	

low-expression group. The correlations of FEZF1-AS1 expression with age, gender, clinical stage and distant metastasis of OSCC patients were analyzed using the chi-square test. As shown in Table I, the expression of FEZF1-AS1 showed no significant correlations with age and gender of OSCC patients. However, FEZF1-AS1 expression was significantly correlated with clinical stage. At the same time, the expression of miR-196a also showed significant correlation with clinical stage. To further explore the correlation between lncRNA FEZF1-AS1 expression and prognosis of OSCC patients, relevant follow-up data were collected. The Kaplan-Meier survival curve indicated that high expression of FEZF1-AS1 was significantly correlated with poor prognosis of OSCC ($p<0.05$, Figure 1D). The above results indicated that FEZF1-AS1 might serve as a novel biological index for predicting the prognosis of OSCC.

Downregulation of FEZF1-AS1 Inhibited the Growth of OSCC Cells

To explore the influence of lncRNA FEZF1-AS1 on the proliferation of OSCC cells, FEZF1-AS1 knockdown model was first successfully established. After transfection of TPTE2P1 lentiviral vector in CAL-27 and Tca8113 cell lines interference efficiency was verified *via* qRT-PCR (Figure 2A). Subsequent experiments revealed that the proliferation ability of cells in FEZF1-AS1 silencing group (sh-FEZF1-AS1) significantly declined compared with that in the NC group (Figure 2B, 2C, 2D).

MiR-196a Was a Direct Target of FEZF1-AS1

To further verify the targeted effect of miR-196a on FEZF1-AS1, FEZF1-AS1 sequence was cloned into Luciferase reporter plasmid pmirGLO, and mutant-type vector pmirGLO-FEZF1-AS1-mut was also constructed. Subsequently, pmirGLO-FEZF1-AS1-WT, pmirGLO-FEZF1-AS1-mut or pmirGLO was co-transfected with miR-196a into CAL-27 and Tca8113 cells. Luciferase reporter gene assay showed that the overexpression of miR-196a could significantly reduce the activity of Luciferase containing wild-type FEZF1-AS1 vector ($p<0.05$) rather than decrease the activity of Luciferase containing mutant-type vector ($p>0.05$) or empty vector ($p>0.05$). The above findings further proved that FEZF1-AS1 could be targeted by miR-196a through this binding site (Figure 3A).

MiR-196a Was Downregulated in Human OSCC Tissues and Cell Lines

The expression of miR-196a in 42 pairs of OSCC tissues and the corresponding para-carcinoma tissues and OSCC cell lines were detected *via* qRT-PCR as well. The results manifested that the expression level of miR-196a in OSCC tissues was significantly lower than that of para-carcinoma tissues, and the difference was statistically significant (Figure 3B). Similarly, the expression of miR-196a in OSCC cells was remarkably lower than that of Hs 680.Tg cells, and there was a statistically significant difference (Figure 3C). Sub-

