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Circular RNA FAT1 (hsa_circ_0001461) Promotes the Progression of Oral Squamous Cell Carcinoma by Regulating miR-181d-5p/miR-199a-5p

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Keywords

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Abstract

Objective: To explore the role and mechanism of circular RNA FAT1 (hsa_circ_0001461)-miR-181d-5p/miR-199a-5p axis in the biological functions of oral squamous cell carcinoma (OSCC) cells. **Methods:** The clinical tissues of 30 OSCC patients were collected, and the expressions of circFAT1, miR-181d-5p and miR-199a-5p in OSCC tissues and cells were detected by real-time quantitative PCR. starBase and dual-luciferase reporter genes were used to verify the targeting relationship between miR-181d-5p/miR-199a-5p and circFAT1. Functional experiments included CCK-8, Transwell, and flow cytometry to determine the effects of circFAT1-miR-181d-5p/miR-199a-5p axis on OSCC cell viability, migration, invasion, apoptosis and cycle. Western blot combined with LY294002 treatment was used to measure the expressions of PI3K/Akt/mTOR signaling pathway-related proteins. **Results:** The circFAT1 expression was increased, and miR-181d-5p and miR-199a-5p expressions were decreased in OSCC ($p < 0.001$). CircFAT1 could target miR-181d-5p and miR-199a-5p simultaneously ($p < 0.01$). CircFAT1 silencing, miR-181d-5p mimic and miR-199a-5p mimic inhibited OSCC cell viability, migration, invasion and cycle progression, induced apoptosis, and suppressed the activation of PI3K/Akt/mTOR pathway, while overexpressed circFAT1, miR-181d-5p inhibitor and miR-199a-5p inhibitor did the opposite ($p < 0.05$). MiR-181d-5p and miR-199a-5p partially reversed the effect of circFAT1 on the biological functions of OSCC cells ($p < 0.05$). LY294002 pretreatment offset the role of circFAT1 overexpression in PI3K/Akt/mTOR pathway and cell viability ($p < 0.05$). **Conclusion:** CircFAT1 promotes the malignant progression of OSCC cells by regulating miR-181d-5p/miR-199a-5p.

1 Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, accounting for over 90% of all head and neck cancers, and is related to poor prognosis and high mortality [1]. The molecular pathogenesis of OSCC is highly complex, involving multiple genetic alterations. Identifying potential biomarkers is of great significance for the diagnosis and treatment of diseases, and the improvement of prognosis.

Circular RNA (circRNA) is considered a promising candidate target for cancer diagnosis and treatment due to its high conservation and tissue specificity. Our project team has previously conducted research on the relevant between OSCC and miR-181, and found the association between miR-181 with OSCC cell growth, invasion and migration [2]. MiR-199a-5p can target and regulate mTOR pathway [3], and also has been reported to act as a tumor suppressor in OSCC [4]. Based on previous basic research and existing literature reports, this study aims to screen for circRNAs that have binding sites for miR-181/miR-199 sponges and are also related to OSCC. With starBase and GSE131182 data set, 7 circRNAs have been identified, including NCOA1, ATXN1, FAT1, TNPO3, FNDC3B, SSH2 and MAP3K4 (Figure 1). Of them, circFAT1 (hsa_circ_0001461) expression has been reported to be elevated in head and neck squamous cell carcinomas, and mediate cancer stemness and tumor immune escape [5]. Therefore, this study explored the role of circFAT1 in OSCC and preliminarily understood its relation with miR-181d-5p/miR-199a-5p, based on the analyses of samples from clinical patients and *in vivo* cell assays.

2 Materials and methods

2.1 Clinical information

30 OSCC patients who have received surgery in our hospital from Jan. 2021 to Oct. 2021 were selected, from which the clinical tissue samples were collected, including cancer tissues and para-carcinoma tissues, which have been confirmed by pathological examination. This study program has been approved by the Ethics Committee of our hospital, with signed written informed consent from all patients. Inclusion criteria: (1) The pathological confirmation of OSCC has been confirmed by three pathologists; (2) No radio/chemo-therapy before surgery. Exclusion criteria: (1) Other systemic diseases; (2) Receiving other anti-tumor treatment before surgery.

2.2 Methods

2.2.1 Cell culture, transfection and treatment

The normal oral keratinocyte cell line hNOK was purchased from Tongpai (Shanghai) Biotechnology Co., Ltd. OSCC cells SCC-25 and CAL-27 were obtained from the American Type Culture Collection, and HSC-2 and HSC-3 were from the Riken Cell Bank. All cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a homothermal incubator at 37 ℃ with 5% CO2. According to the instructions of Lipofectamine 3000 (Invitrogen, USA), small interfering RNA against circFAT1 (Si-FAT1), FAT1 overexpression plasmid, miR-181d-5p mimic, miR-181d-5p inhibitor, miR-199a-5p mimic and miR-199a-5p inhibitor, as well as their corresponding control (Si-NC, NC, MC, IC) were transfected into HSC-3 or SCC-25 cells. The mimic, inhibitor and their negative control were all purchased from RIBOBIO (Guangzhou, China), and siRNA and overexpresion plasmid were synthesized by GenePharma (Shanghai, China).

To verify the effect of tested genes on PI3K/Akt/mTOR signaling pathway, 50 μM LY294002 (PI3K/Akt/mTOR inhibitor; MedChemExpress) was added to pre-treat cells 1 h before transfection.

2.2.2 Real-time quantitative PCR (qRT-PCR)

Total RNA was extracted from OSCC tissues and cells

using TRIzol reagent, and cDNA was synthesized using the cDNA Synthesis Kit (TransGen Biotech, Beijing, China). QRT-PCR was performed using the Applied Biosystems real-time fluorescence quantitative PCR instrument and SYBR Green Supermix reagent to quantify the expression levels of circFAT1, miR-181d-5p, and miR-199a-5p in OSCC tissues and cells. GAPDH and U6 were used as internal reference genes. The relative expression levels of circFAT1, miR-181d-5p, and miR-199a-5p were analyzed using the $2^{-\Delta\Delta Ct}$ method.

The primer sequences are as follows (5′-3′): circFAT1 (forward primer: AACAGAAGAGAACTGGGGCG, reverse primer: GATCAGGGTGCCAATGGTGA); GAPDH (forward primer: TGTTCGTCATGGGTGTGAAC, reverse primer: ATGGCATGGACTGTGGTCAT); miR-181d-5p (forward primer: AACATTCATTGTTGTCGGTGGGTG, reverse primer: TCGTATCCAGTGCGTGTCG); miR-199a-5p (forward primer: CCAGTGTTCAGACTACCTGTTC, reverse primer: GTATCCAGTGCGTGTCGTGG); U6 (forward primer: CTCGCTTCGGCAGCACA, reverse primer: AACGCTTCACGAATTTGCGT).

