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Original Article

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Long non-coding RNA FEZF1-AS1 promotes rectal cancer progression by competitively binding miR-632 with FAM83A

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Abstract

The long non-coding RNA (lncRNA) forebrain embryonic zinc finger protein 1 antisense RNA1 (FEZF1-AS1) was recently identified as an oncogenic gene in several types of tumors. The biological function of FEZF1-AS1 in rectal cancer progression, however, remains unknown. In the present study, we discover that FEZF1-AS1 is significantly upregulated in rectal cancer tissues and cells. Knocking down of FEZF1-AS1 suppresses cell proliferation, migration, and invasion *in vitro*, and tumorigenesis *in vivo*. Furthermore, FEZF1-AS1 functions as a competing endogenous RNA (ceRNA) for miR-632, resulting in the suppression of family with sequence similarity 83, member A (FAM83A). Overall, our findings reveal that FEZF1-AS1/miR-632/FAM83A axis plays an oncogenic role in rectal cancer progression, suggesting that it may be a novel therapeutic target for rectal cancer.

Key words FEZF1-AS1, miR-632, FAM83A, rectal cancer, proliferation, migration, invasion

Introduction

Rectal cancer is one of the malignant tumors of the digestive tract. As a major subtype of colorectal cancer, rectal cancer accounts for approximately 30% of all colorectal cancers [1]. Rectal cancer has a significant potential for metastasis, which accounts for its high morbidity and mortality rates [2,3]. The incidence of the disease is remarkably high in older individuals aged between 40 and 50 years [4]. Rectal cancer is a primary cause of death in the elderly population [5]. Despite significant advances in clinical treatments for rectal cancer, such as laparoscopic surgery [6], minimally invasive treatment [7], adjuvant chemo-radiotherapy, and immunotherapy, the prognosis of patients with rectal cancer remains poor and the overall survival is low, particularly for patients with advanced-stage cancer or with metastatic tumors [1,8,9]. Therefore, it is imperative to understand the mechanisms underlying rectal cancer progression and identify novel therapeutic targets for rectal cancer.

Long non-coding RNAs (lncRNAs), which is a class of non-coding RNAs with a length of more than 200 nucleotides, do not have the ability to code proteins [10]. LncRNAs have been implicated in a variety of biological processes, including cell proliferation, apoptosis, invasion, migration, and tumorigenesis [11,12]. Numerous studies have established that lncRNAs can act as oncogenes or tumor suppressors in a variety of human cancers, including rectal cancer [13–15]. Additionally, it has been observed that some lncRNAs function as competing endogenous RNAs (ceRNAs) for the binding of microRNAs, which regulate the biological functions of cells [16,17]. Therefore, the lncRNA/microRNA network has provided a novel perspective on rectal cancer development. LncRNA forebrain embryonic zinc finger protein 1 antisense RNA1 (FEZF1-AS1) has been reported to promote tumorigenesis in a variety of cancers, including colorectal cancer [18], gastric cancer [19], pancreatic ductal adenocarcinoma [20], and breast cancer [21]. Data from the cancer genome atlas (TCGA) revealed that FEZF1-AS1 expression is significantly upregulated in rectal cancer tissues when compared to that in non-cancer tissues. However, little is known about the biological function and mechanisms of FEZF1-AS1 in rectal cancer.

In the present study, we revealed that FEZF1-AS1 expression was significantly increased in rectal tissues and cell lines. *In vitro* and *in vivo* functional experiments revealed that knocking down FEZF1-AS1 inhibited cell proliferation, migration, invasion, and tumorigenesis. Additional findings demonstrated that FEZF1-AS1 could exert oncogenic effects via sponging miR-632 and regulating the expression of the family with sequence similarity 83, member A (FAM83A). Overall, this study established that the FEZF1-AS1/miR-632/FAM83A signaling axis is implicated in rectal cancer progres-

sion, implying that it may be a target for rectal cancer therapy.

Materials and Methods

Cell culture and transfection

Human rectal cancer cell lines (SW837 and SW1463) and normal human colon epithelial cell lines (fetal human cells FHC) were obtained from the American Type Culture Collection (ATCC, Manassas, USA). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies) and 1% streptomycin-penicillin (Gibco Life Technologies) in a 5% CO_2 atmosphere at 37°C. The coding region of the FAM83A gene was successfully amplified, and cloned into the pcDNA3.1 vector. Short hairpin RNA (shRNA) targeting FEZF1-AS1 (sh-FEZF1-AS1: 5′-GCACGCTTCCGAGTTTCCATT-3′) and negative control (NC: 5′-ACTACCGTTGTATAGGTGT-3′) lentivirus were purchased from GeneChem Biotech Co., Ltd (Shanghai, China). Lentiviruses were used to infect rectal cancer cells in the presence of 8 ng/ml polybrene (Sigma-Aldrich, St-Louis, USA). miR-632 mimics (Sense: 5′-GUGUCUGCUUCCUGUGGGA-3′, antisense: 5′-CACAGACGAAGGACACCCU-3′), control mimics (Cat No: miR01101), miR-632 inhibitors (5′-CACAGACGAAGGACACCCU-3′), control inhibitors (Cat No: miR02201), FAM83A siRNA (siFAM83A: 5′-GTCAAGCACAACAACATCA-3′), and control siRNA (siNC: 5′-ACTACCGTTGTATAGGTGT-3′) were purchased from RiboBio Co., Ltd (Guangzhou, China). Lipofectamine 2000 (Invitrogen, Carlsbad, USA) was used to transiently transfect cell lines according to the manufacturer's instructions.

TCGA data analysis

The TCGA database was used to acquire data on rectal cancer gene expression. Transcriptome profiling with the R program was used to identify differentially expressed genes (DEGs) between rectal cancer and normal tissues. The screening criterion for DEGs was determined as follows: adjusted P-value < 0.05 and $|\log2 \text{ (fold change)}| > 1.5$.

qRT-PCR

Total RNA was extracted from tissues and cells using the TRIzol reagent (Invitrogen). Total RNA was reverse transcribed into complementary DNA (cDNA) using a microRNA Reverse Transcription Kit (Promega, Madison, USA) or a PrimeScript RT Reagent Kit (Takara, Tokyo, Japan) for *miR-632*, *FEZF1-AS1*, and *FAM83A*. qRT-PCR was performed using SYBR Select Master Mix and ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, USA). *β-Actin* and *U6* were selected as the endogenous controls for mRNA and miRNAs, respectively. Relative changes in gene expression were analyzed using the $2^{-\Delta\Delta Ct}$ method. All primer sequences are listed in Table 1.

Western blot analysis

Proteins were extracted using lysis buffer (Beyotime Biotechnology, Shanghai, China). All proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes. After incubation in blocking buffer for 90 min at room temperature, the membranes were incubated with anti-FAM83A antibody (1:1000; Proteintech, Wuhan, China), anti-E-Cadherin (1:1000; Cell Signaling Technology, Beverly, USA), anti-N-Cadherin (1:1000; Cell Signaling Technology), anti-p21 (1:1000; Cell Signaling Technology), anti-p27 (1:1000; Cell Signaling Technology), or anti-GAPDH antibody (1:3000; Cell Signaling Technology) overnight at 4°C. Following that, the membranes were incubated with an HRP-labeled rabbit IgG secondary antibody (1:5000; Cell Signaling Technology). The immunoreactivities were evaluated using enhanced chemiluminescence reagents. GAPDH was used as the loading control.

