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FBXW7 inhibited cell proliferation and invasion regulated by miR-27a through PI3K/AKT signaling pathway and epithelial-to-mesenchymal transition in oral squamous cell carcinoma

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Abstract. – OBJECTIVE: Our purpose was to detect the molecular mechanism of F-box and WD repeat domain containing 7 (FBXW7) in regulating cell growth and metastasis of oral squamous cell carcinoma (OSCC).

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) and Western blot were applied to calculate the messenger ribonucleic acid (mRNA) and protein levels of genes and miR-27a. The proliferation and invasive abilities were measured by methyl thiazolyl tetrazolium (MTT) and transwell assays. The. Kaplan-Meier method was conducted to evaluate the 5-year overall survival of oral squamous cell carcinoma patients.

RESULTS: FBXW7 was downregulated while miR-27a was upregulated in OSCC tissues and cells compared with the corresponding adjacent tissues. Downregulation of FBXW7 or upregulation of miR-27a in OSCC tissues predicted poor outcome of OSCC patients. FBXW7 suppressed the growth through the phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/AKT) signaling pathway in OSCC cell line HSC3. FBXW7 inhibited the invasion-mediated epithelial-mesenchymal transition (EMT) in HSC3 cells. The expression of FBXW7 was mediated by miR-27a by directly binding to the 3'-untranslated region (3'-UTR) of FBXW7 in HSC3 cells. MiR-27a reversed partial roles of FBXW7 on the proliferation and invasion in OSCC cells.

CONCLUSIONS: FBXW7 was mediated by miR-27a and could inhibit the proliferation through the PI3K/AKT pathway and invasion-mediated EMT in OSCC cell line. The newly identified miR-27a/FBXW7/PI3K/AKT axis provides novel insights into the pathogenesis of OSCC.

Key Words:
FBXW7, PI3K/AKT, Oral squamous cell carcinoma, EMT.

Introduction

Oral squamous cell carcinoma (OSCC), a global health problem, is the most frequent head and neck cancer with 500,000 new cases annually[1]. The pathogenesis of OSCC was diverse, including alcohol consumption, smoking, and immunodeficiency[2]. Moreover, the 5-year survival of OSCC is poor due to the great potential for metastasis[3]. Thus, it is urgent to detect the biomarkers for the treatment of OSCC.

F-box and WD repeat domain containing 7 (FBXW7), a member of the F-box protein family, has been reported[4] to act as the substrate recognition component of the SCF E3 ubiquitin ligase. In breast cancer, FBXW7 has been demonstrated[5] to function as a tumor suppressor through suppressing the proliferation and enhancing apoptosis. Similarly, Cai et al[6] demonstrated that the upregulation of FBXW7 inhibited the migration, invasion, and epithelial-mesenchymal transition (EMT) in renal cell carcinoma. In addition, Ishii et al[7] revealed that FBXW7 served as a predictor and was associated with poor prognosis of pancreatic cancer patients.

MicroRNAs (miRNAs) are non-coding small RNAs with 19-25 nucleotides that regulate the messenger ribonucleic acid (mRNA) deadenylation and degradation via directly binding to the

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3'-untranslated region (3'-UTR) of target mRNA[8,9]. MiRNAs mediated several biological progresses including growth, differentiation, and tumorigeness[10]. MiR-27a has been reported[11] to act as an oncomiR by promoting the proliferative, invasive, and the EMT abilities in osteosarcoma. MiR-27a-3p mimic promoted the proliferation, migration, invasion, and suppressed apoptosis in colorectal cancer[12]. MiR-27a has been indicated to promote the proliferation, motility, and metastasis in gastric cancer[13].

In this work, FBXW7 mediated by miR-27a to inhibit the proliferation through phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/AKT) pathway and inhibit the invasion-mediated EMT in OSCC. MiR-27a reversed partial roles of FBXW7 on the proliferation and invasion OSCC cells.

Patients and Methods

Patients and Tissue Samples

We collected 52 cases of oral squamous cell carcinoma patients admitted to the Jinan Stomatological Hospital from January 2016 to July 2018, and obtained pairs of cancer tissues and corresponding adjacent tissues. The samples were instantly frozen in liquid nitrogen and stored at –80°C freezer. None patients had received radiotherapy or chemotherapy before the operation. All of the patients provided written informed consent, and approval for the research was received from the Ethics Committee of Jinan Stomatological Hospital.

Cell Culture and Preparation

One normal oral epithelial cell line CGHNK2 and two OSCC cell lines SCC25 and HSC3 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) maintained with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA) was employed to culture all the cells in an atmosphere with 5% CO_2 at 37°C.

Vectors and Transfection

Plasmid complementary deoxyribonucleic acid-FBXW7 (pcDNA-FBXW7), the miR-27a mimic and the miR-27a inhibitor oligos, as well as the corresponding negative control, were synthesized and purchased from Ribobio (Guangzhou, China). HSC3 cells were seeded in 6-well plate and cultured overnight for transfection. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and equal amounts of oligo fragments were separately diluted using Opti-MEM/Reduced serum medium (Thermo Fisher Scientific, Waltham, MA, USA). Once the two solutions were mixed, the mixture was added into the 6-well plate. For cells, the oligonucleotide fragment was inserted in vector and added in cells, followed by the cells selected by Geneticin (G418; Thermo Fisher Scientific, Waltham, MA, USA) or harvested after 48 h.