2.2.3 Prediction and validation of target gene relationships

The wild-type or mutant sequence of circFAT1 was recombined into the dual-luciferase reporter vector pmirGLO to construct the dual-luciferase plasmid (FAT1-wt or FAT1-mut). SCC-25 and HSC-3 cells were cultured in complete medium and co-transfected with miR-181d-5p mimic or miR-199a-5p mimic and the dual-luciferase recombinant plasmid, with mimic control (MC#1, MC#2) as the control. Subsequently, the relative luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega, USA).

2.2.4 CCK-8 assay for cell viability detection

After transfection, SCC-25 or HSC-3 cells (1×10^3) were seeded into 96-well plates. Following 72-h culture, the CCK-8 solution (Solarbio, Beijing, China) was reacted with the cells for about 3 h. Next, the relative cell viability was analyzed using the SpectraMax iD3 microplate reader.

2.2.5 Transwell assay for cell migration and invasion detection

Transwell assay was performed to determine the effect of target genes OSCC cell migration and invasion. In different invasion assays, Matrigel (Solarbio) was pre-added into Transwell compartment. Then, the transfected cell were digested, resuspended, and incubated in serum-free medium in the upper compartment, while the complete culture media with FBS was added into lower Transwell compartment. 48 h after incubation, the cells were fixed with fixative, and stained with 0.1% crystal violet (Solarbio), followed by microscopic examination and data recording.

2.2.6 Flow cytomery assay for cell apoptosis and cell cycle detection

The cell cycle was examined using a cell cycle and apoptosis detection kit (Beyotime, Shanghai, China), and cell apoptosis was tested using an Annexin V-FITC apoptosis detection kit. For apoptosis detection, cells were digested, placed in EP tubes, and resuspended in the Annexin V-FITC binding buffer from the kit, followed by the sequential addition of Annexin V-FITC and propidium iodide staining solution. The mixture was gently mixed and incubated at room temperature in the dark for about 15 min. Detection was performed using the CytoFLEX flow cytometer (Beckman Coulter, USA). For cell cycle examination, cells were digested, washed, fixed overnight at 4 ℃ with 70% ethanol, and dyed with propidium iodide working solution in the dark at 37 ℃ for 30 min. The results were analyzed using a flow cytometer.

2.2.7 Western blot for protein level detection

Cells were lysed with RIPA lysis buffer (Beyotime). Protein concentration was measured using a BCA kit. Following electrophoresis and separation, proteins were transferred to PVDF membranes (Thermo Scientific, USA). After blocking, the membranes were incubated with the corresponding primary and secondary antibodies. After exposure and development, GAPDH was used as an internal reference to analyze the relative protein expression levels. All antibodies were purchased from Abcam (UK). Goat anti-rabbit IgG H&L (HRP) and goat anti-mouse IgG H&L (HRP) from Abcam were exploited as secondary antibodies.

2.3 Statistical methods

Statistical analysis was performed using GraphPad Prism 8.0. Quantitative data were expressed as mean ± standard deviation. Paired sample *t*-tests were used for comparisons in Figure 1B-C, and independent sample *t*-tests were for comparisons in Figure 1J-M. Multi-group comparisons were completed with one-way ANOVA. $p < 0.05$ indicated statistical significance.

3 Results

3.1 Expressions and relationships of circFAT1, miR-181d-5p, and miR-199a-5p in OSCC tissues and cells

The expression of circFAT1 was upregulated in OSCC tissues and cells, while miR-181d-5p and miR-199a-5p expressions were downregulated ($p < 0.001$, Figure 1B-G). Figure 1H-I showed the binding sites of circFAT1 with miR-181d-5p and miR-199a-5p. Dual-luciferase reporter assays confirmed that miR-181d-5p and miR-199a-5p could both serve as target miRNA of circFAT1 ($p < 0.01$, Figure 1J-M).

3.2 CircFAT1 impacted OSCC cell viability via regulating miR-181d-5p/miR-199a-5p

Figure 2A showed that siFAT1 significantly reduced circFAT1 levels ($p < 0.001$), while miR-181d-5p inhibitor barely impacted circFAT1 levels ($p > 0.05$). The relative expression of miR-181d-5p was evidently increased in the siFAT1+IC#1 group ($p < 0.001$) and decreased in the siNC+miR-181d-5p inhibitor group ($p < 0.01$). The combination of siFAT1 and miR-181d-5p inhibitor reversed the trends of miR-181d-5p levels in the above two groups ($p < 0.001$, Figure 2B). FAT1 overexpression plasmid significantly elevated circFAT1 expression ($p < 0.001$), while miR-181d-5p mimic had no apparent effect on circFAT1 levels ($p > 0.05$, Figure 2C). The relative expression of miR-181d-5p was significantly decreased in the FAT1+MC#1 group ($p < 0.001$) and increased in the NC+miR-181d-5p mimic group ($p < 0.001$). The combination of FAT1 and miR-181d-5p mimic reversed the trend of miR-181d-5p in the above two groups ($p < 0.001$, Figure 2D). MiR-199a-5p inhibitor downregulated while miR-199a-5p mimic upregulated miR-199a-5p level. MiR-199a-5p had no effect on circFAT1 expression but reversed the regulatory effect of circFAT1 on miR-199a-5p expression ($p < 0.01$, Figure 2E-H).

The CCK-8 assay data indicated that siFAT1 inhibited cell viability, while overexpression of circFAT1 had the opposite effect. MiR-181d-5p inhibitor and miR-199a-5p inhibitor promoted cell viability, while miR-181d-5p mimic and miR-199a-5p mimic exerted opposite effects. Both miR-199a-5p and miR-181d-5p counteracted the regulatory effect of circFAT1 on cell viability ($p < 0.01$, Figure 2I-L).