Table 1. Sequences of primers used in this study

Name	Primer sequence (5′→3′)
miR-632-RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTCCCAC
miR-632 forward	AAGCGTGTCTGCTTCCT
miR-632 reverse	GTGCAGGGTCCGAGGT
FEZF1-AS1 forward	GCTATGACTCAGGGTTGGAC
FEZF1-AS1 reverse	CAATTGCACGAAAACAGGTT
FAM83A forward	ACTGACGGCTGAGATGAGGT
FAM83A reverse	GCTTGGGAGCTTCTGTGAC
U6 forward	GCAAGGATGACACGCACAA
U6 reverse	TGTGCGTGTCATCCTTGC
β-Actin forward	GAGGGAAATCGTGCGTGAC
β-Actin reverse	TTCTGACCCATTCCCACC

Cell counting kit-8 assay

Cell viability was quantified using the cell counting kit-8 (CCK-8) assay kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Briefly, cells were seeded into 96-well plates (4000 cells/well), and cell viability was recorded every 24 h. The plate was incubated at 37°C for 2 h after the addition of 10 μL CCK-8 solution to each well. A microplate reader (Thermo Fischer Scientific, Waltham, USA) was used to determine the absorbance at a wavelength of 450 nm.

Colony formation assay

Transfected cells were seeded into a 6-well plate at a density of 2000 cells per well and cultured for 14 days in media supplemented with 10% FBS. The colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution. Then the number of colonies was counted under an optical microscope.

Wound healing assay

A total of 1×10^5 cells were seeded into each well of 6-well plates and cultured to confluence. A 200-μL pipette tip was used to create linear scratch wounds. After the cells were rinsed with phosphate-buffered saline, they were maintained for 24 h. Images were captured at 0 and 24 h, and wound areas were estimated using the ImageJ public domain software (NIH, Bethesda, USA).

Transwell invasion assay

A total of 1×10^5 cells were seeded into the upper chamber of the transwell membranes coated with Matrigel (BD Bioscience, San Jose, USA) in 200 μL serum-free medium. As an attractant, a total of 600 μL medium supplemented with 10% FBS was added to the lower chamber. After 24 h of culture, cells on the membrane surface were removed with a cotton swab. The chambers were fixed with 4% paraformaldehyde and then stained with 0.1% crystal violet

solution. An optical microscope (Nikon, Tokyo, Japan) was used to observe and count the invaded cells.

Dual-luciferase reporter assay

The putative miR-632 binding sites from wild-type (wt) and mutant (mut) FEZF1-AS1 (FEZF1-AS1-wt/mut), as well as the 3′-untranslated region (UTR) of FAM83A (FAM83A-3′UTR-wt/mut) were cloned into the psiCHECK-2 luciferase vector (Promega), respectively. Using lipofectamine 2000 (Invitrogen), the SW837 and SW1463 cells were co-transfected with the FAM83A-3′UTR-wt/mut or FEZF1-AS1-wt/mut psiCHECK-2 plasmid and miR-632 mimics, or control mimics. The Dual-Luciferase Reporter Assay System (Promega) was used to determine the luciferase activities.

RNA immunoprecipitation assay

Cells were lysed in RNA immunoprecipitation (RIP) lysis buffer (EMD Millipore, Billerica, USA). The extract was mixed with magnetic beads (EMD Millipore) conjugated with control anti-IgG or anti-Ago2 antibody (EMD Millipore), and incubated at 4°C for 6–8 h. To remove the protein, the beads were washed with wash buffer and then incubated for 30 min at 55°C with proteinase K. Finally, qRT-PCR was performed to determine the abundance of FEZF1-AS1 and miR-632.

Mouse xenograft experiments

SW837 cells were subcutaneously injected with sh-FEZF1-AS1 or NC lentivirus into the dorsal flanks of 5-week-old female BALB/c nude mice. Every five days, the volume (V) was measured using a caliper and calculated using the formula, $V = 1/2 \times \text{width}^2 \times \text{length}$. All mice were euthanized 30 days after implantation. All animal experiments were approved and conducted in accordance with the protocols of the Animal Care and Use Committee of Nanhua Hospital Affiliated to Nanhua University (Approval No. 2019-ky-28).

Immunohistochemical staining

Immunohistochemical staining was performed on 4 μm tumor tissue sections. The section was deparaffinized, rehydrated, and autoclaved in a 0.01 M citrate buffer solution (pH 6.0). Section was later incubated overnight with anti-Ki-67 antibody (1:500; Cell Signaling Technology), followed by incubation with biotin-labeled secondary antibody (1:5000; Thermo Scientific, Waltham, USA) at room temperature for 20 min. The section was detected using 3,3′-diaminobenzidine tetrahydrochloride, counterstained with hematoxylin, and visualized under a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

IBM SPSS Statistics 22.0 (IBM Corp., Armonk, USA) and GraphPad Prism 9.0 (GraphPad Software Inc., La Jolla, USA) were used to perform statistical analysis. The results are expressed as the mean ± standard deviation (SD). To compare the means between two groups, the Student's *t*-test was used. $P < 0.05$ was considered statistically significant.

Results

Upregulation of FEZF1-AS1 in rectal cancer tissues and cells

DEGs in rectal cancer and normal tissues were analyzed using data from the TCGA database. Figure 1A depicts the top 20 differentially expressed lncRNAs between the two groups. When rectal cancer tissues were compared to normal tissues, *FEZF1-AS1* expression was significantly upregulated at the transcript level (Figure 1B). *FEZF1-AS1* expression was significantly higher at the transcript level in rectal cancer SW837 and SW1463 cell lines than in FHC cells, which was consistent with the results in tissues (Figure 1C). These results suggest that *FEZF1-AS1* is highly expressed in rectal cancer tissues and cell lines.

Knockdown of *FEZF1-AS1* suppresses cell proliferation, migration, and invasion

To investigate the biological functions of FEZF1-AS1 in rectal cancer cells, we used lentivector-mediated FEZF1-AS1 shRNA to knock down FEZF1-AS1 in SW837 and SW1463 cell lines, and qRT-PCR was performed to assess the knockdown efficiency (Figure 2A). CCK-8 assay results indicated that silencing of *FEZF1-AS1* significantly decreased cell proliferation ability (Figure 2B). Similarly, downregulation of FEZF1-AS1 expression significantly reduced the ability of SW837 and SW1463 cell lines to form colonies (Figure 2C). Downregulation of FEZF1-AS1 expression resulted in an increase in p21 and p27 expression, which correlates with cell growth (Figure 2F). Additionally, transwell invasion assay results demonstrated that knockdown of *FEZF1-AS1* decreased the invasion capacity of rectal cancer cells (Figure 2D). In wound healing assay, inhibiting FEZF1-AS1 expression attenuated the migration rate of rectal cancer cells (Figure 2E). As demonstrated by western blot analysis, knockdown of FEZF1-AS1 increased the expression of epithelial marker protein (E-cadherin), and decreased the expression of mesenchymal marker protein (N-cadherin) (Figure 2F). Overall, these findings suggest that shFEZF1-AS1 can inhibit the proliferation, migration, and invasion of rectal cancer cells.

FEZF1-AS1 directly interacts with miR-632 in rectal cancer cells

It has been revealed that FEZF1-AS1 can act as a ceRNA for miRNAs [22]. To elucidate the molecular mechanisms by which FEZF1-AS1 promotes rectal cancer progression, the DIANA tools online software program was used to predict whether miR-632 is a potential target for FEZF1-AS1 (Figure 3A). According to bioinformatics analysis of TCGA rectal cancer data, miR-632 expression was downregulated in rectal cancer tissues, implying that miR-632 may act as a tumor suppressor gene (Supplementary Table S1). Additionally, the expression of miR-632 was significantly decreased in rectal cancer cell lines when compared to that in normal cells (Figue 3B). qRT-PCR results demonstrated that knockdown of *FEZF1-AS1* significantly upregulated the expression of miR-632 in rectal cancer cells (Figure 3C). Meanwhile, overexpression of miR-632 significantly downregulated *FEZF1-AS1* expression in rectal cancer cells (Figure 3D,E). To investigate the interaction between FEZF1-AS1 and miR-632, two luciferase reporter plasmids with either FEZF1-AS1-wt or FEZF1-AS1-mut were constructed and co-transfected with miR-632 or control mimics into SW837 and SW1463 cell lines. Forced expression of miR-632 significantly lowered the fluorescence intensity of FEZF1-AS1-wt plasmids when compared to FEZF1-AS1-mut plasmids, as demonstrated by dual-luciferase reporter assay (Figure 3F). Additionally, the RIP assay demonstrated that FEZF1-AS1 and miR-632 were significantly enriched in the immunoprecipitates from the Ago2 group, implying that the Ago2 protein was bound to FEZF1-AS1 and miR-632 in SW837 and

