European Review for Medical and Pharmacological Sciences 2020; 24: 2281-2293

LncRNA FEZF1-AS1 promoted chemoresistance, autophagy and epithelial-mesenchymal transition (EMT) through regulation of miR-25-3p/ITGB8 axis in prostate cancer

Z.-H. WANG[1], J.-H. WANG[1], K.-Q. WANG[2], Y. ZHOU[3], J. WAN

[1]Department of Urology, West China Hospital, Sichuan University, Cheng Chi
[2]Department of Urology, Daguan Hospital of Jinjiang District, Chen C
[3]Department of Urology, The People's Hospital of Kaizhou District ongqing a

RETRACTED

Abstract. – OBJECTIVE: Accumulating evidence determined that lncRNA plays important roles in the development and occurrence of cancers. Prostate cancer is the second most common type of cancer and one of the top five cancers for the cause of male death in the world. Therefore, this study was to explore the regulatory mechanism of lncRNA in chemoresistance of PC.

MATERIALS AND MATERIALS: qRT-PC used to detect the mRNA expression of F AS1, miR-25-3p and ITGB8. Western blo applied to measure the protein expressio ITGB8 E-cadherin, N-cadherin, Vimentin, LC LC3II, ATG5 and Beclin-1. In on, CCK assay was used to assess ation o transfected cells. Luciferas porte say and RIP assay were used to mine t elation- ship among FEZF1-AS1, 5-3p

RESULTS: In this udy ression FEZF1-AS1 and ITG was up ated, where- as the expression iR-25-3p ownregu- lated in PC tum es and PC/ lls. Lu- ciferase repo a and RIP as y deter- mined that mi 5-3p target of FEZF1-AS1 and ITGB8 s a target f of miR-25-3p. In- teresting knockdown of AS1 could in- hibit c ability and EMT and promoted cell au- toph in PC/ cells, but inhibition of miR- 25 pro on of ITGB8 could reverse the effec ZF1-AS PC/PTX cells.

CON NS: I s study, we found that IA FE S omoted chemoresistance, au agy a thelial-mesenchymal tran- siti EMT) th gh regulation of miR-25-3p/ ITG in PC, providing a new regulatory m PC and a novel therapeutic tar- .

rds:
1-AS1, Chemoresistance, Autophagy, EMT, Prostate cancer.

Introduction

ostate cancer (PC) is an epithelial malig- cy that occ in the prostate, which is the nd most co n type of cancer and one of t five ca for the cause of male death in t hemoresistance was the main problem e treatment of PC. While alleviat- ing the development of PC, drugs also increase oresistance of cancer cells to drugs and du e effect of drug treatment on cancers[2]. Paclitaxel (PTX) is a natural anti-cancer drug that has a significant alleviated effect on PC, but chemoresistance is also occurred[3-6]. However, chemoresistance studies for PC cells are still very limited.

LncRNA is a type of non-coding RNA of more than 200 bp in length[7]. LncRNAs play an important regulatory role in the treatment and diagnosis of cancer, and its regulation mechanism has also improved the understanding of cancer occurrence and development[8-10]. It is well known that lncRNA affects the expression of mRNA by binding to its target miRNA, thereby affecting a series of physiological metabolism, immunity, and cell cycle processes[11-14], including PC[15]. Such as lncRNA PCAT-1 could contribute to cell proliferation *via* regulating cMyc in PC[16]. Growing evidence determined that FEZF1-AS1 played multiple roles in cancers, including colorectal carcinoma, gastric cancer, osteosarcoma and PC[17-20]. In this study, we found that FEZF1-AS1 is highly expressed in PC, but its role in drug resistance is not fully understood.

MiRNAs are also important regulators of the development, progression, chemoresistance of cancer cells[21,22]. In PC, miR-29b has been shown to

Corresponding Author: Jia Wang, MD; e-mail: hjxwoq@163.com

be involved in cell proliferation, metastasis and EMT[23,24]. With the development of sequencing technology, more and more miRNAs involved in cancer cell progression have been discovered, and miR-25-3p is one of them[25,26]. miR-25-3p is a tumor suppressor that is low in cancers and inhibits the development and progression of cancer cells[27,28]. However, research on the mechanism of drug resistance in PC cells needs further exploration.

Integrin beta-8 (ITGB8), a member of the integrin beta chain family, encodes a single-pass type I membrane protein containing a VWFA domain and four cysteine-rich repeats[29]. ITGB8 is shown to be involved in cancer cell invasion, metastasis and chemoresistance[30,31], but its function in PC has not been fully explored.