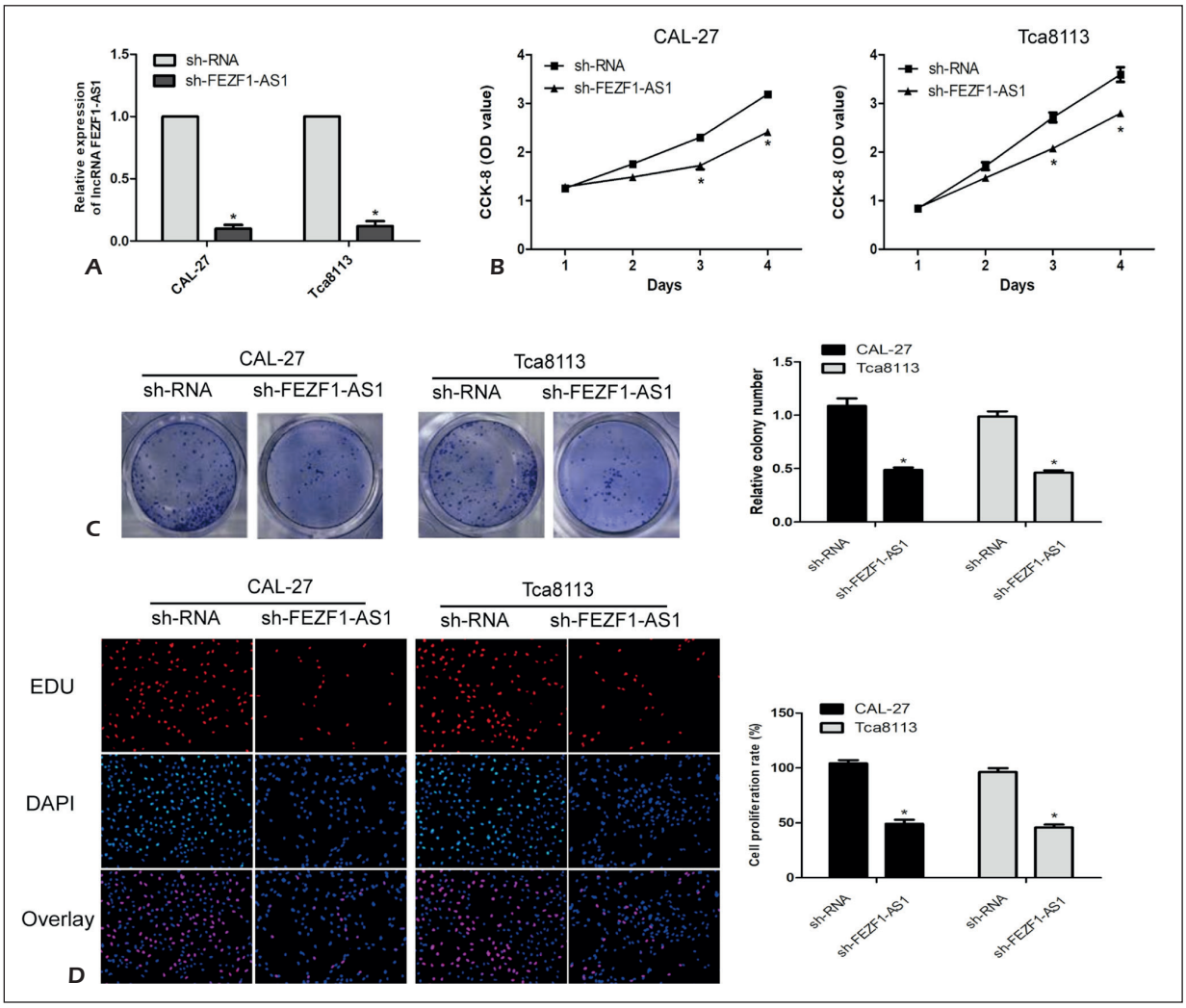

Figure 2. Silencing FEZF1-AS1 inhibits the proliferation of OSCC cells. ***A***, Interference efficiency of FEZF1-AS1 in CAL-27 and Tca8113 cell lines verified *via* qRT-PCR. ***B***, Knock-down of FEZF1-AS1 on the proliferation of CAL-27 and Tca8113 cells detected *via* CCK-8 assay. ***C***, Knock-down of FEZF1-AS1 on the proliferation of CAL-27 and Tca8113 cells detected *via* colony formation assay (Magnification × 40). ***D***, Knock-down of FEZF1-AS1 on the proliferation of CAL-27 and Tca8113 cells detected *via* EdU assay (Magnification × 40). The data are expressed as mean ± standard deviation, *$p<0.05$, **$p<0.01$.

sequently, 12 paired OSCC tissues were selected to detect the expression of FEZF1-AS1 and miR-196a *via* qRT-PCR. It was found that the mRNA expression level of FEZF1-AS1 was negatively correlated with miR-196a in OSCC tissues (Figure 3D).