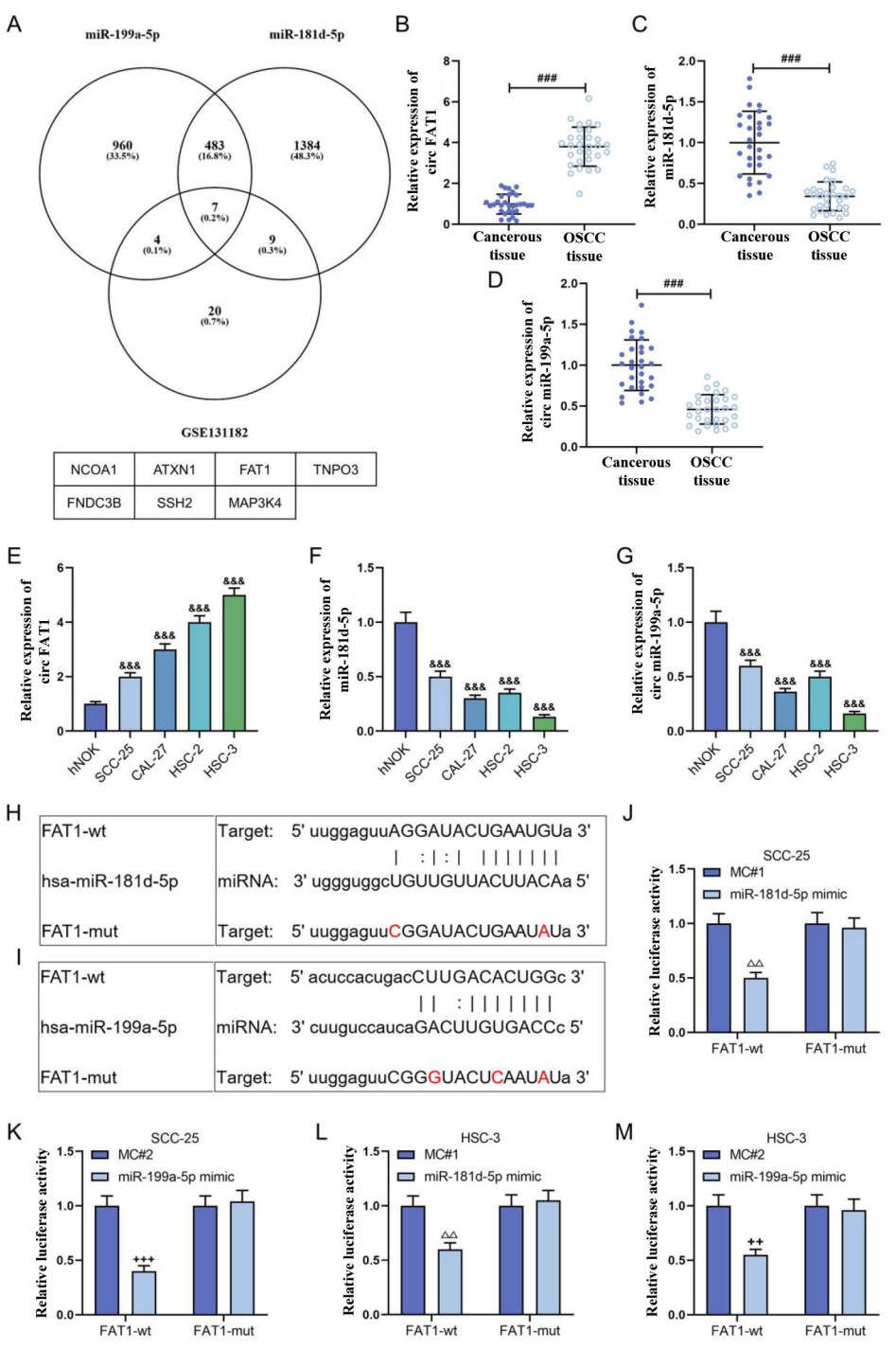

Figure 1 Expressions and relationships of circFAT1, miR-181d-5p and miR-199a-5p in OSCC tissues and cells. (A) Venn diagram. (B-G) Results of qRT-PCR. (H-I) Binding sites. (J-M) Dual-luciferase reporter assay results. $^{###}p < 0.001$ vs. Para-carcinoma tissue; $^{\&\&\&}p < 0.001$ vs. hNOK; $^{\triangle\triangle}p < 0.01$ vs. MC#1 (mimic control); $^{++}p < 0.01$, $^{+++}p < 0.001$ vs. MC#2.