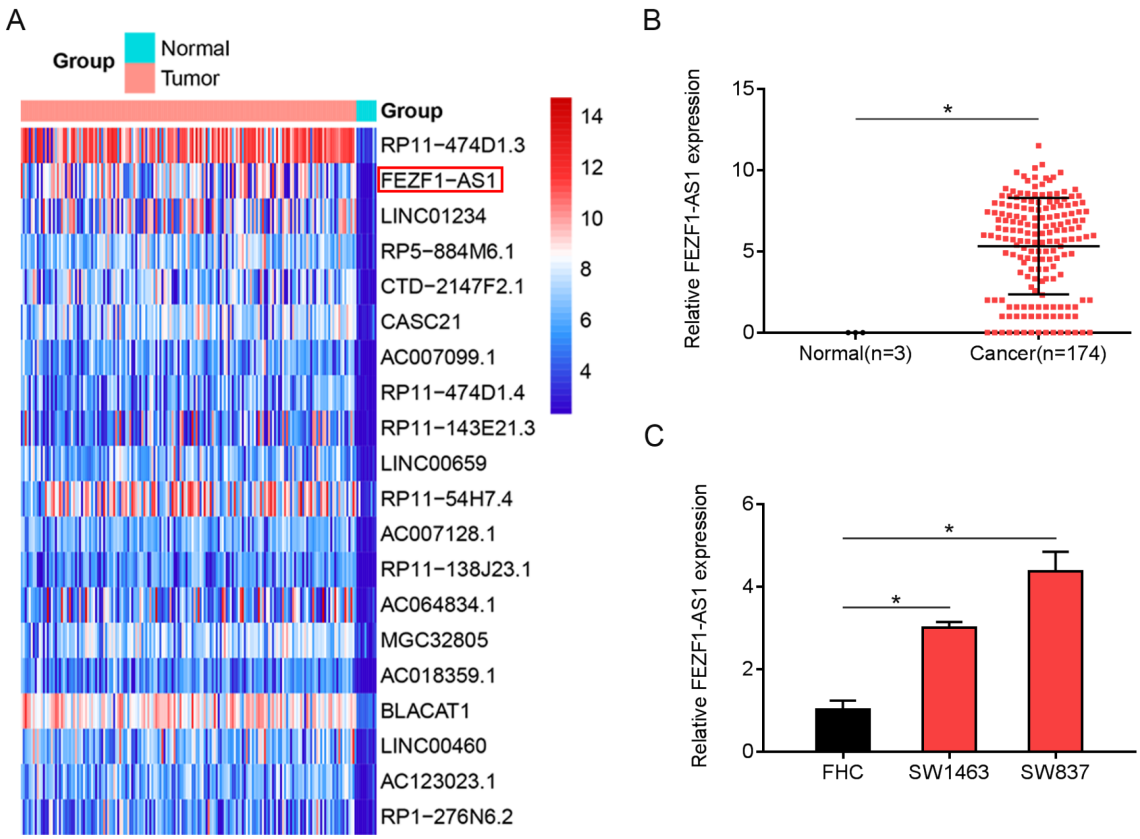

Figure 1. Upregulation of FEZF1-AS1 expression in rectal cancer tissues and cells (A) Heatmap plot showing the top 20 significantly upregulated lncRNAs based on data obtained from the TCGA database. (B) FEZF1-AS1 expression in TCGA rectal cancer cells. (C) qRT-PCR analysis of FEZF1-AS1 expression in rectal cancer cell lines (SW837 and SW1463) and normal human colon epithelial cell line (fetal human cell [FHC]). $*P < 0.05$.

SW1463 cell lines (Figure 3G). Overall, these results suggest that FEZF1-AS1 can bind to miR-632.

Depletion of miR-632 reverses FEZF1-AS1 inhibition-mediated effects on cell proliferation, migration, and invasion

To further explore the biological interactions between FEZF1-AS1 and miR-632 in rectal cancer, SW837 cell lines were co-transfected with sh-FEZF1-AS1 lentivirus and miR-632 inhibitor. The qRT-PCR results demonstrated that downregulation of FEZF1-AS1 expression significantly increased the expression of miR-632, which was partially inhibited by the miR-632 inhibitor in rectal cancer cells (Figure 4A). Notably, the inhibitory effects on cell proliferation, migration, and invasion mediated by FEZF1-AS1 knockdown were partially rescued by the miR-632 inhibitor (Figure 4B–F). These results imply that FEZF1-AS1 promotes rectal cancer cell functions through miR-632.

FEZF1-AS1 regulates FAM83A expression by competitively binding to miR-632

To investigate the ceRNA networks among FEZF1-AS1, miR-632, and their target genes in rectal cancer cells, we used TargetScan (http://www.targetscan.org/vert_72/) to identify whether FAM83A is a potential target for miR-632 (Figure 5A). Based on TCGA rectal cancer database, we found that the FAM83A expression level was significantly increased in rectal cancer tissues compared to that in the normal tissues (Supplementary Table S2). Consistently, the mRNA and protein expression levels of FAM83A were significantly upregulated in rectal cancer cell lines compared to those in FHC cells (Figure 5B,C). Subsequently, a dual-luciferase reporter assay was used to determine whether there is direct interaction between miR-632 and FAM83A. The luciferase reporter plasmids with FAM83A 3′UTR wt or mut (FAM83A-3′UTR-wt or FAM83A-3′UTR-mut) were constructed and co-transfected with miR-632 or control mimics into SW837 and SW1463 cell lines. Ectopic expression of miR-632 significantly reduced luciferase activity in the FAM83A-3′UTR-wt plasmids but had no effect on FAM83A-3′UTR-mut vectors (Figure 5D). In the rescue experiments, downregulation of miR-632 counteracted the corresponding suppression of FAM83A expression at mRNA or protein level induced by knockdown of *FEZF1-AS1* in SW837 and SW1463 cell lines (Figure 5E,F). Overall, these findings demonstrate that FEZF1-AS1 can bind competitively to miR-632, hence facilitating FAM83A expression.

FAM83A reverses the suppressive effects of FEZF1-AS1 silencing on cell proliferation, migration, and invasion

To establish whether FAM83A regulates FEZF1-AS1, we examined the expression of FAM83A in SW837 cells co-transfected with sh-FEZF1-AS1 lentivirus and FAM83A plasmid. The downregulated expression of FAM83A at mRNA and protein levels through the silencing of *FEZF1-AS1* was reversed by an increase in FAM83A expression (Figure 6A,B). Ectopic expression of FAM83A rescued FEZF1-AS1 inhibition-mediated effects on cell growth invasion, and migration (Figure 6C–G). These results suggest that FEZF1-AS1