In this paper, we focus on the functional study of lncRNA resistance in PC cells, further exploring its molecular regulation mechanism in drug resistance, improving the understanding of cancer cell resistance mechanism.

Materials and Methods

Patients, Tissues and Serum Sampl

A total of 47 PC tissues and matched ac nt tissues, and PC serum samples were obta from the West China Hospital, Sichuan Uni sity with the informed consent ients. Th study was approved by the I Review Board of the West China H tal, Si an University. All patients were nosed and had not undergone chemot or ra before specimen col on. rmal serum samples were colle from 42 p who were not diagnosed All tissue serum were stored a C.

Cell Cul and Transf n

PC lines (DU145 and CaP) were purchas om th merican Type Culture Collec (ATC Manass , VA, USA). DU145/PTX aP/PTX s were obtained from U145 a CaP treatment with paclitaxel ure m l cell lines were seeded in six- plates cultured in Dulbecco's modified 's medium (DMEM; Life Technologies, , USA) contained with 10% fetal ine serum (FBS; Atlanta Biologicals, Lawe ville, GA, USA) at 37°C in a humidified at here with 5% CO_2.

A plasmids and oligos, Si-FE2F1-AS1, si-ITGB8, anti-miR-25-3p, pcDNA-ITGB8 (ITGB8), miR-25-3p mimic (miR-25-3p) and their negative control, were purchased from Ribobio (Guangzhou, China). A negative control siRNA R, Life Technologies) was also utilize ing up to 70% confluence, all plasm and oligos were transfected into DU145/P d LNCaP/PTX using Lipofectamine 2000 o-Fisher Scientific, Waltham, MA, U) acco to the manufacturer's instructio

qRT-PCR

Total RNA was ex d f all tis and cells by using T l re nvitro Carlsbad, CA, USA llowing t nu urer's instructions. F -25-3p, RN anscripted to cDNA s TaqMan m NA Reverse Transcript n Kit ied Biosystems, Foster City, C USA). For 1 or ITGB8, RNA wa ripted to cD by using M-MLV R rse Transcriptase (Invitrogen). U6 and Glyceraldehyde pho te dehydrogenase (GAPDH) used as ref ce genes for miR-25-3p and F or ITGB spectively. SYBR Green Real-ti (Invitrogen) was used for quantitative R , according to the manufacturer's rotocol. Each sample had three replications. were calculated and analyzed using e 2 method. The primers are as follows: U6-F 5'-CTCGCTTCGGCAGCACA-3'; U6-R 5'-AACGCTTCACGAATTTGCGT-3'; miR-25-3p-F 5'-CATTGCACTTGTCTCGGTCTGA-3'; miR-25-3p-R 5'-GCTGTCAACGATACGCTACGTAACG-3'; ITGB8-F 5'-CCCGTGACTTTCGTCTTGGA-3'; ITGB8-R 5'-CCTTTCGGGGTGGATGCTAA-3'; FE2F1-F 5'-TTAGGAGCCTTGTTCTGTGT-3; FE2F1-R 5'-GCGCATGTACTTAAGAAAGA-3'; GAPDH-F 5'-GGAGCGAGATCCCTCCAAAAT-3'; GAPDH-R 5'-GGCTGTTGTCATACTTCTCATGG-3'.

Western Blot

Cell samples were washed with Tris-buffered saline (TBS) and lysed using a lysis buffer (Beyotime, Shanghai, China) on ice for protein extraction. Then, a BCA protein assay kit (Beyotime) was used to detect the protein concentration. Subsequently, 50 μg of proteins were added onto 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to separation and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with primary antibodies, including anti-ITGB8 anti-E-cadherin, anti-N-cadherin, anti-Vimentin, anti-LC3I, anti-LC3II, anti-ATG5,

RETRACTED

anti-Beclin-1 and anti-GAPDH (1:2000, Cell Signaling Technology, Danvers, MA, USA). After that, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody solution (goat anti-mouse IgG, 1:2000 dilution, Cell Signaling Technology). The ECL Western blotting substrate (Promega, Madison, WI, USA) was applied to detect and visualize the blot signals.

Cell Viability

CCK-8 Kit (Dojindo Molecular Technologies, Kumamoto, Japan) was used to measure the cell viability according to the manufacturer's protocol. Briefly, all cells were seeded into 96-well plates at a density of 5000 cells/cm². Next, each well was added into 10 uL CCK-8 solutions and incubated at 37°C for 0 h, 24 h, 48 h, and 72 h. The absorbance was detected at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA) at 0 h, 24 h, 48 h, 72 h. DU145, LNCaP, DU145/PTX and LNCaP/PTX cells were treated at concentrations of 0, 2, 4, 8, 16 paclitaxel for 24 h, and the half-maximal inhibitory concentration IC50 was cal- ed as the concentration of paclitaxel t creased cell viability by 50% using sigi al dose-responses curves (IC50 Calculator ware; AAT Bioquest, Sunnyvale, CA, USA).