Upregulation of MiR-196a Inhibited the Growth of OSCC Cells

To study the function of miR-196a in OSCC, lentiviral vector overexpressing miR-196a was constructed and transfected into CAL-27 and Tca8113 cells. Interference efficiency was verified *via* qRT-PCR, and the difference was statistically significant (Figure 4A). After miR-196a overexpression in CAL-27 and Tca8113 cell lines, the proliferation of cells was detected *via* CCK-8 assay, EdU assay and colony formation assay. The results showed that the proliferation ability of cells in the miR-196a overexpression group (transfected with miR-196a mimics) was significantly declined when compared with the NC group (Figure 4B, 4C and 4D).

FEZF1-AS1 Regulated MiR-196a Expression in Human OSCC Cells

To further investigate the interaction between FEZF1-AS1 and miR-196a in OSCC cells, miR-196a was silenced in OSCC cells with FEZF1-AS1 silencing. Transfection efficiency of FEZF1-

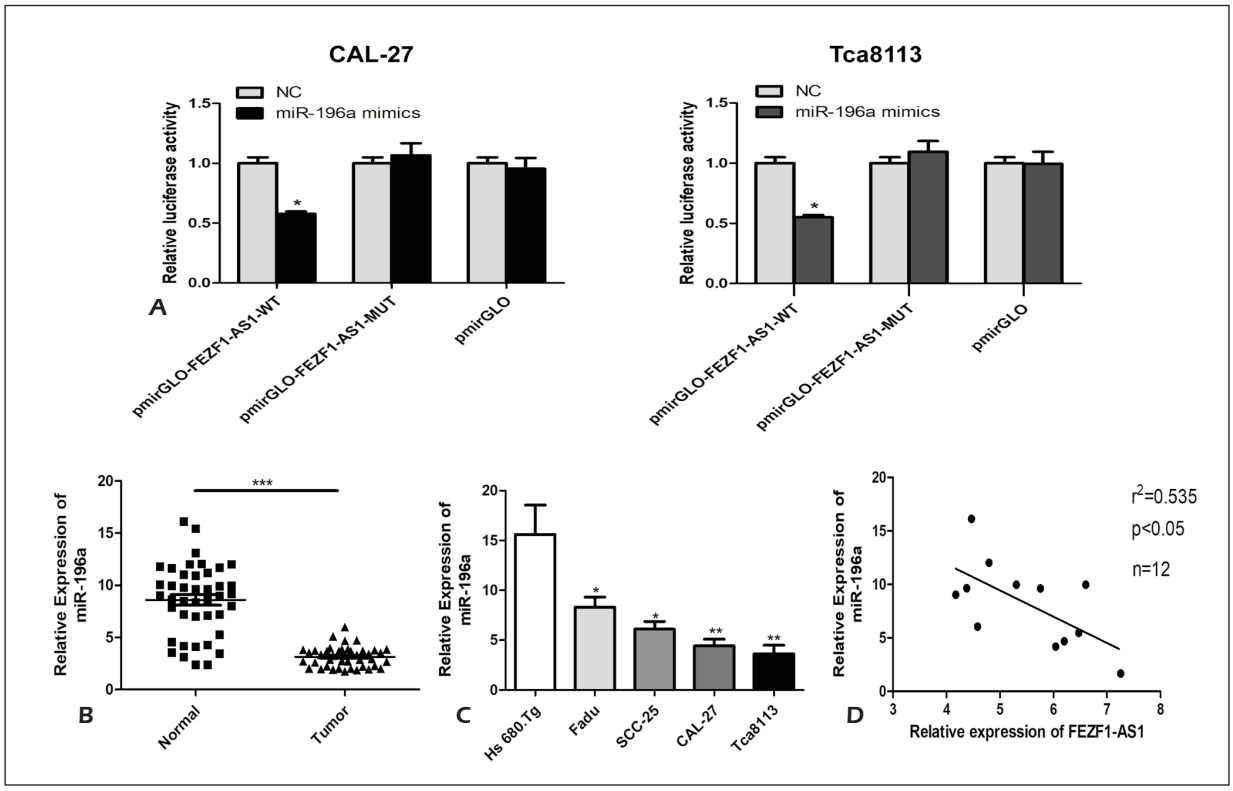

Figure 3. Direct targeted effect of FEZF1-AS1 on miR-196a. ***A***, Direct targeted effect of FEZF1-AS1 on miR-196a verified *via* Dual-Luciferase reporter gene assay. Dual-Luciferase reporter gene assay for CAL-27 and Tca8113 cell lines reveals that overexpression of miR-196a significantly reduces the activity of Luciferase containing wild-type FEZF1-AS1 vector ($p<0.05$) without lowering the activity of Luciferase containing mutant-type vector ($p>0.05$) or empty vector ($p>0.05$). ***B***, Difference in miR-196a expression between OSCC tissues and para-carcinoma tissues detected *via* qRT-PCR. ***C***, Expression of miR-196a in OSCC cell lines detected *via* qRT-PCR. ***D***, There is a significant negative correlation between the expression levels of FEZF1-AS1 and miR-196a in OSCC tissues. The data are expressed as mean ± standard deviation, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