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

The reverse transcription of miR-27a was calculated by using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; Foster City, CA, USA). QRT-PCR was performed using the TaqMan MicroRNA assay.

The first complementary deoxyribose nucleic acid (cDNA) strand of FBXW7 was synthesized using the Reverse Transcription System (Thermo Fisher Scientific; Waltham, MA, USA). Power SYBR Green PCR Master Mix (Applied Biosystems; Foster City, CA, USA) was conducted to carry out PCR reaction using the ABI 7300HT system (Applied Biosystems; Foster City, CA, USA). The mRNA levels of miR-27a and FBXW7 were normalized to U6 snRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers were as follows: FBXW7 Forward 5'-GTCCCGAGAAGCGGTTTGATA-3', Reverse 5'-TGCTCAGGCACGTCAGAAAAG-3'; GAPDH Forward 5'-AGCCCAAGATGCCCTTCAGT-3', Reverse 5'-CCGTGTTCCTACCCCCAATG-3'; miR-27a Forward 5'-GCGCATTCACAGTGGCTAAG-3', Reverse 5'-CGGCCCAGTGTTCAGACTAC-3'; U6 Forward 5'-CTCGCTTCGGCAGCACA-3', Reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Western Blot Assay

Radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing 1% phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich, St. Louis, MO, USA) was utilized to lyse HSC3 cells. The concentration of total proteins was measured by bicinchoninic acid (BCA) Protein Quantification Kit (Solarbio, Beijing, China). Once separated through 10% dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) through electrophoresis, the proteins were transferred on poly-

vinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). Next, the membranes were blocked by 5% skimmed milk for 1 h at room temperature. The membranes were incubated using the primary antibodies, which were FBXW7 (1:1,000; Abcam, Cambridge, MA, USA), E-cadherin (1:1000; Abcam, Cambridge, MA, USA), N-cadherin (1:1000; Abcam, Cambridge, MA, USA), Vimentin (1:1000; Abcam, Cambridge, MA, USA), p-PI3K (1:1000; Abcam, Cambridge, MA, USA), PI3K (1:1000; Abcam, Cambridge, MA, USA), p-AKT (1:1000; Abcam, Cambridge, MA, USA), AKT (1:1000; Abcam, Cambridge, MA, USA) and GAPDH (1:3,000 dilution; Sigma-Aldrich, St Louis, MO, USA). Subsequently, the membranes were incubated by the anti-rabbit or mouse horseradish peroxidase (HRP)-conjugated secondary antibody, and the signal was measured by enhanced chemiluminescence (ECL).

Proliferation Assay

Methyl thiazolyl tetrazolium (MTT) assay was conducted to determine the cell proliferative ability using MTT (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and dimethyl sulfoxide (DMSO) solutions (Amresco, Solon, OH, USA). Before transfection, HSC3 cells with a density of 5×10^3 cells/well were seeded into 96-well plates and were then cultured at 37°C in an atmosphere of 5% CO_2. After cultured 24, 48, 72, and 96 h, 10 μL of MTT were added into each well and incubated for 4 h at 37°C. Once the medium contained with MTT was removed, and once 150 μL DMSO were added into each well, the absorbance of cell proliferation was measured at 490 nm.

Transwell Assay

The cell invasive ability was assessed by transwell chamber (8 μm; Biosciences, San Jose, CA, USA), which was covered with Matrigel (Clontech; Mountain View, CA, USA). We seeded 100 μL cell suspension into the upper chamber, which were suspended in Dulbecco's Modified Eagle's Medium (DMEM) without serum. Meanwhile, 600 μL DMEM medium with 15% fetal bovine serum (FBS) were placed into the lower chamber. After incubated 48 h at 37°C in an atmosphere with 5% CO_2, the cells still at the upper surface were removed by a cotton swab, and the invaded cells were fixed and stained using methanol and 0.1% crystal violet. The ability of cells to invade is represented by the number of cells passing through the membrane.

Luciferase Reporter Assays

TargetScan predicted that miR-27a targeted to FBXW7. To clarify that miR-27a was directly binding to the 3'-UTR of FBXW7 mRNA, the binding sequences were mutated from ACUGUGAA to UGACACUU. The 3′-UTR fragment of FBXW7 mRNA containing the wild type or mutated sequence was inserted into the pmirGlo vector, which was designated as pmirGlo-FBXW7-WT (WT) and pmirGlo-FBXW7-MUT (MUT), respectively. Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was utilized to perform the transfection in HSC3 cells. After transfection for 48 h, the firefly luciferase activity was calculated by Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) with *Renilla* luciferase as normalization.