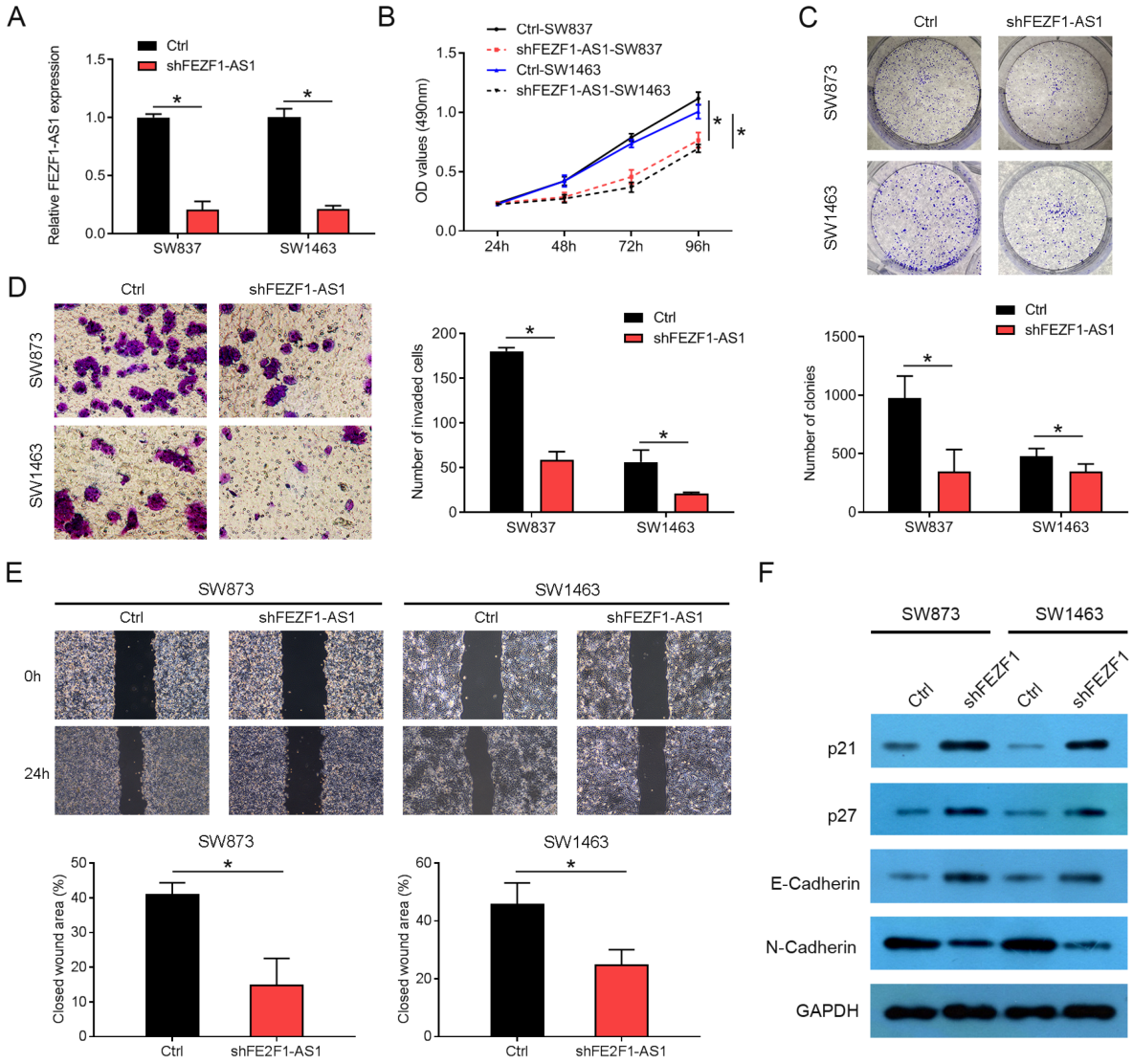

Figure 2. Knockdown of *FEZF1-AS1* suppresses cell proliferation, migration, and invasion (A) Relative expression of FEZF1-AS1 in SW837 and SW1463 cell lines after infection with sh-FEZF1-AS1 lentivirus. Analysis of cell growth by (B) CCK-8 and (C) colony formation assays. (D) Evaluation of cell invasion ability by transwell invasion assay. Magnification fold: ×100. (E) Analysis of cell migration by wound-healing assay. Magnification fold: ×40. (F) The protein expressions of p21, p27, E-cadherin, and N-cadherin were assessed by western blot analysis. $^*P < 0.05$.

competitively binds to miR-632 to stimulate the expression of FAM83A, thereby facilitating the progression of rectal cancer.

FAM83A knockdown represses the proliferation, migration, and invasion of rectal cancer cells

We also studied the roles of FAM83A in rectal cancer cells. SW837 cells were transfected with FAM83A siRNA, and RT-qPCR and assays were then performed to assess the interference efficiency of FAM83A in rectal cancer cells (Supplementary Figure S1A,B). FAM83A silencing could weaken cell proliferation, migration, and invasion of rectal cancer cells (Supplementary Figure S1C–G). Knockdown of *FAM83A* causes the same effects on rectal cancer cells as knockdown of *FEZF1-AS1*. Taken together, these results further confirm that FEZF1-AS1 facilitates the growth, migration, and invasion of rectal cancer cells by regulating the miR-632/FAM83A axis.

Silencing of FEZF1-AS1 inhibits xenograft tumor growth *in vivo*

To further investigate the involvement of FEZF1-AS1 in rectal cancer *in vivo*, SW837 cell lines, which were stably infected with NC or sh-FEZF1-AS1 lentivirus, were subcutaneously inoculated into nude mice. Tumors in the FEZF1-AS1 knockdown group were significantly smaller than those in the control group (Figure 7A). Similarly, FEZF1-AS1-depleted cells developed more slowly than the control cells (Figure 7B), and the average tumor weight of the FEZF1-AS1-silenced group was much less than that of the control group (Figure 7C). The results of qRT-PCR and western blot analysis revealed that the expressions of FEZF1-AS1 and FAM83A were significantly reduced, whereas miR-632 expression was increased in