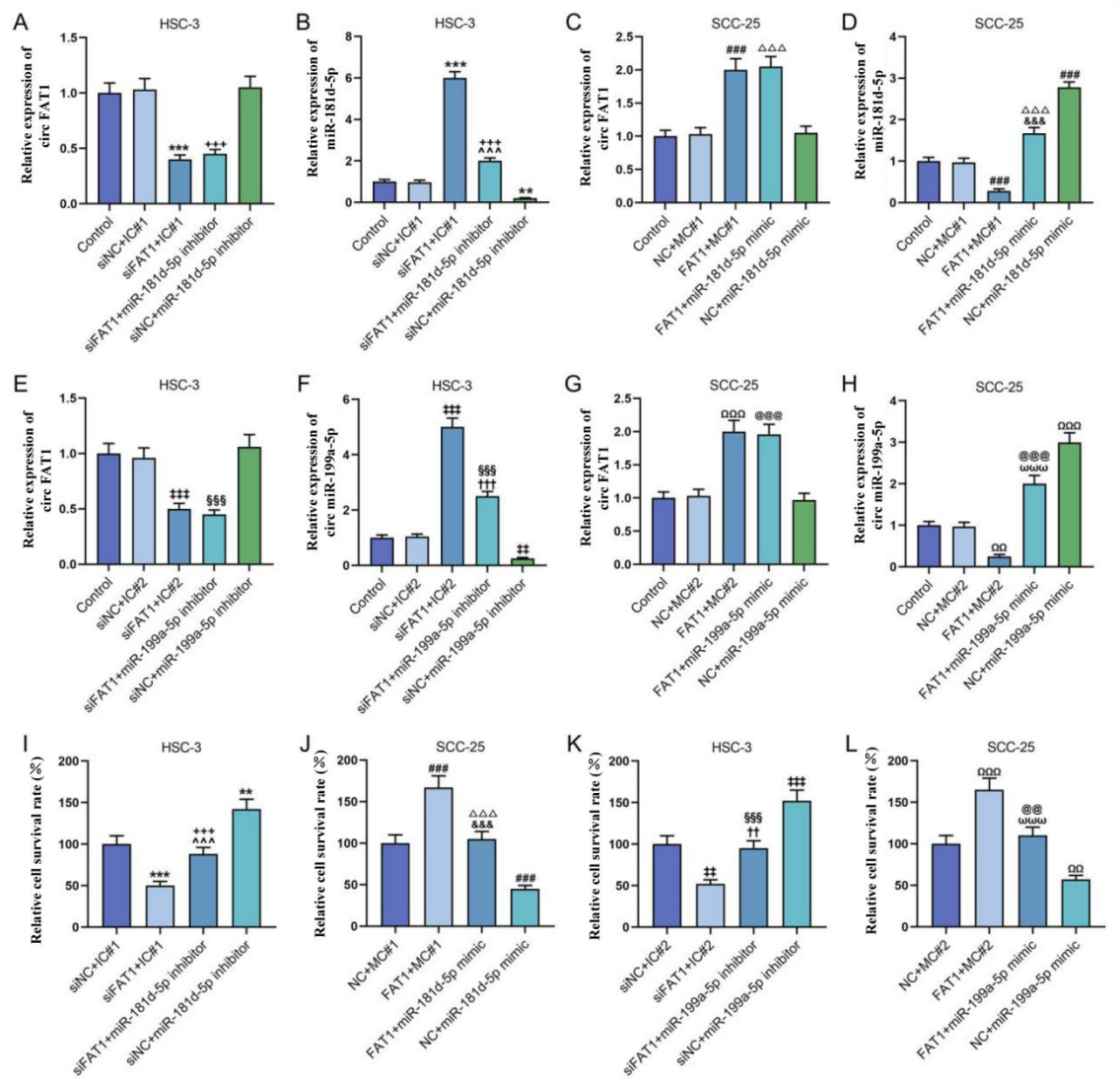

Figure 2 circFAT1 affects OSCC cell viability by regulating miR-181d-5p/miR-199a-5p. (A-H) Results of qRT-PCR. (I-L)CCK-8 results. * $p < 0.05$, ** $p < 0.05$, *** $p < 0.001$, other statistical symbols are handled in the same way. * vs. siNC+IC#1 (inhibitor control); ^ vs. siFAT1)+IC#1; + vs. siNC+miR-181d-5p inhibitor; # vs. NC+MC#1; & vs. FAT1+MC#1; △ vs. NC+miR-181d-5p mimic; ‡ vs. siNC+IC#2; † vs. siFAT1+IC#2; § vs. siNC+miR-199a-5p inhibitor; Ω vs. NC+MC#2; ω vs. FAT1+MC#2; @ vs. NC+miR-199a-5p mimic.

3.3 CircFAT1 mediated OSCC cell migration and invasion via miR-181d-5p/miR-199a-5p

Transwell migration assay results showed that siFAT1, miR-181d-5p mimic, and miR-199a-5p mimic reduced cell migration, while overexpression of FAT1, miR-181d-5p inhibitor, and miR-199a-5p inhibitor accelerated cell migration ($p < 0.01$). Notably, miR-181d-5p inhibitor and miR-199a-5p inhibitor partially abrogated siFAT1-induced decrease of relative migration rate, while miR-181d-5p mimic and miR-199a-5p mimic partially reduced FAT1 overexpression-induced increase of relative migration rate ($p < 0.01$, Figure 3A-E). The invasion assay showed similar results. CircFAT1 silencing, miR-181d-5p inhibitor, and miR-199a-5p inhibitor dampened cell invasion, while circFAT1 overexpression, miR-181d-5p mimic, and miR-199a-5p mimic enhanced cell invasion ($p < 0.05$). Both miR-199a-5p and miR-181d-5p reversed the regulatory effects of circFAT1 on cell invasion ($p < 0.01$, Figure 3F-J).