Statistical Analysis

Data are presented as means ± SD (standard deviation), and the statistical analyses were performed by Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA). Comparison between two or multiple groups was performed using Student's *t*-test and One-way analysis of variance (ANOVA) test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

FBXW7 Was Downregulated in OSCC Tissues and Downregulation of FBXW7 Predicted Poor 5-year Overall Survival

RT-qPCR was applied to calculate the expression of FBXW7 in OSCC tissues and the corresponding adjacent non-tumor tissues. As expected, we calculated the FBXW7 expression in tissues and cells by RT-qPCR. Also, FBXW7 was discovered to be downregulated in OSCC tissues compared with the corresponding non-tumor tissues ($p<0.05$; Figure 1A). Besides, downregulation of FBXW7 predicted poor 5-year overall survival of OSCC patients ($p<0.05$, Figure 1B). In addition, the expression of miR-27a had an inverse connection with lymph node metastasis ($p<0.05$), TNM stage ($p<0.05$; Table I). The expression of miR-27a was higher in OSCC tissues than the corresponding non-tumor tissues ($p<0.05$; Figure 1C). The Kaplan-Meier method revealed that the overall survival was associated

with miR-27a expression, and over-expression of miR-27a predicted poor prognosis of OSCC patients ($p<0.05$; Figure 1D).

FBXW7 Suppressed the Proliferation Through PI3K/AKT Signal Pathway in OSCC

MRNA levels of FBXW7 were evaluated in cell lines. Also, FBXW7 was discovered to be low expressed in OSCC cell lines HSC3 ($p<0.01$) and SCC25 compared to the CGHNK2 cell line ($p<0.05$; Figure 2A). To explore the function of FBXW7 in OSCC, pcDNA3.1-FBXW7 was conducted to up-regulate the expression of FBXW7 in HSC3 cells ($p<0.05$; Figure 2B). The proliferative ability was evaluated using the MTT assay after transfection of FBXW7 overexpressed plasmid (pcDNA3.1-FBXW7), as well as the negative control. Cell proliferation was inhibited by transfecting with pcDNA3.1-FBXW7 in HSC3 cells ($p<0.05$; Figure 2C). In addition, the expressions of p-PI3K and p-AKT were inhibited by re-expressing FBXW7 in HSC3 cells (Figure 2D).

FBXW7 Inhibited the Invasion-Mediated EMT of OSCC cells

Transwell assay was used to calculate the invasion of HSC3 cells. Similar to proliferation, the invasive ability was reduced by pcDNA3.1-FBXW7 ($p<0.05$; Figure 3A). To explore the mechanism of FBXW7 on OSCC the invasion, Western blot was utilized to assess the protein levels of EMT markers. As expected, FBXW7 inhibited the protein levels of N-cadherin and Vimentin, whereas it reduced the expression of E-cadherin in HSC3 cells (Figure 3B), which suggested that FBXW7 suppressed the invasion-mediated EMT in HSC3 cells.

FBXW7 Was a Target Gene of MiR-27a in OSCC Cells

TargetScan was conducted to predict the target genes of miR-27a. Also, FBXW7 was predicted as a potential target of miR-27a. To verify miR-27a directly binding to FBXW7, the binding sequences on the 3'-UTR of FBXW7 mRNA were mutated from ACUGUGAA (WT) to UG-