AS1 was detected *via* qRT-PCR (Figure 5A). The results demonstrated that silencing miR-196a could offset the effect of FEZF1-AS1 knockdown on OSCC cell proliferation (Figure 5B).

Discussion

Currently, a consensus about the occurrence and development of cancer has emerged. After normal cells are stimulated by various factors, related genes are mutated through multiple steps. This may further lead to the activation of a growth signal, insensitivity of growth inhibition signal, activation of unrestricted replication potential, sustained angiogenesis, tissue invasion and metastasis and other malignant phenotypes. Ultimately, malignant tumor occurs[22,23]. This is a multi-step, multi-factor and long-term development process. Its molecular events include multigene mutations, such as gene amplification, loss of heterozygotes, as well as methylation-induced gene inactivation. The imbalance between the loss of the function of cancer suppressor gene and activation of function of oncogene leads to the occurrence of diseases[22]. In recent studies, it has been demonstrated that both lncRNA and miRNA play important roles in a variety of diseases, including tumors. Multiple abnormally-expressed lncRNAs and miRNAs can be found in OSCC. They exert crucial roles in the diagnosis, treatment and prognosis of OSCC[11,14,20]. Therefore, searching for abnormally-expressed lncRNAs and miRNAs in OSCC and analyzing their correlations with clinical prognosis may help to increase the diagnosis and treatment levels of OSCC, thereby improving the clinical prognosis of patients.

LncRNA is a type of RNA with more than 200 nt in transcription length, which contains no initiation codon and termination codon[11,20]. LncRNA has a very long primary structure, which can act as a platform for integration with other DNAs and