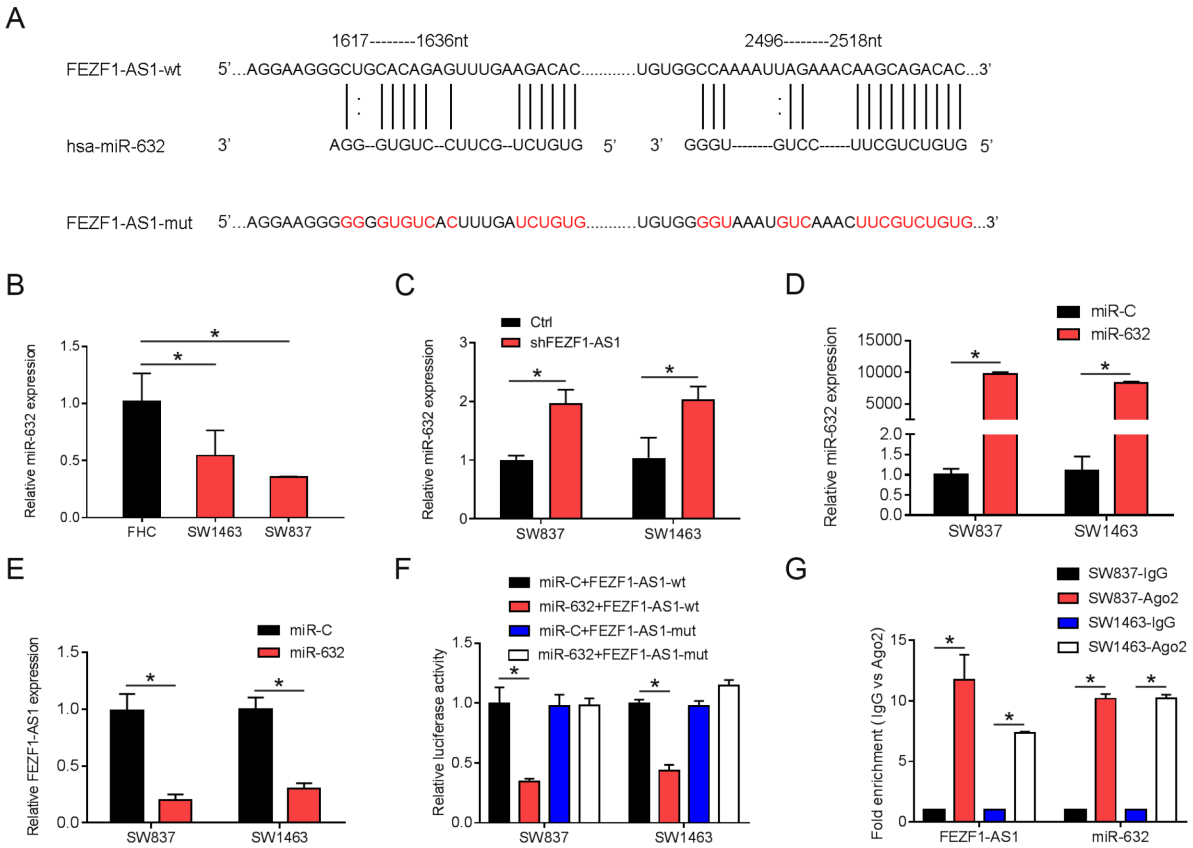

Figure 3. FEZF1-AS1 directly interacts with miR-632 in rectal cancer cells (A) A schematic representation of the binding sites between FEZF1-AS1 and miR-632. (B) Analysis of the effect of FEZF1-AS1 on the expression of miR-632 in SW837 and SW1463 cell lines by qRT-PCR. (C) The expression of miR-632 in sh-FEZF1-AS1-transfected cells. (D) The expressions of miR-632 and (E) FEZF1-AS1 in the SW837 and SW1463 cells transfected with miR-632 or control mimics. (F) Determination of the binding ability of FEZF1-AS1 and miR-632 by the dual-luciferase reporter assay. (G) RIP assay was performed to assess the interactions among FEZF1-AS1, miR-632, and Ago2. *$P < 0.05$.

the FEZF1-AS1-knockdown group (Figure 7D–F). Moreover, the tumors were assessed by immunohistochemical staining, which revealed a decrease in the Ki67 expression in the FEZF1-AS1 shRNA-treated group (Figure 7G). Additionally, sh-FEZF1-AS1 decreased the expression of N-cadherin, and increased the expressions of p21, p27, and E-cadherin, which corroborated the *in vitro* results (Figure 7F). These results suggest that silencing of *FEZF1-AS1* can delay tumor growth in nude mice.

Discussion

This study aimed to investigate the functions and mechanisms of FEZF1-AS1 in rectal cancer. FEZF1-AS1 expression was found to be markedly upregulated in rectal cancer. Additionally, downregulation of FEZF1-AS1 inhibited rectal cancer cell growth, invasion, and migration *in vitro*, as well as tumor growth *in vivo*. FEZF1-AS1 promoted rectal cancer progression by acting as a ceRNA and modulating the miR-632/FAM83A axis.

Numerous studies have established a link between FEZF1-AS1 and tumor progression [23,24]. For example, Bian et al. [18] demonstrated that FEZF1-AS1 expression was upregulated in colorectal cancer tissues and that it promoted colorectal cancer growth and metastasis both *in vitro* and *in vivo*. Liu *et al.* [19] established that the upregulation of FEZF1-AS1 was associated with tumor size, stage, and poor survival of patients with gastric cancer. Sun *et al.* [23] demonstrated that FEZF1-AS1 promoted ovarian cancer cell metastasis and proliferation by targeting miR-130a-5p and its downstream SOX4 expression. In this study, we established that FEZF1-AS1 expression was significantly upregulated in rectal cancer tissues and cells, which is consistent with the findings of Bian *et al* [18]. Silencing of *FEZF1-AS1* inhibited rectal cancer cell proliferation, invasion and migration capacity *in vitro*, and tumor growth *in vivo*, indicating that FEZF1-AS1 plays an oncogenic role in rectal cancer progression.

Recent studies have demonstrated that lncRNAs can act as ceRNAs of miRNAs to regulate target gene expression in rectal cancer [25,26]. For example, LINC00461 promotes rectal cancer progression by functioning as a ceRNA of miR-593-5p to regulate the expression of *CCND1* [26]. However, no research has been conducted to determine the role of FEZF1-AS1 as a ceRNA of miR-632. In this study, bioinformatics analysis and luciferase activity assays revealed that lncRNA FEZF1-AS1 sponged miR-632. miR-632 has been implicated in tumor suppression. According to Jin *et al.* [27], miR-632 expression was significantly downregulated in renal cell carcinoma (RCC) tissues, and overexpression of miR-632 suppressed RCC cell proliferation, migration, and invasion, and induced cell apoptosis. Deniz *et al.* [28] established that overexpression of miR-632 reduced cell viability and induced apoptosis in human testicular germ cell tumors. However, the impact of miR-632 on rectal cancer

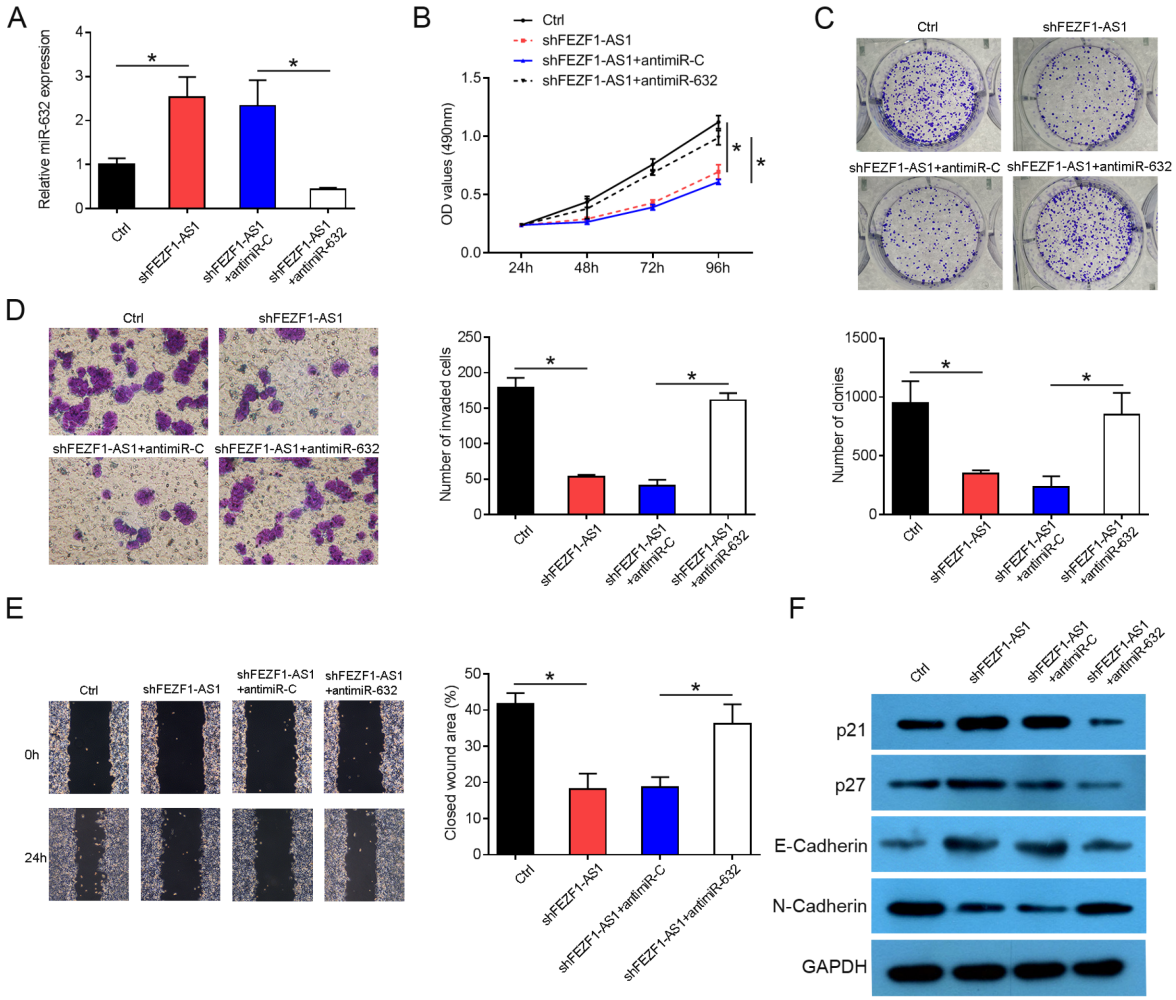

Figure 4. Depletion of miR-632 reverses FEZF1-AS1 inhibition-mediated effects on cell proliferation, migration, and invasion (A) qRT-PCR analysis of miR-632 expression in SW837 cell lines co-transfected with sh-FEZF1-AS1 lentivirus and miR-632 inhibitor. (B) CCK-8 and (C) colony formation assays were used to evaluate cell proliferation. Evaluation of cell invasion and migration by (D) transwell invasion (Magnification fold: ×100) and (E) wound healing assays (Magnification fold: ×40), respectively. (F) The protein expressions of p21, p27, E-cadherin, and N-cadherin detected by western blot analysis. $*P < 0.05$.