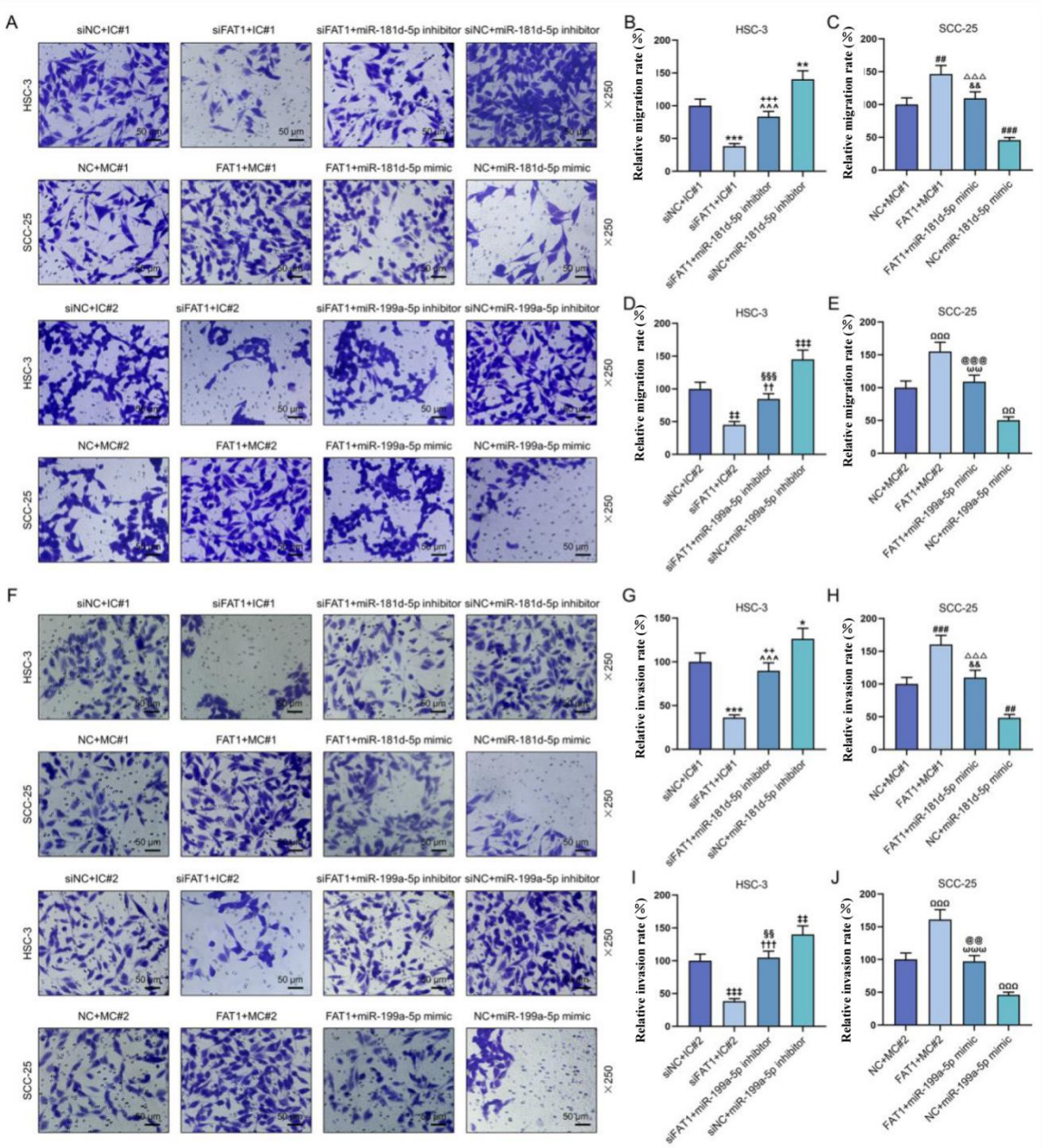

Figure 3 CircFAT1 affected OSCC cell migration and invasion by regulating miR-181d-5p/miR-199a-5p. (A-E) Transwell staining and statistical graph for migration. (F-J) Transwell staining and statistical graph for invasion. * $p < 0.05$, ** $p < 0.05$, *** $p < 0.001$, other statistical symbols are handled in the same way. * vs. siNC+IC#1; ^ vs. siFAT1+IC#1; + vs. siNC+miR-181d-5p inhibitor; # vs. NC+MC#1; & vs. FAT1+MC#1; △ vs. NC+miR-181d-5p mimic; ‡ vs. siNC+IC#2; † vs. siFAT1+IC#2; § vs. siNC+miR-199a-5p inhibitor; Ω vs. NC+MC#2; ω vs. FAT1+MC#2; @ vs. NC+miR-199a-5p mimic.

3.4 CircFAT1 mediated OSCC cell apoptosis via impacting miR-181d-5p/miR-199a-5p

Compared with the siNC+IC#1 group, the apoptosis rate in the siFAT1+IC1 group was significantly increased ($p < 0.001$), while that in the siNC+miR-181d-5p inhibitor group was decreased ($p < 0.05$). The siFAT1+miR-181d-5p inhibitor reversed the changes of apoptosis in the siFAT1+IC#1 and siNC+miR-181d-5p inhibitor groups ($p < 0.001$, Figure 4A-B). Conversely, circFAT1 overexpression reduced apoptosis, while miR-181d-5p mimic promoted apoptosis and partially reversed the effects of overexpressed circFAT1 on apoptosis ($p < 0.01$, Figure 4A, 4C). MiR-199a-5p had the same effect as

miR-181d-5p and could partially offset the effects of circFAT1 on apoptosis in HSC-3 or SCC-25 cells (p < 0.05, Figure 4A, 4D-E).

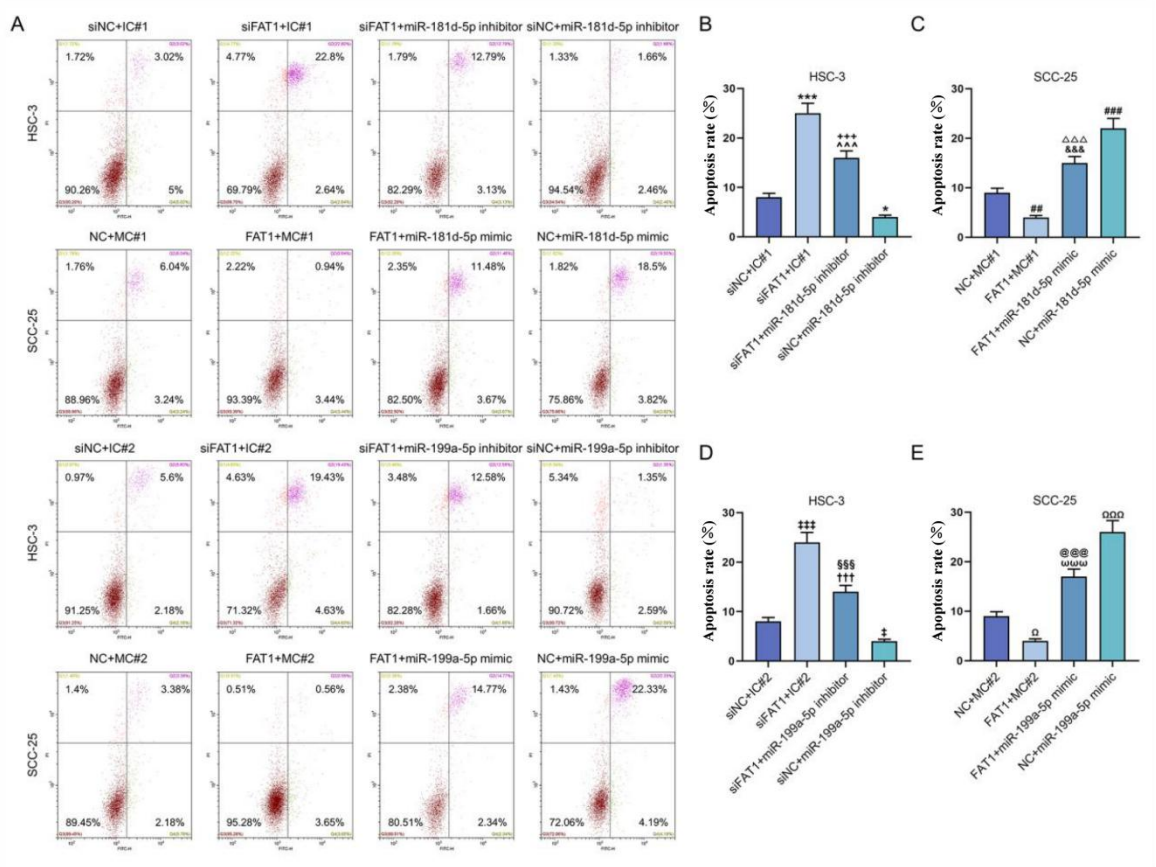

Figure 4 CircFAT1 regulated OSCC cell apoptosis by regulating miR-181d-5p/miR-199a-5p. (A-E) Results of apoptosis detected by flow cytometry. * p < 0.05, ** p < 0.05, *** p < 0.001, other statistical symbols are handled in the same way. * vs. siNC+IC#1; $^{\wedge}$ vs. siFAT1+IC#1; $^{+}$ vs. siNC+miR-181d-5p inhibitor; $^{\#}$ vs. NC+MC#1; $^{\&}$ vs. FAT1+MC#1; $^{\triangle}$ vs. NC+miR-181d-5p mimic; ‡ vs. siNC+IC#2; † vs. siFAT1+IC#2; § vs. siNC+miR-199a-5p inhibitor; $^{\Omega}$ vs. NC+MC#2; $^{\omega}$ vs. FAT1+MC#2; $^{@}$ vs. NC+miR-199a-5p mimic.