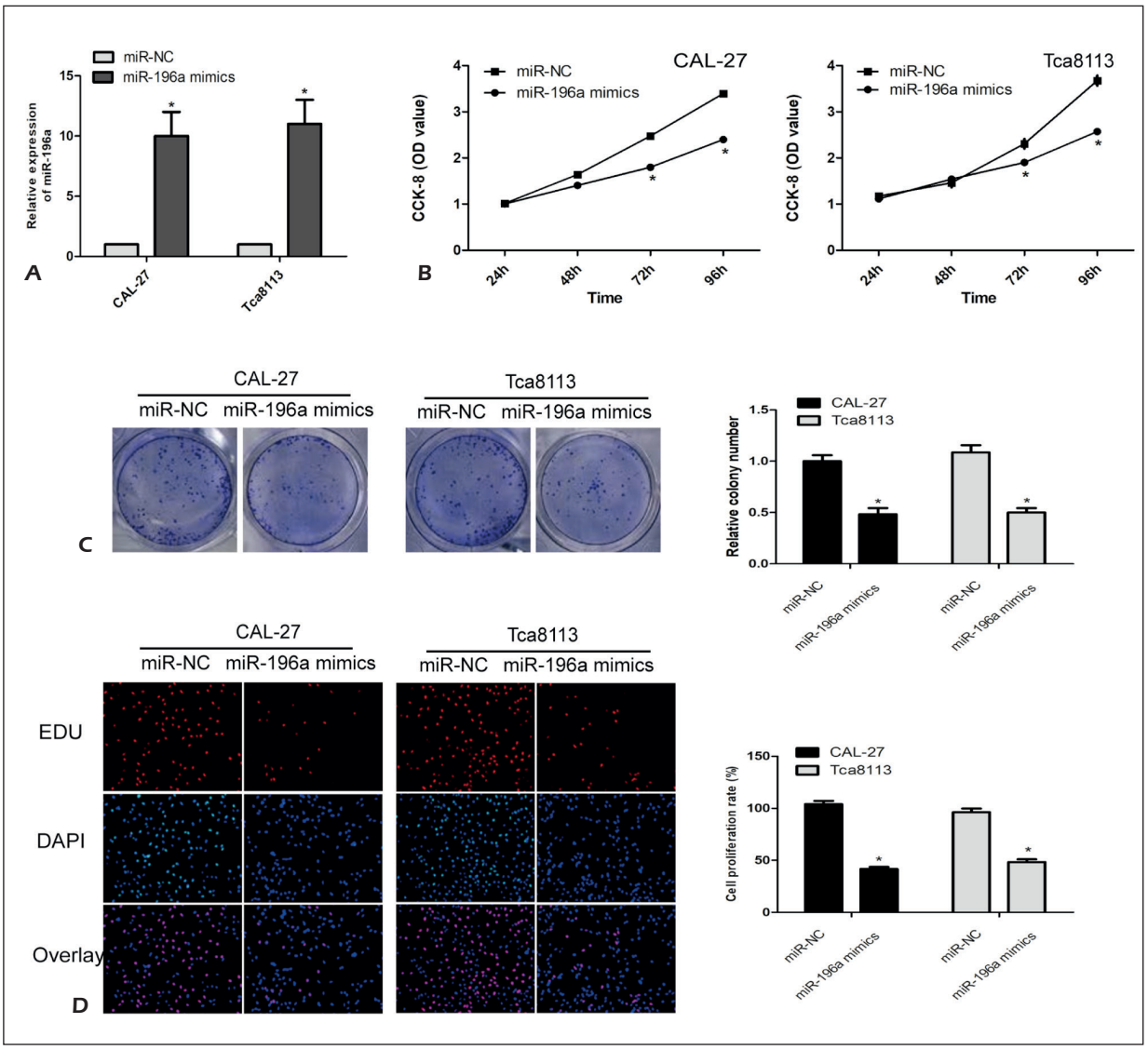

Figure 4. Overexpression of miR-196a inhibits the proliferation of OSCC cells. ***A***, Interference efficiency of miR-196a in CAL-27 and Tca8113 cells verified *via* qRT-PCR. ***B***, Overexpression of miR-196a on the proliferation of CAL-27 and Tca8113 cells detected *via* CCK-8 assay. ***C***, Overexpression of miR-196a on the proliferation of CAL-27 and Tca8113 cells detected *via* colony formation assay (Magnification × 40). ***D***, Overexpression of miR-196a on the proliferation of CAL-27 and Tca8113 cells detected *via* EdU assay (Magnification × 40). The data are expressed as mean ± standard deviation, *$p<0.05$, **$p<0.01$.