has not been eported. TCGA rectal cancer database shows that miR-632 is downregulated in rectal cancer. Our results also demonstrated that miR-632 expression levels were significantly downregulated in rectal cancer cell lines, and miR-632 inhibitor partially rescued the inhibition of rectal cancer cell growth, invasion, and migration regulated by knockdown of *FEZF1-AS1*. Therefore, we infer that FEZF1-AS1 is a critical element in rectal cancer growth via miR-632 sponging.

miRNA target genes play a critical role in the ceRNA network [29]. FAM83A, also known as bj-tsa-9, belongs to a recently discovered oncogenic FAM83 family and is located on chromosome 8q24 [30]. Several studies have shown that FAM83A is considerably overexpressed in a variety of tumor types and enhances cell growth and metastasis [31,32]. However, the expression and function of FAM83A in rectal cancer have not yet been described. We identified FAM83A as the potential biological target for miR-632 by using TargetScan Database. TCGA rectal cancer database reveals that FAM83A expression is significantly higher in rectal cancer tissues than in normal tissues. Additionally, this study established that the expression of FAM83A was increased in rectal cancer cell lines. Moreover, we validated FAM83A as a direct target of miR-632 by dual-luciferase reporter assay. Subsequent functional experiments showed that overexpression of FAM83A can counteract the inhibition of cell proliferation, invasion, and migration that is mediated by sh-FEZF1-AS1. Inhibition of miR-632 had the same effects on rectal cancer cells as overexpression of FAM83A, further confirming that FAM83A is a direct target of miR-632. Additionally, silencing of *FAM83A* can suppress the proliferation, invasion, and migration of rectal cancer cells. Similar results were obtained in rectal cancer cells infected with sh-FEZF1-AS1 lentivirus. By influencing the miR-632/FAM83A axis, FEZF1-AS1 acts as an oncogene. Future studies will examine the growth, migration, and invasion of rectal cancer cells under complete inhibition of gene expression.

In conclusion, this study revealed that FEZF1-AS1, a lncRNA, can promote rectal cancer cell proliferation, migration, invasion, and tumorigenesis by acting as a ceRNA of miR-632 to regulate the expression of FAM83A (Figure 7H). Our findings suggest that FEZF1-AS1 may be a potential novel diagnostic and therapeutic target for

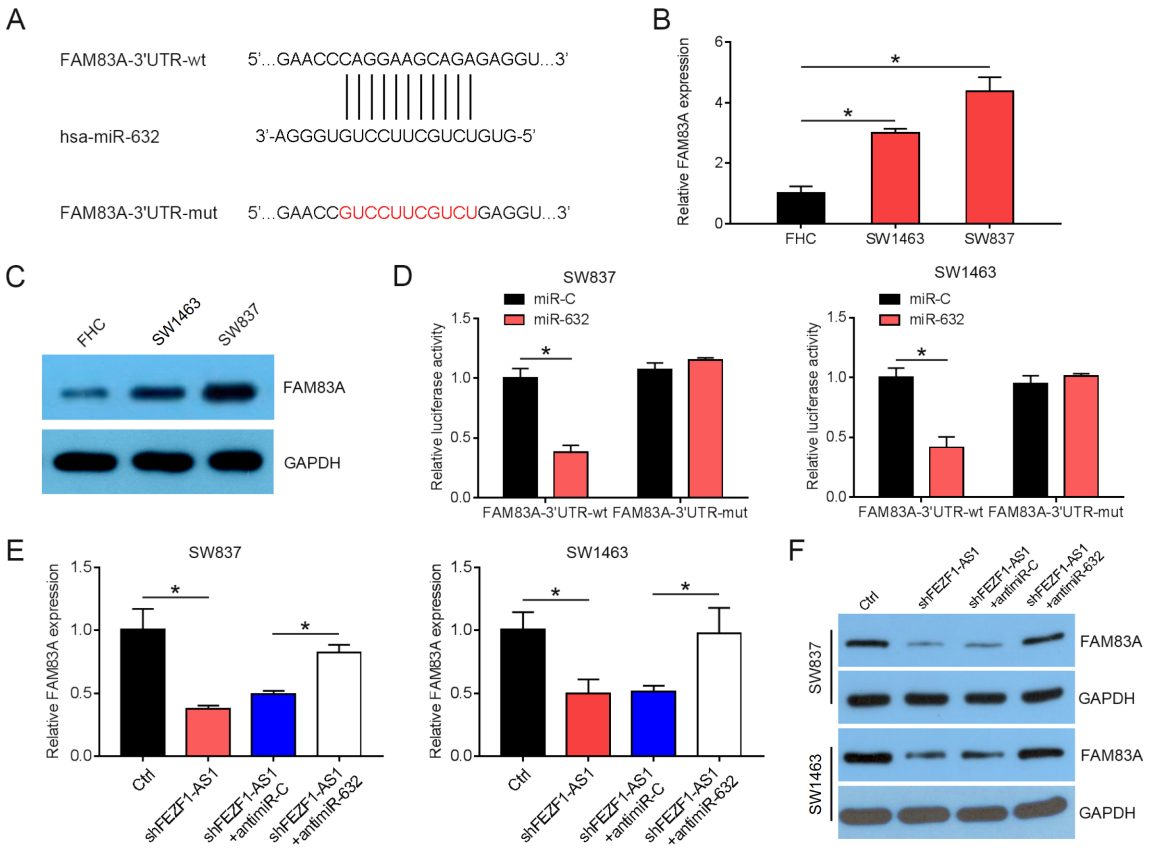

Figure 5. FEZF1-AS1 regulates FAM83A expression by competitively binding to miR-632 (A) The structure of the putative miR-632 binding site in wild-type or mutant FAM83A 3′UTR. Evaluation of FAM83A expression at mRNA and protein levels in rectal cancer cell lines by (B) qRT-PCR and (C) western blot analysis, respectively. (D) Analysis of luciferase activity after co-transfection of FAM83A-3′UTR-wt or FAM83A-3′UTR-mut, and miR-632 or control mimics into SW837 and SW1463 cell lines. (E) Relative mRNA and (F) protein expression levels of FAM83A in rectal cancer cells co-transfected with sh-FEZF1-AS1 lentivirus and miR-632 inhibitors. *$P < 0.05$.

rectal cancer treatment.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA-Cancer J Clin* 2018, 68: 7–30
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA-Cancer J Clin* 2018, 68: 394–424
3. Bailey CE, Hu CY, You YN, Bednarski BK, Rodriguez-Bigas MA, Skibber JM, Cantor SB, ***et al.*** Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. *JAMA Surg* 2015, 150: 17–22
4. Oronsky B, Reid T, Larson C, Knox SJ. Locally advanced rectal cancer: The past, present, and future. *Semin Oncol* 2020, 47: 85–92
5. Farhan F, Fazeli MS, Samiei F, Aghili M, Haddad P, Gholami S, Nabavi M. Morbidity and Mortality Following Short Course Preoperative Radiotherapy in Rectal Carcinoma. *Acta Med Iran* 2015, 53: 627–632
6. Suzumura H, Tsuruta M, Hasegawa H, Okabayashi K, Ishida T, Asada Y, Makino A, ***et al.*** The impact of the mesorectal apparent diffusion coefficient value on surgical difficulty in laparoscopic anterior resection for rectal cancer. *Surg Today* 2019, 49: 239–244
7. Fleshman J, Branda ME, Sargent DJ, Boller AM, George VV, Abbas MA, Peters WR, ***et al.*** Disease-free survival and local recurrence for laparoscopic resection compared with open resection of stage II to III rectal cancer: follow-up results of the ACOSOG Z6051 randomized controlled trial. *Ann Surg* 2019, 269: 589–595
8. Franke AJ, Parekh H, Starr JS, Tan SA, Iqbal A, George Jr. TJ. Total