Luciferase Reporter Assa

Wild-type and mutated F1-AS -UTRs (FE2F1-AS1-WT and F1-AS1 or ITGB8 3'-UTRs (ITGB8- W 3'UTR-MUT) were d in al-luciferase reporter vector (P ega, Mad WI, USA). The wild-type o -AS1 3'-UT ITGB8 3'-UTRs cont b sites with miR-25-3p, the mutated o wild-typ FE2F1-AS1 lacked binding with miR-25 U145/PTX and LNCaP X cells were see d into 24-well plate he F -AS1-WT and FE2F1-AS1-MU ITG UTR VT and ITGB8-3'UTR-MUT -transf d with miR-125-3p or R-NC U145 X and LNCaP/PTX cells ipofe 000 (Thermo-Fisher Scientific altham, , USA).

RNA Immunoprecipitation (RIP)

PC/PTX (DU145/PTX and LNCaP/PTX) cells used as the EZ-Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) to perform RIP experiments according to the manufacturer's protocol. Anti-Ago2 or anti-IgG antibodies were used to incubate with cells according to the manufacturer's protocol. qRT-PCR was applied to d expression of FEZF1-AS1.

Tumor Xenograft Model

Female 6-week-old BALB/c n ice were purchased from Vital Rive nc. (B Chi-na) and injected subcu ously in t flank regions with 2 10^4 DU 5/PTX stably transfected w sh-NC d sh-FEZF1-AS1. Tumor volume e sured in-jection for 7 d, 21 and 2 After injection for , tumor ts e calculated. Volu (width × le (width + length) × mal experiments were approved by he Ethi mmittee of West China Hospital Sichuan Un ty.

Statistical Analysis

ll experime were repeated three times. tailed Stude *t*-test was used to analyze all c isons in ous groups. All data represent the ard deviation (SD) from three indepen xperiments. All statistical analyses ere displayed and analyzed using GraphPad 7.0 (GraphPad Software, San Diego, CA, SA) < 0.05 was considered as statistically significant.

Results

FEZF1-AS1 Was Upregulated In Pc Tumor Tissues And Associated With Paclitaxel Sensitivities In PC

As shown in Figure 1A, FEZF1-AS1 was up-regulated in PC tumor tissues compared with normal tissues. Moreover, FEZF1-AS1 is associated with PTX resistance sensitivity of PC (Figure 1B). In addition, with the increase of paclitaxel concentration, the cell viability of DU145/PTX and LNCaP/PTX cells was significantly higher than that of DU145 and LNCaP cells (Figure 1C and 1D). Additionally, the IC50 of IDU145/PTX and LNCaP/PTX cells were sharply higher than that of DU145 and LNCaP cells (Figure 1C and 1D). Interestingly, the expression level of FEZF1-AS1 in DU145/PTX and LNCaP/PTX cells was also significantly higher than that of DU145 and LNCaP (Figure 1E and 1F). These results indicated that abnormal expression of FEZF1-AS1 in PC is associated with paclitaxel resistance of PC cells.