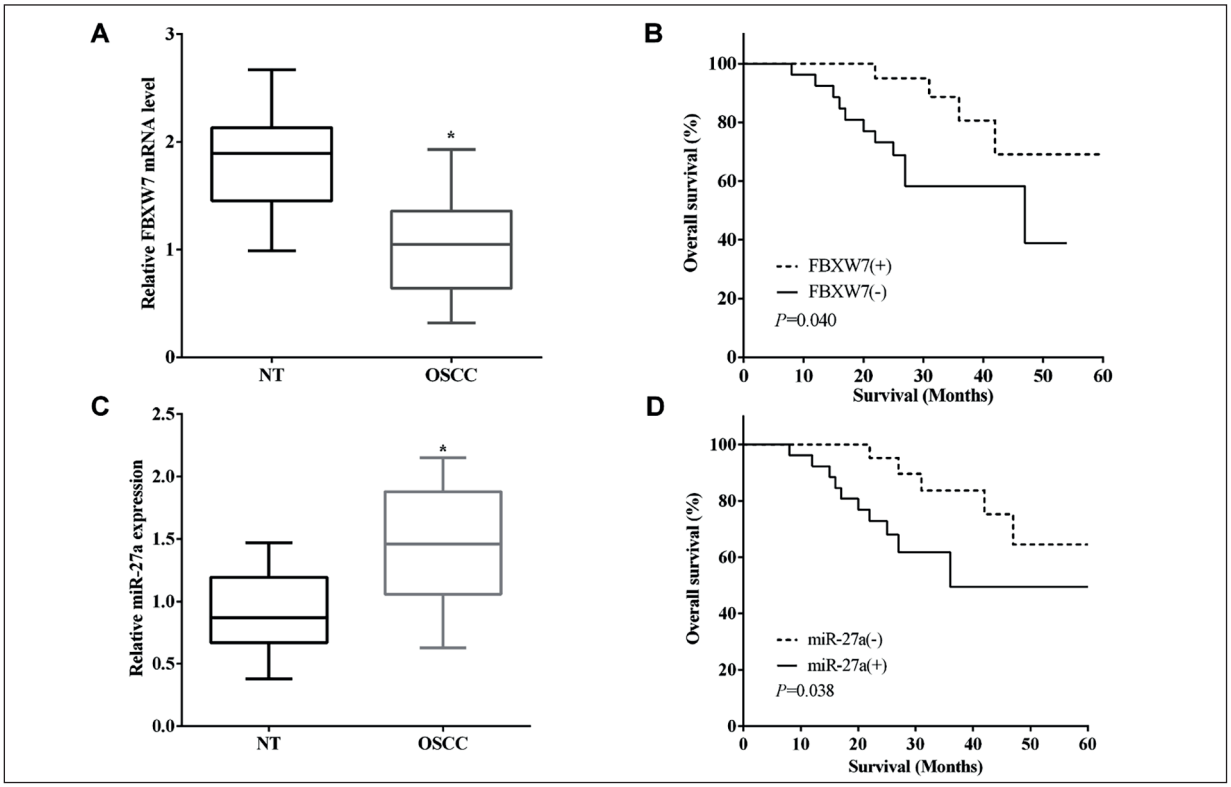

Figure 1. FBXW7 was downregulated in OSCC tissues and downregulation of FBXW7 predicted poor 5-year overall survival **A,** FBXW7 was downregulated in OSCC tissues compared with the corresponding non-tumor tissues. **B,** Downregulation of FBXW7 predicted poor 5-year survival of OSCC patients. **C,** Expression of miR-27a was higher in OSCC tissues than the corresponding non-tumor tissues. **D,** Kaplan-Meier method revealed that the overall survival was associated with the expression of miR-27a and overexpression of miR-27a predicted poor prognosis

Table I. Relationship between miR-223 expression and their clinic-pathological characteristics of 52 OSCC patients.

Characteristics	Cases	MiR-223		*p*-value
		29 High	23 Low	
Age (years)				0.438
≥ 60	28	17 (60.7)	11 (39.3)	
< 60	24	12 (50.0)	12 (50.0)	
Gender				0.598
Male	27	16 (59.3)	11 (40.7)	
Female	25	13 (52.0)	12 (48.0)	
Differentiation				0.112
Well/moderately	29	19 (65.5)	10 (34.5)	
Poorly	23	10 (43.5)	13 (56.5)	
Tumor location				0.105
Buccal cancer	6	3 (50.0)	3 (50.0)	
Oropharyngeal cancer	11	6 (54.5)	5 (45.5)	
Tongue cancer	21	12 (57.1)	9 (42.9)	
Gingival cancer positive	14	9 (64.3)	5 (35.7)	
Lymph node metastasis				0.031*
No	29	20 (69.0)	9 (31.0)	
Yes	23	9 (39.1)	14 (60.9)	
TNM stage				0.035*
I-II	31	21 (67.7)	10 (32.3)	
III-IV	21	8 (38.1)	13 (61.9)	

Statistical analyses were performed by the χ^2-test. *p<0.05 was considered significant.