RNAs. Meanwhile, it forms a diverse and complex secondary spatial structure that can interact with protein factors to exert biological functions[14,20,21]. Compared with mRNA, most lncRNAs are produced by RNA polymerase II catalysis. However, its sequence is not highly conserved and its expression abundance is low, displaying strong specificity in tissues and cells[16,17]. In the past, lncRNA was regarded as "garbage" in genomic transcription. Meanwhile, it was also considered as a by-product in RNA polymerase II transcription without biological functions[16,17]. However, recent studies have indicated that lncRNA is involved in many regulatory processes, such as X chromosome silencing, genomic imprinting, chromatin modification, chromatin transcription activation, chromatin transcription interference and intranuclear transport. The regulatory effect of lncRNA has attracted much attention from researchers[11,16,19,21]. In this work, the expressions of lncRNA FEZF1-AS1 and miR-196a in 42 pairs of OSCC tissues and para-carcinoma tissues were detected. The results revealed that the expression of lncRNA FEZF1-AS1 was markedly up-reg-

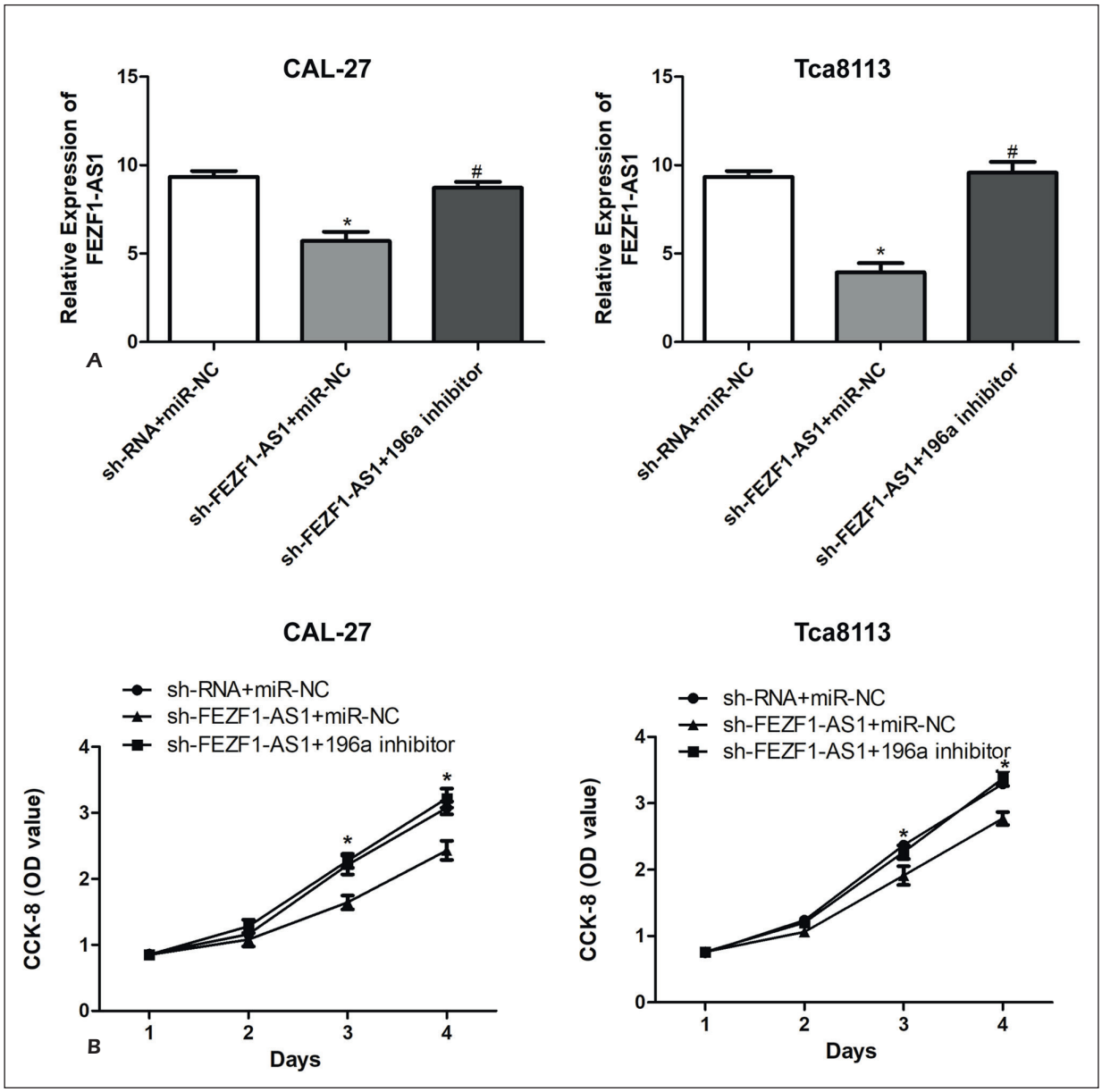

Figure 5. FEZF1-AS1 regulates the expression of miR-196a in OSCC tissues and cell lines. ***A***, Expression level of miR-196a in cells co-transfected with FEZF1-AS1 and miR-196a detected *via* qRT-PCR. ***B***, Co-transfection of FEZF1-AS1 and miR-196a on the proliferation of CAL-27 and Tca8113 cells detected *via* CCK-8 assay. Data are expressed as mean ± standard deviation, **p*<0.05.

ulated in OSCC tissues, while the expression of miR-196a was significantly down-regulated. Meanwhile, the expression of FEZF1-AS1 was positively correlated with pathological stage and poor prognosis of OSCC patients. Therefore, it is believed that FEZF1-AS1 and miR-196a exert cancer-promoting and anti-tumor effect in OSCC, respectively. To further explore the influences of FEZF1-AS1 and miR-196a on the biological functions of OSCC, FEZF1-AS1 knockdown model was established using lentivirus transfection. CCK-8 assay, colony formation assay and EdU assay all demonstrated that FEZF1-AS1 could significantly promote the occurrence and development of OSCC cells. However, the potential molecular mechanism remains unclear.