3.5 CircFAT1 regulated OSCC cell cycle progression via miR-181d-5p/miR-199a-5p

Relative to the siNC+IC#1 group, the cell cycle was signally arrested in the siFAT1+IC#1 group (p < 0.05), while the cell cycle was promoted in the siNC+miR-181d-5p inhibitor group (p < 0.05). The siFAT1+miR-181d-5p inhibitor reversed the regulatory effects on the cell cycle in the siFAT1+IC1 and siNC+miR-181d-5p inhibitor groups (p < 0.001, Figure 5A-B). CircFAT1 overexpression shortened the G0/G1 phase and relatively prolonged the S phase of SCC-25 cells, while miR-181d-5p mimic induced G0/G1 phase arrest in SCC-25 cells and partially reversed the promoting effects of overexpressed circFAT1 on the cell cycle (p < 0.05, Figure 5A, 5C). Similarly, miR-199a-5p inhibitor boosted cell cycle progression and partially offset the inhibitory effects of siFAT1 on the cell cycle, while miR-199a-5p mimic induced G0/G1 phase arrest in SCC-25 cells and partially reversed the promoting effects of overexpressed circFAT1 on the cell cycle (p < 0.05, Figure 5A, 5D-E).

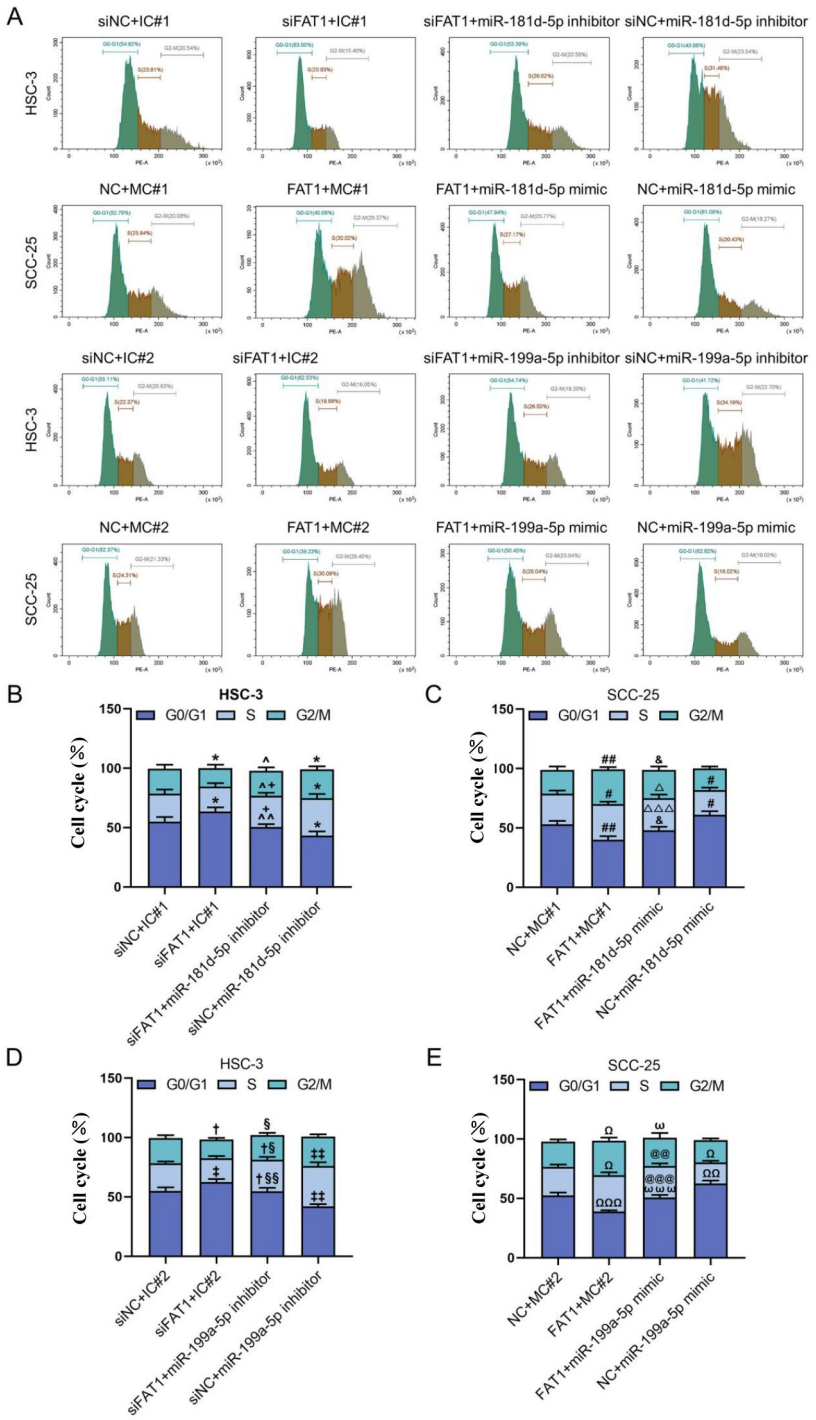

Figure 5 circFAT1 alters OSCC cell cycle progression by regulating miR-181d-5p/miR-199a-5p. (A-E) Results of OSCC cell cycle detected by flow cytometry. * $p < 0.05$, ** $p < 0.05$, *** $p < 0.001$, other statistical symbols are handled in the same way. * vs. siNC+IC#1; ^ vs. siFAT1+IC#1; + vs. siNC+miR-181d-5p inhibitor; # vs. NC+MC#1; & vs. FAT1+MC#1; △ vs. NC+miR-181d-5p mimic; ‡ vs. siNC+IC#2; † vs. siFAT1+IC#2; § vs. siNC+miR-199a-5p inhibitor; Ω vs. NC+MC#2; ω vs. FAT1+MC#2; @ vs. NC+miR-199a-5p mimic.

3.6 CircFAT1 mediated the PI3K/Akt/mTOR signaling pathway via miR-181d-5p/miR-199a-5p

Western blot analysis outcomes revealed that siFAT1, miR-181d-5p mimic, and miR-199a-5p mimic inhibited the phosphorylation of the PI3K/Akt/mTOR pathway, while FAT1 overexpression, miR-181d-5p inhibitor, and miR-199a-5p inhibitor had the opposite effect ($p < 0.05$). Of note, miR-181d-5p inhibitor and miR-199a-5p inhibitor partially reversed the inhibitory

effects of siFAT1 on the PI3K/Akt/mTOR pathway, while miR-181d-5p mimic and miR-199a-5p mimic partially weakened the activation of the PI3K/Akt/mTOR pathway triggered by FAT1 overexpression ($p < 0.01$, Figure 6A-P).