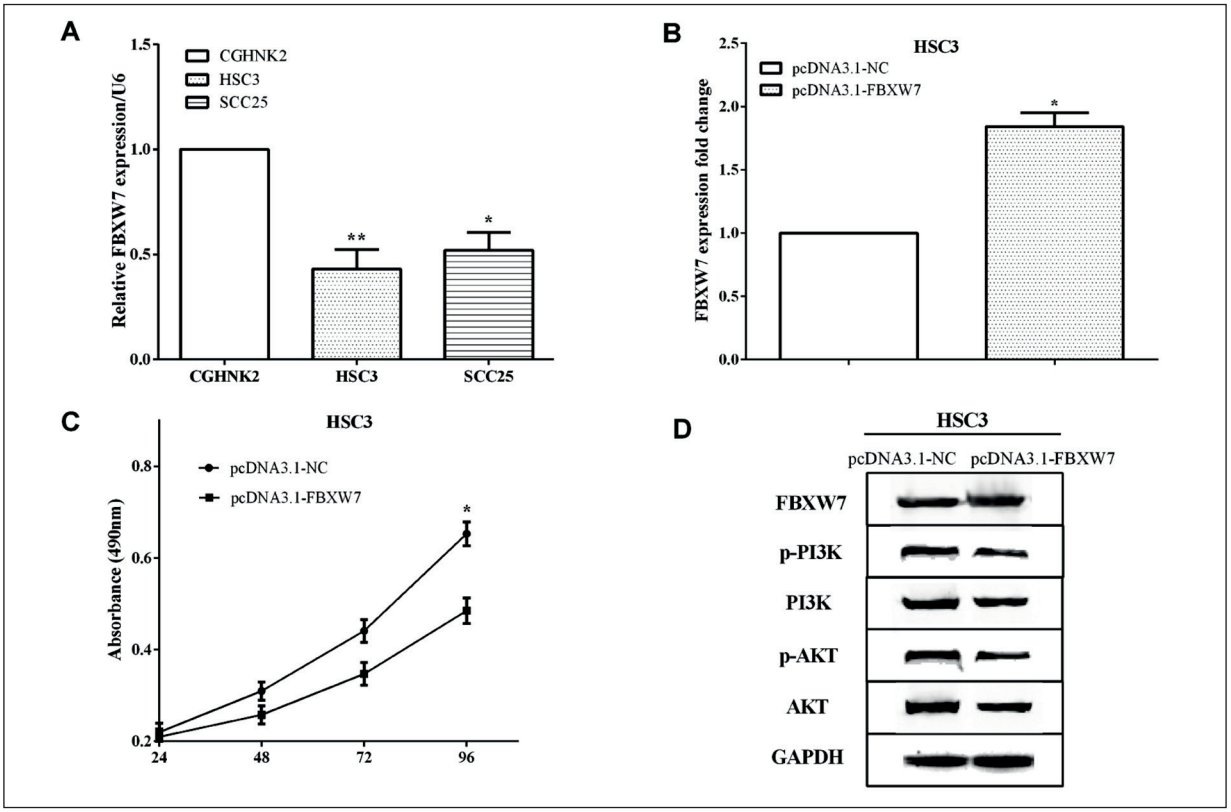

Figure 2. FBXW7 suppressed the proliferation through PI3K/AKT signal pathway in OSCC **A,** FBXW7 was low expressed in OSCC cells HSC3 and SCC25 compared with the CGHNK2 cell line. **B,** pcDNA3.1-FBXW7 was conducted to up-regulate FBXW7 in HSC3 cells. **C,** Proliferation was inhibited by pcDNA3.1-FBXW7 in HSC3 cells. **D,** FBXW7 inhibited the EMT of HSC3 cells.

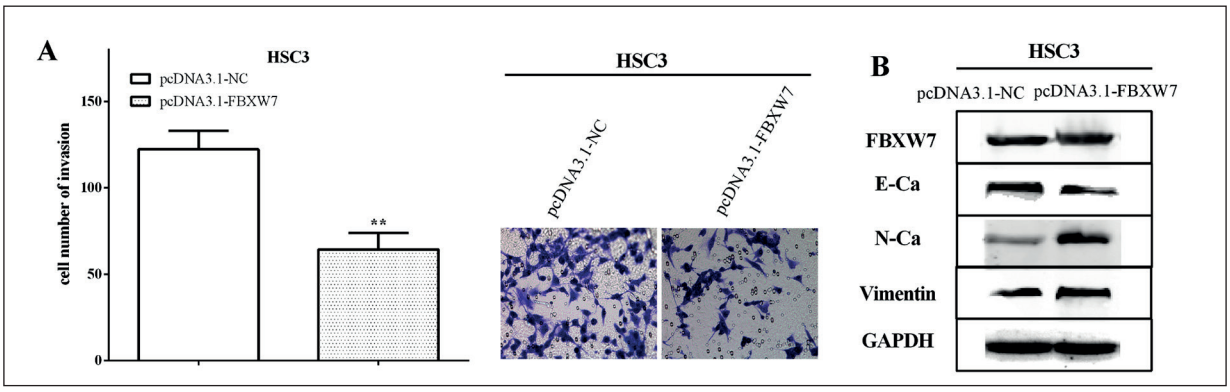

Figure 3. FBXW7 inhibited OSCC the invasion-mediated EMT **A,** Invasive ability was reduced by transfecting pcDNA3.1-FBXW7 (magnification × 40). **B,** FBXW7 suppressed the invasion-mediated EMT in HSC3 cells.

ACACUU (MUT) (Figure 4A). Followed, HSC3 cells co-transfected the miR-27a mimic and WT or MUT. Then we calculated the luciferase activities after transfection. Compared to the control, the miR-27a mimic reduced the luciferase activity of wild type FBXW7 3'-UTR ($p<0.05$), whereas exhibited no inhibitory effects on the luciferase activity of mutant FBXW7 3'-UTR in HSC3 cells ($p>0.05$) (Figure 4B). In addition, the mRNA level of FBXW7 was reduced by miR-27a mimic ($p<0.05$), while it was increased by miR-27a inhibitor in HSC3 cells ($p<0.01$; Figure 4C).

MiR-27a Reversed Partial Function of FBXW7 on the Proliferation and Invasion of OSCC

Since FBXW7 was downregulated by miR-27a, to verify the important roles of FBXW7, the miR-27a mimic was transfected in FBXW7