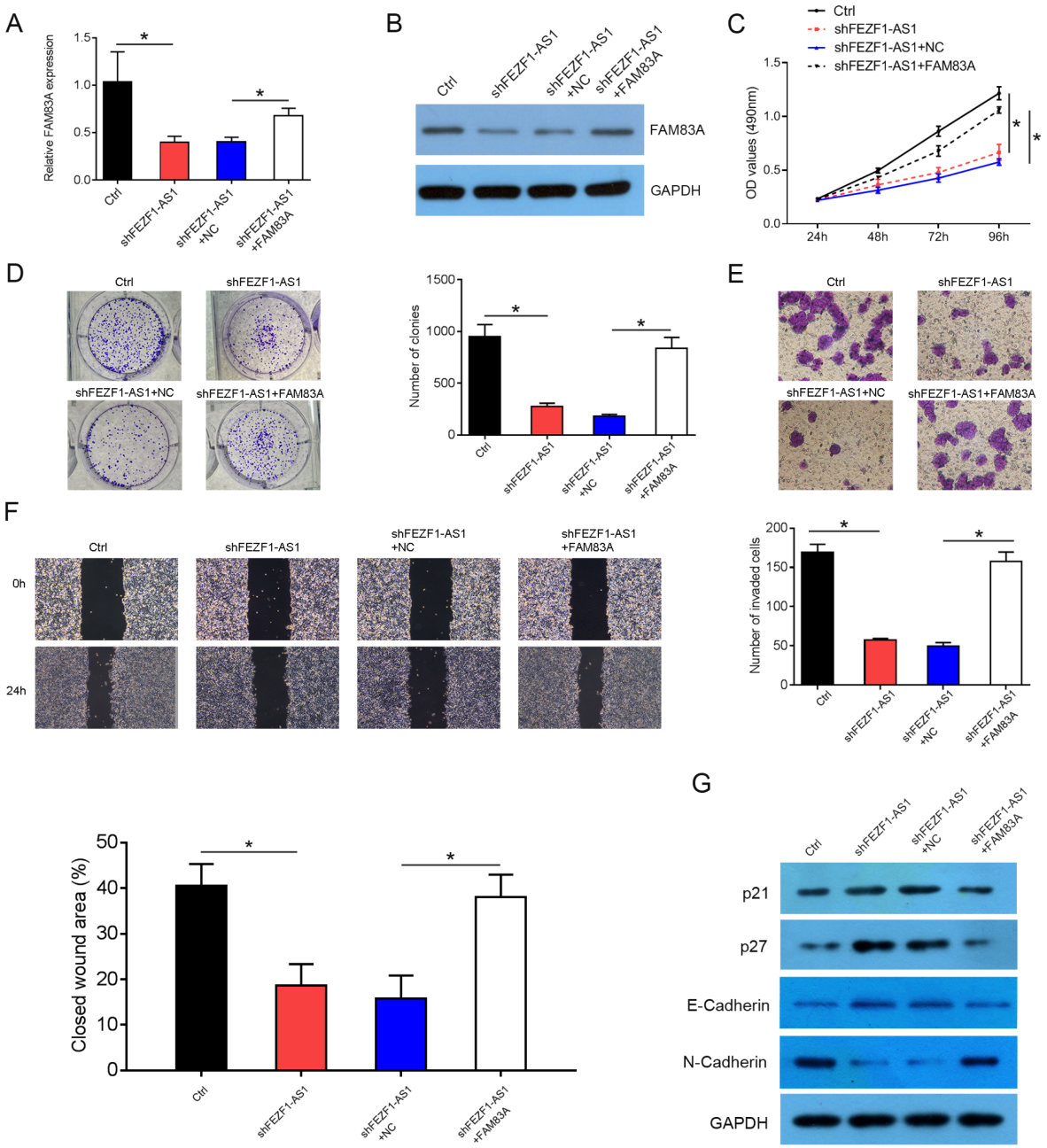

Figure 6. FAM83A reverses the inhibitory effects of FEZF1-AS1 silencing on cell proliferation, migration, and invasion Analysis of FAM83A expression in SW837 cell lines co-transfected with sh-FEZF1-AS1 lentivirus and FAM83A vector by (A) qRT-PCR and (B) western blot analysis, respectively. (C–F) Evaluation of growth, invasion, and migration ability of cells by CCK-8, colony formation, transwell invasion (Magnification fold: ×100), and wound healing assays (Magnification fold: ×40). (G) p21, p27, E-cadherin and N-cadherin protein expressions detected by western blot analysis. *$P < 0.05$.

neoadjuvant therapy: a shifting paradigm in locally advanced rectal cancer management. *Clin Colorectal Cancer* 2018, 17: 1–12

9. Du D, Su Z, Wang D, Liu W, Wei Z. Optimal interval to surgery after neoadjuvant chemoradiotherapy in rectal cancer: a systematic review and meta-analysis. *Clin Colorectal Cancer* 2018, 17: 13–24
10. Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. *Br J Cancer* 2013, 108: 2419–2425
11. Jia YJ, Liu ZB, Wang WG, Sun CB, Wei P, Yang YL, You MJ, *et al.* HDAC6 regulates microRNA-27b that suppresses proliferation, promotes apoptosis and target MET in diffuse large B-cell lymphoma. *Leukemia* 2018, 32: 703–711
12. Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. *Oncogene* 2017, 36: 5661–5667
13. Evans JR, Feng FY, Chinnaiyan AM. The bright side of dark matter: lncRNAs in cancer. *J Clin Invest* 2016, 126: 2775–2782
14. Tao F, Xu Y, Yang D, Tian B, Jia Y, Hou J, Dong M. LncRNA NKILA correlates with the malignant status and serves as a tumor-suppressive role in rectal cancer. *J Cell Biochem* 2018, 119: 9809–9816