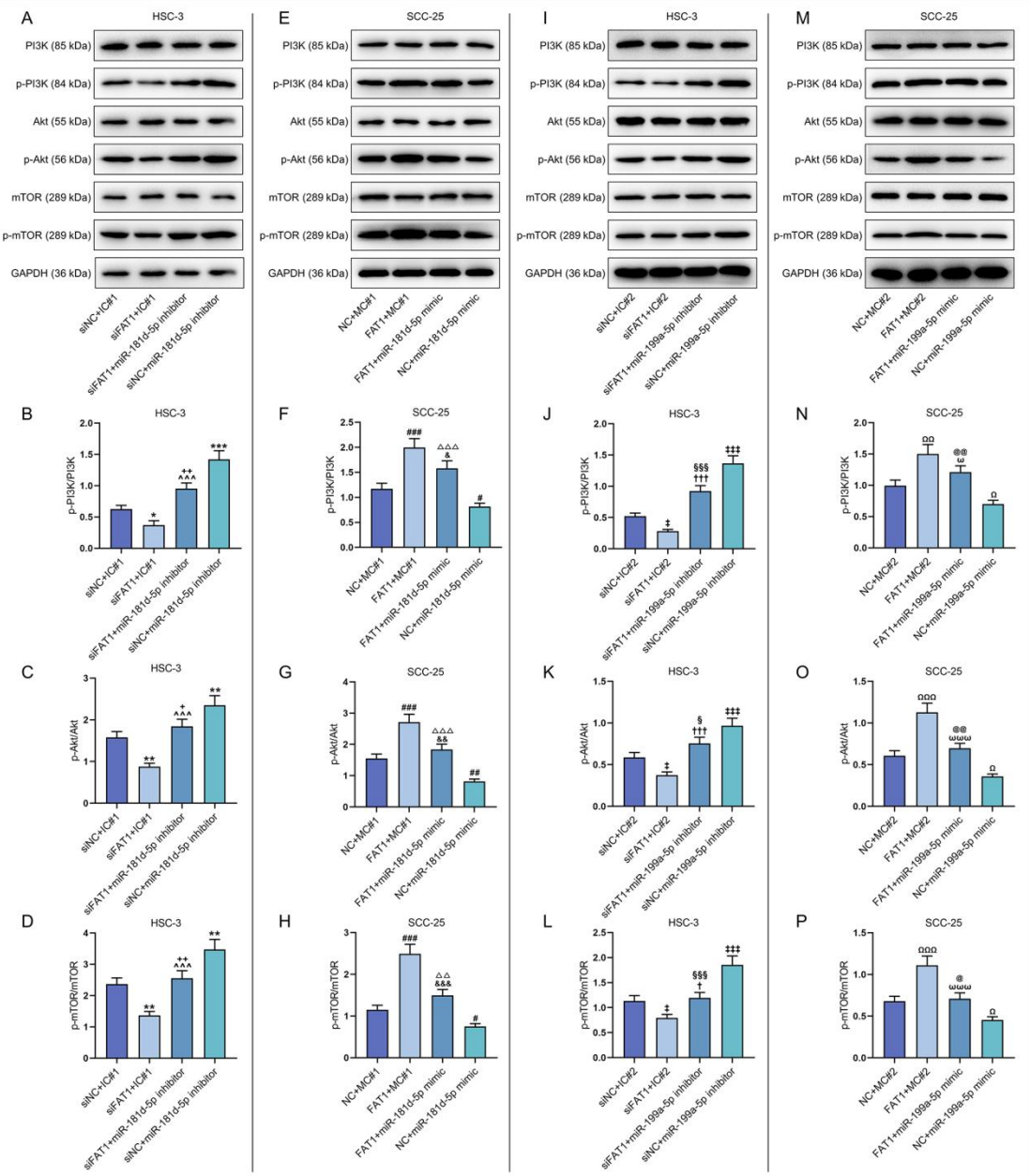

Figure 6 circFAT1 regulates PI3K/Akt/mTOR signaling pathway through miR-181d-5p/miR-199a-5p. (A-P) Western blot results of proteins related to the PI3K/Akt/mTOR signaling pathway. * $p < 0.05$, ** $p < 0.05$, *** $p < 0.001$, other statistical symbols are handled in the same way. * vs. siNC+IC#1; ^ vs. siFAT1+IC#1; + vs. siNC+miR-181d-5p inhibitor; # vs. NC+MC#1; & vs. FAT1+MC#1; △ vs. NC+miR-181d-5p mimic; ‡ vs. siNC+IC#2; † vs. siFAT1+IC#2; § vs. siNC+miR-199a-5p inhibitor; Ω vs. NC+MC#2; ωvs. FAT1+MC#2; @ vs. NC+miR-199a-5p mimic.

3.7 Effects of LY294002 pretreatment on the circFAT1/miR-181d-5p/miR-199a-5p axis

Western blot analysis data indicated that pretreatment of SCC-25 cells with LY294002 attenuated the activation of the PI3K/Akt/mTOR pathway caused by circFAT1 overexpression and had a synergistic effect with miR-181d-5p mimic and miR-199a-5p mimic (p

< 0.05, Figure 7A-H). The CCK-8 assay outcomes confirmed that pretreatment of SCC-25 cells with LY294002 partially inhibited the enhanced cell viability caused by overexpression of circFAT1 and had a synergistic effect with miR-181d-5p mimic and miR-199a-5p mimic, further reducing cell viability (p < 0.01, Figure 7I-J).