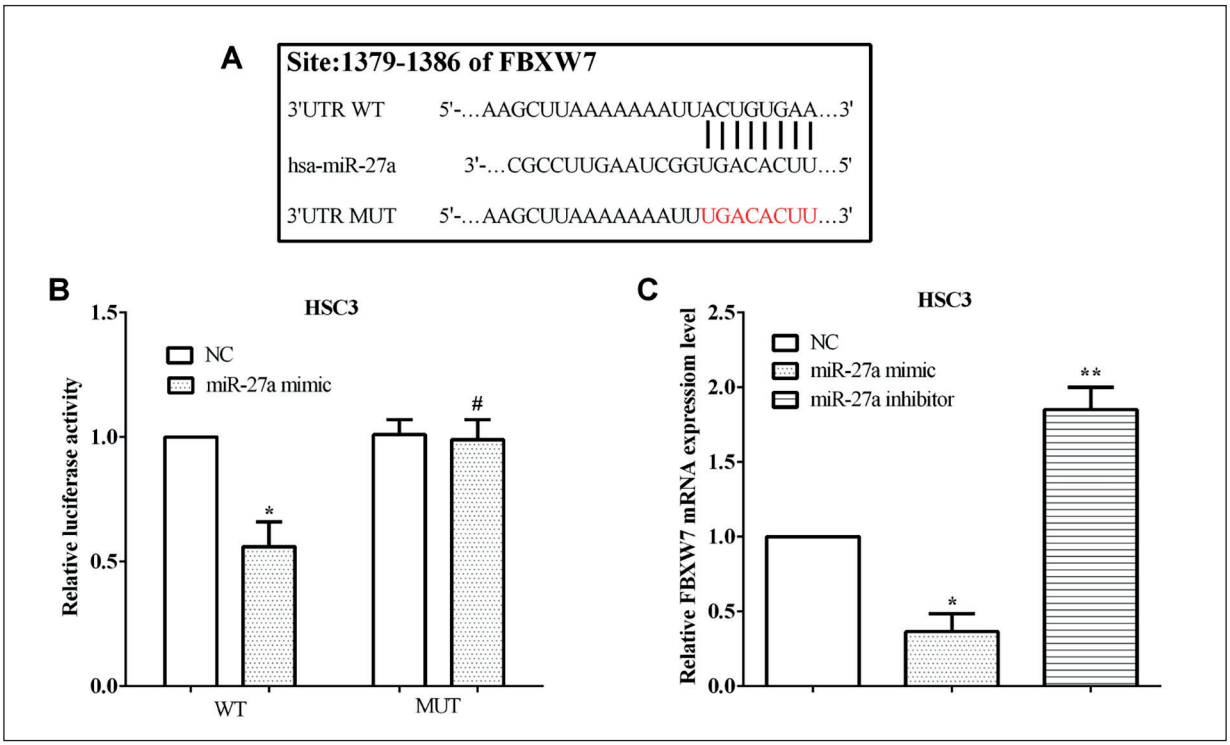

Figure 4. FBXW7 was a target gene of miR-27a in OSCC cells. **A,** TargetScan was conducted to predict FBXW7 was a potential target of miR-27a. **B,** MiR-27a mimic reduced the luciferase activity of wild type FBXW7 3'-UTR. **C**, MRNA level of FBXW7 was reduced by the miR-27a mimic, while it was increased by miR-27a inhibitor in HSC3 cells.

overexpressed HSC3 cells. As results, the expression of FBXW7 could re-express through transfected miR-27a mimic in FBXW7 overexpressed HSC3 cells (p<0.05; Figure 5A). The proliferation and invasion were assessed using MTT and transwell assays. The proliferative ability was promoted by transfecting the miR-27a mimic in FBXW7 overexpressed HSC3 cells (p<0.05; Figure 5B). Transwell assay revealed that the invasive ability was increased when transfected the miR-21 mimic in FBXW7 overexpressed cells than only transfected FBXW7, suggesting that miR-27a reversed the partial function of FBXW7 (p<0.05; Figure 5C).

Discussion

Oral squamous cell carcinoma is the most common oral cancer in India which has a high incidence rate of 30-40%[14]. OSCC originates from oral epithelial dysplasia (OED), which was characterized by an altered change of epithelium squamous dysplastic cells[15,16]. The 5-year survival of OSCC patients was poor due to great potential for metastasis[3]. Thus, it is urgent to detect the biomarkers for the treatment of OSCC.

FBXW7 is one of the most commonly deregulated ubiquitin-proteasome system proteins in human cancer and controlled proteasome-mediated degradation of oncoproteins[4]. Downregulation of FBXW7 was associated with poor prognosis in gastric adenocarcinoma and non-small-cell lung cancer[17,18]. Consistent with all the findings, we showed that the expression of FBXW7 was lower in OSCC tissues than the corresponding non-tumor tissues, and the downregulation of FBXW7 predicted poor overall survival of OSCC patients. FBXW7 inhibited the migration and invasion *via* suppressing EMT in renal cell carcinoma[19]. In our work, we discovered that overexpression of FBXW7 inhibited the proliferation of HSC3 cells through the PI3K/AKT signaling pathway. Moreover, FBXW7 impaired the invasion and EMT in HSC3 cells.

MiRNAs, 19-25 nucleotides RNAs, bind the 3'-UTR of target mRNA, leading to the mRNA degradation or translation repression[8,20]. FBXW7