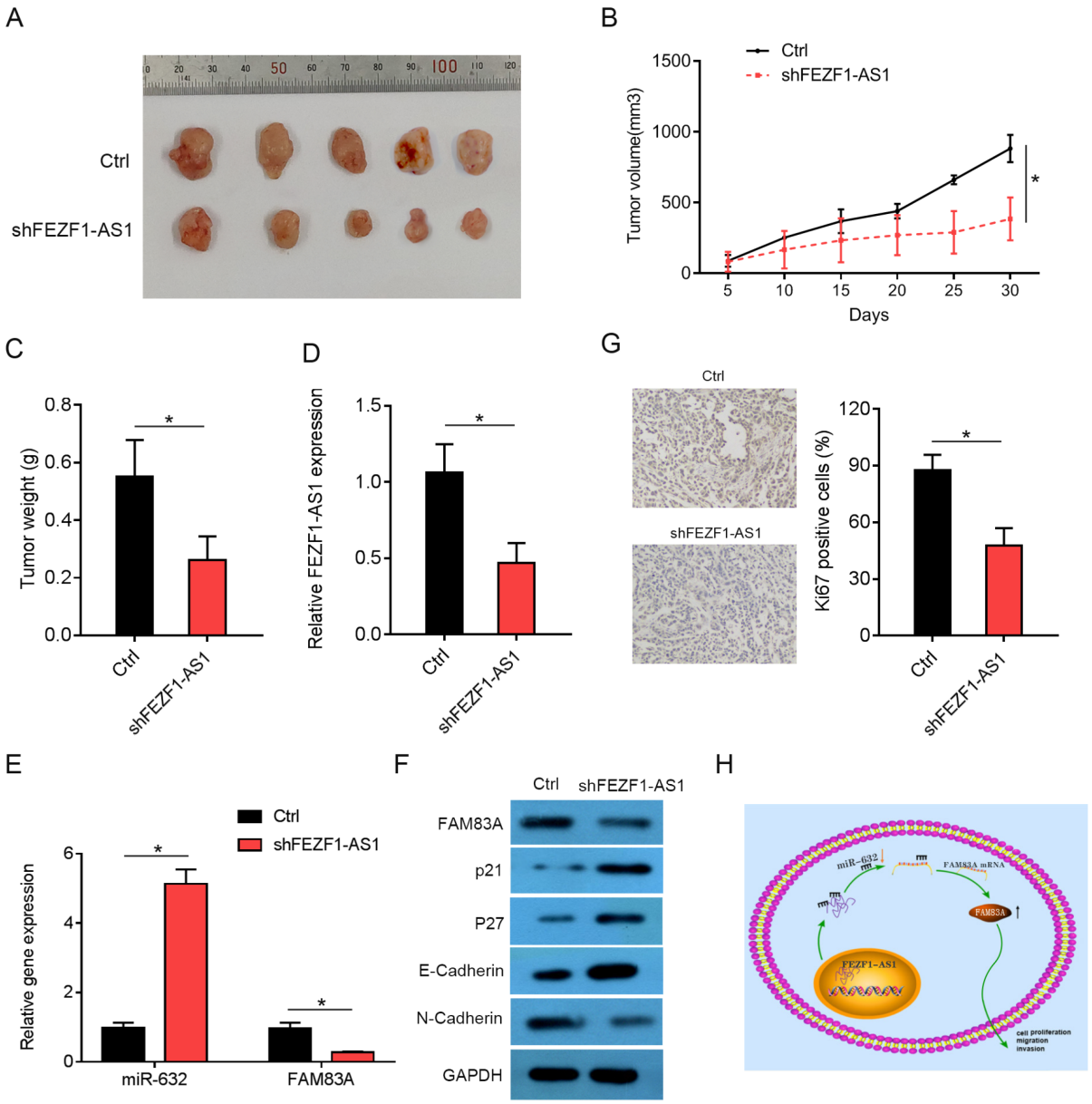

Figure 7. Silencing FEZF1-AS1 inhibits xenograft tumor growth *in vivo* (A) Representative image of indicated xenograft tumors. (B) Tumor growth curve. (C) Tumor weight of indicated xenograft tumors. (D) Relative expression of FEZF1-AS1 in the tumors formed by FEZF1-AS1-silenced or control cells. (E) miR-632 and FAM83A mRNA expressions in indicated xenograft tumors. (F) The protein expressions of FAM83A, p21, p27, E-cadherin, and N-cadherin in indicated xenograft tumors. (G) Representative images and quantification of Ki-67 through immunohistochemical staining in the different groups. Magnification fold: ×100. (H) A schematic diagram of the molecular mechanism of the FEZF1-AS1/miR-632/FAM83A axis in regulating malignant progression of rectal cancer. *$P < 0.05$.

15. Yu X, Yang Y, Li Y, Cao Y, Tang L, Chen F, Xia J. Baicalein inhibits cervical cancer progression via downregulating long noncoding RNA BDLNR and its downstream PI3K/Akt pathway. *Int J Biochem Cell Biol* 2018, 94: 107–118
16. Chen X, Chen Z, Yu S, Nie F, Yan S, Ma P, Chen Q, *et al.* Long noncoding RNA LINC01234 functions as a competing endogenous RNA to regulate CBFB expression by sponging miR-204-5p in gastric cancer. *Clin Cancer Res* 2018, 24: 2002–2014
17. Zheng ZQ, Li ZX, Zhou GQ, Lin L, Zhang LL, Lv JW, Huang XD, *et al.* Long noncoding RNA FAM225A promotes nasopharyngeal carcinoma tumorigenesis and metastasis by acting as ceRNA to sponge miR-590-3p/miR-1275 and upregulate ITGB3. *Cancer Res* 2019, 79: 4612–4626
18. Bian Z, Zhang J, Li M, Feng Y, Wang X, Zhang J, Yao S, *et al.* LncRNA–FEZF1-AS1 promotes tumor proliferation and metastasis in colorectal cancer by regulating PKM2 signaling. *Clin Cancer Res* 2018, 24: 4808–4819
19. Liu YW, Xia R, Lu K, Xie M, Yang F, Sun M, De W, *et al.* LincRNAFEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-Mediated H3K4me2 demethylation. *Mol Cancer* 2017, 16: 39
20. Ye H, Zhou Q, Zheng S, Li G, Lin Q, Ye L, Wang Y, *et al.* FEZF1-AS1/miR-

107/ZNF312B axis facilitates progression and Warburg effect in pancreatic ductal adenocarcinoma. *Cell Death Dis* 2018, 9: 34
21. Zhang Z, Sun L, Zhang Y, Lu G, Li Y, Wei Z. Long non-coding RNA FEZF1-AS1 promotes breast cancer stemness and tumorigenesis via targeting miR-30a/Nanog axis. *J Cell Physiol* 2018, 233: 8630–8638
22. Wang ZH, Wang JH, Wang KQ, Zhou Y, Wang J. LncRNA FEZF1-AS1 promoted chemoresistance, autophagy and epithelial-mesenchymal transition (EMT) through regulation of miR-25-3p/ITGB8 axis in prostate cancer. *Eur Rev Med Pharmacol Sci* 2020, 24: 8250
23. Sun Z, Gao S, Xuan L, Liu X. Long non-coding RNA FEZF1-AS1 induced progression of ovarian cancer via regulating miR-130a-5p/SOX4 axis. *J Cell Mol Med* 2020, 24: 4275–4285
24. Li J, Zhao LM, Zhang C, Li M, Gao B, Hu XH, Cao J, *et al.* The lncRNA FEZF1-AS1 promotes the progression of colorectal cancer through regulating OTX1 and targeting miR-30a-5p. *Oncol Res* 2020, 28: 51–63
25. Qi X, Lin Y, Liu X, Chen J, Shen B. Biomarker discovery for the carcinogenic heterogeneity between colon and rectal cancers based on lncRNA-associated ceRNA network analysis. *Front Oncol* 2020, 10: 535985
26. Qu W, Huang W, Yang F, Ju H, Zhu G. Long noncoding RNA LINC00461 mediates cisplatin resistance of rectal cancer via miR-593-5p/CCND1 axis. *Biomed PharmacoTher* 2020, 124: 109740
27. Jin L, Li Y, Zhang Z, He T, Hu J, Liu J, Chen M, *et al.* miR-514a-3p functions as a tumor suppressor in renal cell carcinoma. *Oncol Lett* 2017, 14: 5624
28. Özata DM, Li X, Lee L, Liu J, Warsito D, Hajeri P, Hultman I, *et al.* Loss of miR-514a-3p regulation of PEG3 activates the NF-kappa B pathway in human testicular germ cell tumors. *Cell Death Dis* 2017, 8: e2759
29. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature* 2014, 505: 344–352
30. Li Y, Dong X, Yin Y, Su Y, Xu Q, Zhang Y, Pang X, *et al.* BJ-TSA-9, a novel human tumor-specific gene, has potential as a biomarker of lung cancer. *Neoplasia* 2005, 7: 1073–1080
31. Zheng YW, Li ZH, Lei L, Liu CC, Wang Z, Fei LR, Yang MQ, *et al.* FAM83A promotes lung cancer progression by regulating the wnt and hippo signaling pathways and indicates poor prognosis. *Front Oncol* 2020, 10: 180
32. Zhou C, Liang Y, Zhou L, Yan Y, Liu N, Zhang R, Huang Y, *et al.* TSPAN1 promotes autophagy flux and mediates cooperation between WNT-CTNNB1 signaling and autophagy via the *MIR454-FAM83A-TSPAN1 axis in pancreatic cancer.* *Autophagy* 2021, 17: 3175–3195