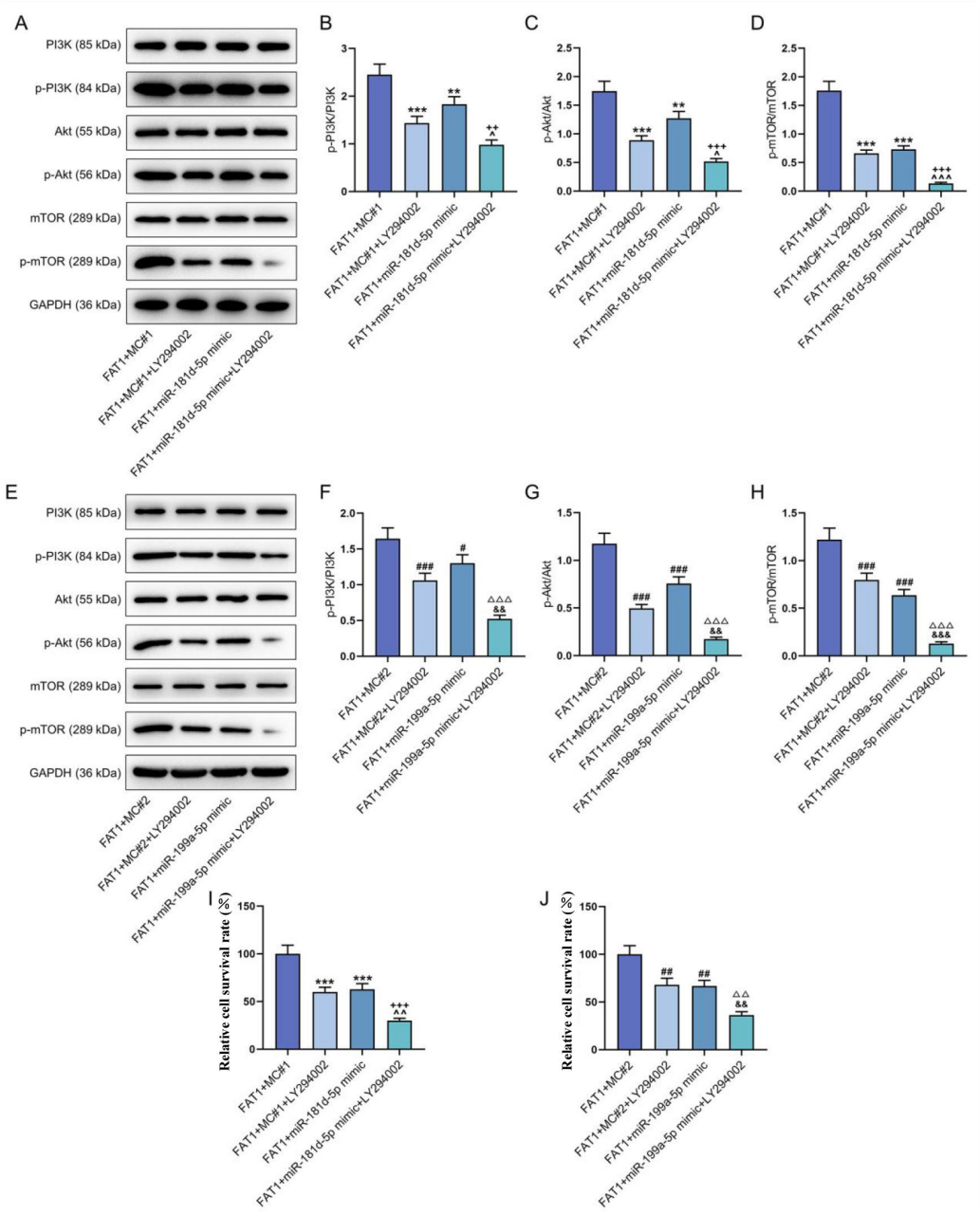

Figure 7 The effect of LY294002 pretreatment on the circFAT1/miR-181d-5p/miR-199a-5p axis. (A-H) LY294002 treatment 1 h before cell transfection (PI3K/Akt/mTOR inhibitor), Immunoblot assay for detecting PI3K/Akt/mTOR signaling pathway. (I-J) CCK-8 results. * p < 0.05, ** p < 0.05, *** p < 0.001, other statistical symbols are handled in the same way. * vs. FAT1+MC#1; ^ vs. FAT1+MC#1+LY294002; + vs. FAT1+miR-181d-5p mimic; # vs. FAT1+MC#2; & vs. FAT1+MC#2+LY294002; △ vs. FAT1+miR-199a-5p mimic.

4 Discussion

On the basis of previous research, our research group has discovered a circRNA that plays a role in OSCC, namely circFAT1. In addition, our research group

revealed the mechanism by which circFAT1 participates in the malignant progression of OSCC cells by regulating the PI3K/Akt/mTOR pathway through the miR-181d-5p/miR-199A-5p axis.

CThe research on circRNA in OSCC has gradually attracted attention in recent years. Circ_0000140 acts as a "molecular sponge" that competitively binds to miR-31 and upregulates the LATS2 axis to exert tumor suppressive effects in OSCC through the Hippo signaling pathway [6]. CircIGHG-induced epithelial mesenchymal transition promotes the progression of oral squamous cell carcinoma through miR-142-5p/IGF2BP3 signaling pathway [7]. Our research group have experimentally demonstrated that circFAT1, as an oncogene, is overexpressed in OSCC and accelerates the migration, invasion, and progression of OSCC cell lines, while reducing cell apoptosis. Consistently, a newly published literature suggested that circ_0001461 promotes OSCC progression through the miR-145/TLR4/NF-κB axis [8]. Differently, our research group also found that miR-181d-5p and miR-199a-5p levels are downregulated in OSCC tissues and cells, and circFAT1 could affect miR-181d-5p and miR-199a-5p, respectively, thereby regulating the biological functions of OSCC cells.

PI3Ks are a protein family closely related to tumors, which can induce the conversion of PIP2 to PIP3, thereby activating AKT and mTOR pathway and ultimately promoting the progression of OSCC. At present, the PI3K/Akt/mTOR pathway has attracted much attention as a potential therapeutic target. Per2 stimulates autophagy, inhibites cell proliferation, and promotes apoptosis in a PI3K/Akt/mTOR pathway-dependent manner, ultimately repressing OSCC progression [9]. The circEPSTI1/miR-942-5p/LTP-2 axis affects OSCC cell proliferation and invasion by accelerating the phosphorylation of EMT and PI3K/Akt/mTOR signaling pathway components [10]. This study found that circFAT1 is implicated in regulating the PI3K/Akt/mTOR pathway, while PI3K inhibitors LY294002, miR-181d-5p, and miR-199a-5p all block the PI3K/Akt/mTOR pathway activated by circFAT1. Besides, we found that LY294002 significantly inhibited the viability of OSCC cells overexpressing circFAT1 and had a synergistic effect with miR-181d-5p and miR-199a-5p. Therefore, circFAT1 may promote OSCC progression by activating the PI3K/Akt/mTOR pathway via the miR-181d-5p/miR-199a-5p axis.

In conclusion, circFAT1 expression is upregulated, while miR-181d-5p and miR-199a-5p expressions are downregulated in OSCC tissues and cells. Overexpression of circFAT1 accelerates the malignant progression of OSCC cells, while circFAT1 silencing has the opposite effect. Additionally, circFAT1 regulates the biological functions of OSCC cells by targeting the miR-181d-5p/miR-199a-5p axis to phosphorylate proteins related to the PI3K/Akt/mTOR pathway. This study reveals a new regulatory pathway in OSCC, providing potential biomarkers for the diagnosis and treatment of OSCC.

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Not applicable.

Conflicts of Interest

All authors declare that the research was conducted in the absence of any commercial or fnancial relationships that could be construed as a potential confict of interest.

Author Contributions

Investigation, J.C.; Data collection, L.W.; Validation, D.M.; Supervision, H.G.; Writing—original draft, Y.S.; Writing—review and editing, Y.S. All authors have read and agreed to the published version of the manuscript.

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Availability of Data and Materials

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Supplementary Materials

Not applicable.

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