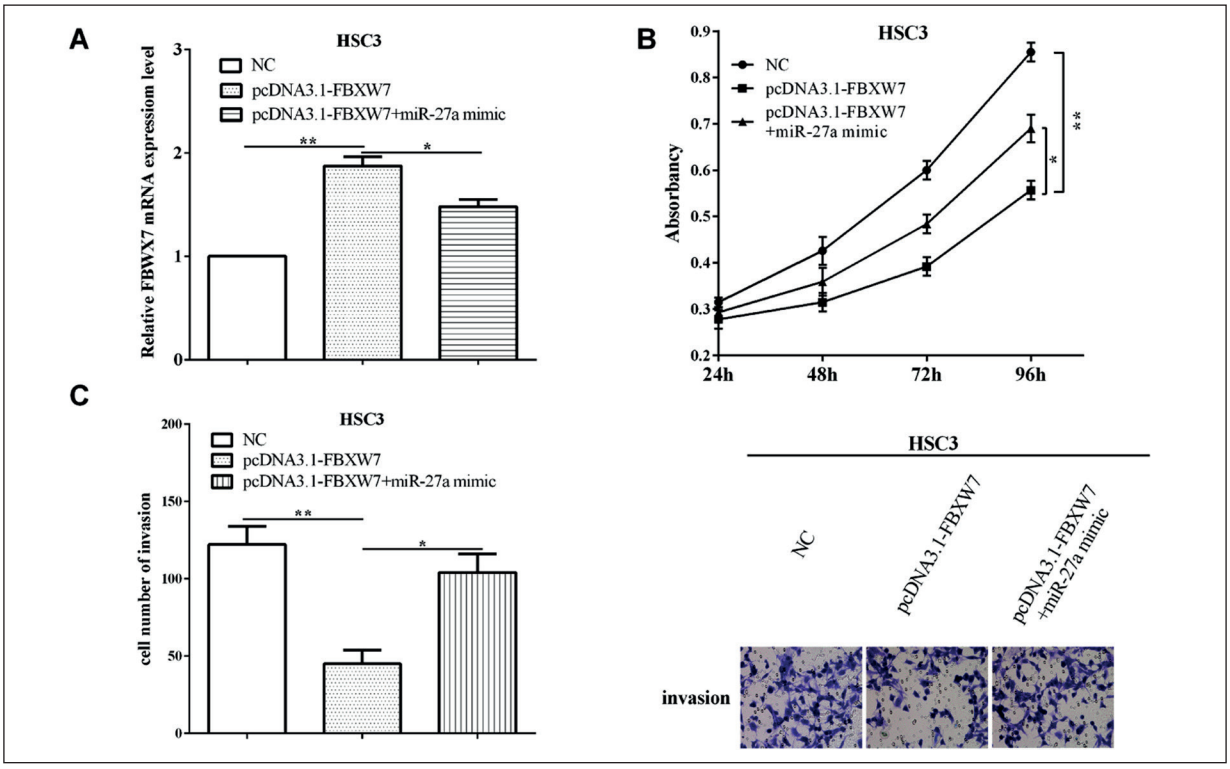

Figure 5. MiR-27a reversed partial function of FBXW7 on the proliferation and invasion of OSCC. **A,** Expression of FBXW7 could re-express through transfecting the miR-27a mimic in FBXW7 overexpressed HSC3 cells. **B,** MiR-27a mimic promoted the proliferative ability in FBXW7 overexpressed HSC3 cells. **C,** Transwell assay revealed that the invasive ability was increased when transfected with the miR-21 mimic in FBXW7 overexpressed cells (magnification × 40).

was a target gene of several MiRNAs, including miR-182, miR-223, miR-92b, and miR-25[21-24]. MiR-27a acted as a biomarker to enhance the growth and colon formation in prostate cancer[25]. Similarly, miR-27a enhanced the migration, invasion, and EMT *via* the Wnt signal in ovarian cancer[26]. Also, we found that miR-27a promoted the proliferation and migration by directly targeting to the 4'-UTR of FBXW7 in OSCC cells, which were consistent with the findings in breast cancer and esophageal cancer [27,28].