At present, the following modes of action have been confirmed in the mechanism research of lncRNA exerting biological functions, such as regulation and modification. LncRNA can affect the activity and expression of proteins. It altered its position in cells by binding to some specific pro-

teins, thus regulating encoding genes to exert effects. Meanwhile, lncRNA can competitively bind to miRNAs, thereby affecting the regulatory effect on target mRNAs. Previous studies have indicated that lncRNA can also exert an enhancer-like effect on some encoding genes. Moreover, lncRNA may play a regulatory role by affecting the expression of adjacent genes, namely through the physical positional relation with target genes[24-26]. MiR-196a is a key molecule in the miRNA family. Experimental results in this study showed that miR-196a was lowly expressed in OSCC tissues when compared with para-carcinoma tissues. Meanwhile, miR-196a could significantly inhibit the proliferation of OSCC cells. Bioinformatics analysis predicted that there was a binding site of miR-196a in FEZF1-AS1 sequence. Direct binding of FEZF1-AS1 to downstream miR-196a was verified *via* Dual-Luciferase reporter gene assay. Meanwhile, miR-196a failed to be enriched in the absence of the mutation of FEZF1-AS1 vector in the miR-196a binding site. This further verified the binding site of FEZF1-AS1 to miR-196a. The above results indicated that there might be a positive feedback regulation loop. Due to the "sponge adsorption" mechanism of lncRNA FEZF1-AS1, the concentration of miR-196a rapidly declined, thereby promoting the malignant progression of OSCC.

Conclusions

The expression of lncRNA FEZF1-AS1 is markedly up-regulated in OSCC tissues and cells. Meanwhile, it is significantly correlated with pathological stage and poor prognosis of OSCC. Therefore, lncRNA FEZF1-AS1 may promote the malignant progression of OSCC by regulating miR-196a.

Conflict of Interests

The authors declare that they have no conflict of interest.

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