Conclusions

We revealed that FBXW7 was downregulated while miR-27a was upregulated in OSCC tissues and cell lines, and the downregulation of FBXW7 or upregulation of miR-27a in OSCC tissues predicted poor outcome of OSCC patients. FBXW7 suppressed the growth through the PI3K/AKT signaling pathway in OSCC cell line HSC3. FBXW7 inhibited the invasion-mediated EMT in HSC3 cells. The expression of FBXW7 was mediated by miR-27a by directly binding to the 3'-UTR of FBXW7 in HSC3 cells. MiR-27a reversed partial roles of FBXW7 on the proliferation and invasion in OSCC cell.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45: 309-316.
2) Shaw RJ, Pace-Balzan A, Butterworth C. Contemporary clinical management of oral squamous cell carcinoma. Periodontol 2000 2011; 57: 89-101.
3) Jerjes W, Upile T, Petrie A, Riskalla A, Hamdoon Z, Vourvachis M, Karavidas K, Jay A, Sandison A, Thomas GJ, Kalavrezos N, Hopper C. Clinicopathological parameters, recurrence, locoregional and distant metastasis in 115 T1-T2 oral squamous cell carcinoma patients. Head Neck Oncol 2010; 2: 9.
4) Yeh CH, Bellon M, Nicot C. FBXW7: a critical tumor suppressor of human cancers. Mol Cancer 2018; 17: 115.
5) Chen X, Li XY, Long M, Wang X, Gao ZW, Cui Y, Ren J, Zhang Z, Liu C, Dong K, Zhang H. The FBXW7 tumor suppressor inhibits breast cancer proliferation and promotes apoptosis by targeting MTDH for degradation. Neoplasma 2018; 65: 201-209.
6) Cai Y, Zhang M, Qiu X, Wang B, Fu Y, Zeng J, Bai J, Yang G. Upregulation of FBXW7 suppresses renal cancer metastasis and epithelial mesenchymal transition. Dis Markers 2017; 2017: 8276939.
7) Ishii N, Araki K, Yokobori T, Gantumur D, Yamanaka T, Altan B, Tsukagoshi M, Igarashi T, Watanabe A, Kubo N, Hosouchi Y, Kuwano H, Shirabe K. Reduced FBXW7 expression in pancreatic cancer correlates with poor prognosis and chemotherapeutic resistance via accumulation of MCL1. Oncotarget 2017; 8: 112636-112646.
8) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
9) Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge CB, Bartel DP. The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. Science 2005; 310: 1817-1821.
10) Gao H, Deng H, Xu H, Yang Q, Zhou Y, Zhang J, Zhao D, Liu F. MicroRNA-223 promotes mast cell apoptosis by targeting the insulin-like growth factor 1 receptor. Exp Ther Med 2016; 11: 2171-2176.
11) Liu J, Li M, Liu X, Liu F, Zhu J. MiR-27a-3p promotes the malignant phenotypes of osteosarcoma by targeting ten-eleven translocation 1. Int J Oncol 2018; 52: 1295-1304.
12) Liang J, Tang J, Shi H, Li H, Zhen T, Duan J, Kang L, Zhang F, Dong Y, Han A. MiR-27a-3p targeting RXRalpha promotes colorectal cancer progression by activating Wnt/beta-catenin pathway. Oncotarget 2017; 8: 82991-83008.
13) Wang J, Guan X, Zhang Y, Ge S, Zhang L, Li H, Wang X, Liu R, Ning T, Deng T, Zhang H, Jiang X, Ba Y, Huang D. Exosomal miR-27a derived from gastric cancer cells regulates the transformation of fibroblasts into cancer-associated fibroblasts. Cell Physiol Biochem 2018; 49: 869-883.
14) Geetha KM, Leeky M, Narayan TV, Sadhana S, Saleha J. Grading of oral epithelial dysplasia: points to ponder. J Oral Maxillofac Pathol 2015; 19: 198-204.
15) Siriwardena SBSM, Tsunematsu T, Qi G, Ishimaru N, Kudo Y. Invasion-related factors as potential diagnostic and therapeutic targets in oral squamous cell carcinoma-A review. Int J Mol Sci 2018; 19. pii: E1462.
16) Xu XJ, Yuan J, Sun WJ, Chen QY, Lin Y, Tang L, Liang LZ. Inhibition of microRNA-218 promotes oral squamous cell carcinoma growth by targeting GLUT1 to affect glucose metabolism. Eur Rev Med Pharmacol Sci 2018; 22: 7726-7734.
17) Li MR, Zhu CC, Ling TL, Zhang YQ, Xu J, Zhao EH, Zhao G. FBXW7 expression is associated with prognosis and chemotherapeutic outcome in Chinese patients with gastric adenocarcinoma. BMC Gastroenterol 2017; 17: 60.
18) Xiao G, Li Y, Wang M, Li X, Qin S, Sun X, Liang R, Zhang B, Du N, Xu C, Ren H, Liu D. FBXW7 sup-

presses epithelial-mesenchymal transition and chemo-resistance of non-small-cell lung cancer cells by targeting snai1 for ubiquitin-dependent degradation. Cell Prolif 2018; 51: e12473.

19) He H, Dai J, Xu Z, He W, Wang X, Zhu Y, Wang H. Fbxw7 regulates renal cell carcinoma migration and invasion via suppression of the epithelial-mesenchymal transition. Oncol Lett 2018; 15: 3694-3702.

20) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.

21) Chang H, Liu YH, Wang LL, Wang J, Zhao ZH, Qu JF, Wang SF. MiR-182 promotes cell proliferation by suppressing FBXW7 and FBXW11 in non-small cell lung cancer. Am J Transl Res 2018; 10: 1131-1142.

22) Mansour MR, Sanda T, Lawton LN, Li X, Kreslavsky T, Novina CD, Brand M, Gutierrez A, Kelliher MA, Jamieson CH, von Boehmer H, Young RA, Look AT. The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. J Exp Med 2013; 210: 1545-1557.

23) Gong L, Ren M, Lv Z, Yang Y, Wang Z. MiR-92b-3p promotes colorectal carcinoma cell proliferation, invasion, and migration by inhibiting FBXW7 in vitro and in vivo. DNA Cell Biol 2018; 37: 501-511.

24) Gong J, Cui Z, Li L, Ma Q, Wang Q, Gao Y, Sun H. MicroRNA-25 promotes gastric cancer proliferation, invasion, and migration by directly targeting F-box and WD-40 Domain Protein 7, FBXW7. Tumour Biol 2015; 36: 7831-7840.

25) Gao W, Hong Z, Huang H, Zhu A, Lin S, Cheng C, Zhang X, Zou G, Shi Z. MiR-27a in serum acts as biomarker for prostate cancer detection and promotes cell proliferation by targeting Sprouty2. Oncol Lett 2018; 16: 5291-5298.

26) Zhang LY, Chen Y, Jia J, Zhu X, He Y, Wu LM. MiR-27a promotes EMT in ovarian cancer through active Wnt/-catenin signalling by targeting FOXO1. Cancer Biomark 2019; 24: 31-42.

27) Jiang G, Shi W, Fang H, Zhang X. MiR27a promotes human breast cancer cell migration by inducing EMT in a FBXW7dependent manner. Mol Med Rep 2018; 18: 5417-5426.

28) Wu XZ, Wang KP, Song HJ, Xia JH, Jiang Y, Wang YL. MiR-27a-3p promotes esophageal cancer cell proliferation via F-box and WD repeat domain-containing 7 (FBXW7) suppression. Int J Clin Exp Med 2015; 8: 15556-15562.