RETRACTED

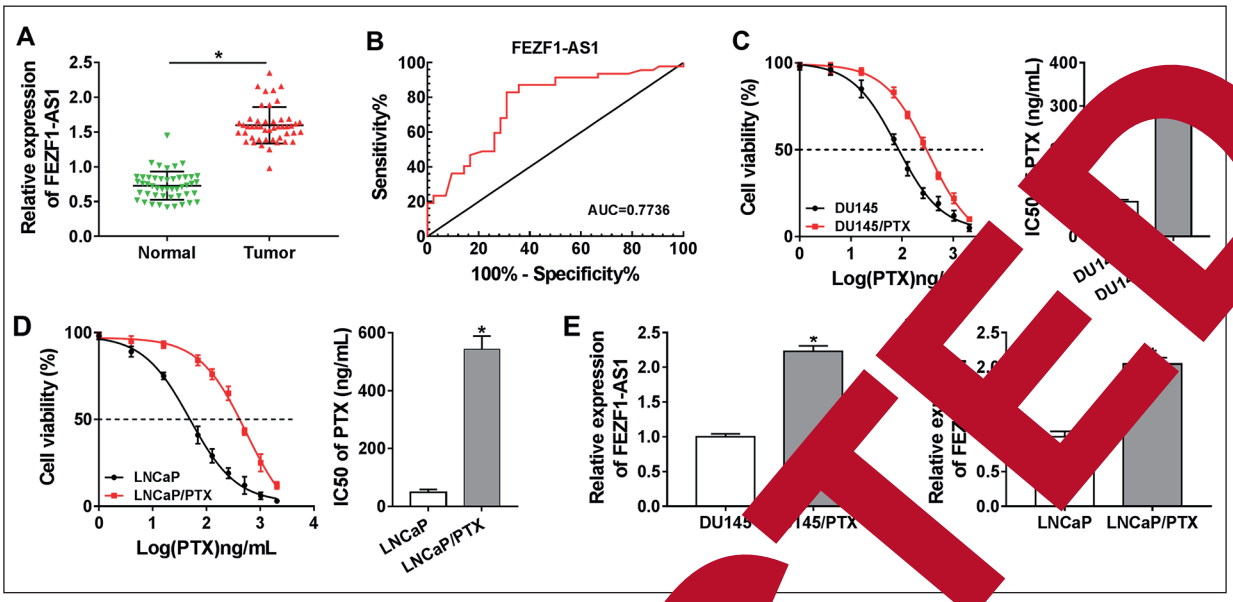

Figure 1. FEZF1-AS1 was up-regulated in PC tumor tissues a sociated with paclitaxel sensitivities in PC. **(A)** The expression of FEZF1-AS1 in normal tissues and PC tumor tissues detected with PCR. **(B)** ROC curve analysis of the diagnostic value of FEZF1-AS1 for drug resistance in PC. **(C** and C50 values of 45/PTX, DU145, LNCaP/PTX and LNCaP cells treated with paclitaxel of different concentrations (0, 2, 16 ng/mL) we tected. **(E** and **F)** The expression of FZEF1-AS1 in DU145/PTX, DU145, LNCaP/PTX and LNCaP cells cted us RT-PCR. *$p < 0.05$.

RETRACTED

Knockdown of FEZF1-AS1 Could Inhibit Cell Viability and EMT and Promoted Cell Autophagy in PC/PTX Cells

To further clarify the func ZF1-AS1 in PC resistance, DU145/P and L aP/PTX cells transfected with si-N si-FEZ and si-FEZE1-AS1 transfecti fic the expression of FE AS 45/PTX and LNCaP/PTX cells ure 2A). I ion, we also found that de the expressi EZE1-AS1 significa re the IC50 DU145/PTX and L P/PTX Figure 2C and 2D). Moreover cell prolifera si-FEZE1-AS1 group significantly reduce compared to si-NC p in D /PTX and LNCaP/PTX cells (Fi 2D 2E). C3I, LC3II, ATG5 and Beclin portant ulators of autophagy in ells wh lect t tophagy state of cells. As in Fig 2H, the protein expression of I II/LC3I, G5 and Beclin-1 were significan hibited by si-FEZE1-AS1 transfection in D d LNCaP/PTX cells. In addition, ZE1-AS1 also affected the EMT process of 45/PTX and LNCaP/PTX cells. E-cadherin, adherin and Vimentin have proven to be important biomarkers for EMT. In this paper, a decreased FEZE1-AS1 expression significantly the protein expression of N-cadherin d entin and promoted the expression of E-cadherin protein in DU145/PTX and LNCaP/PTX cells (Figure 2I to 2K). Therefore, all data determined that a decreased expression of FEZE1-AS1 inhibited proliferation and EMT as well as promoted autophagy in DU145/PTX and LNCaP/PTX cells, revealing that the low expression of FEZE1-AS1 contributed to reducing the paclitaxel resistance of PC cells and inhibited the growth and metastasis of PC/PTX cells.

MiR-25-3p Was a Target MiRNA of FEZF1-AS1

Target genes for FEZF1-AS1 candidates were predicted by starBase 3.0. The results showed that miR-25-3p was the target miRNA of FEZF1-AS1 (Figure 3A). To further validate the relationship between FEZF1-AS1 and miR-25-3p in DU145/PTX and LNCaP/PTX cells, analysis of the luciferase reporter assay showed that luciferase activity was significantly reduced when miR-25-3p was combined with FEZF1-AS1-WT DU145/PTX and LNCaP/PTX cells (Figure 3B and 3C). At the same time, miR-25-3p was shown to be underexpressed in PC tissues and negatively correlated with expression of FEZF1-AS1 (Figure 3D and 3E). In addition, the expression of miR-25-3p

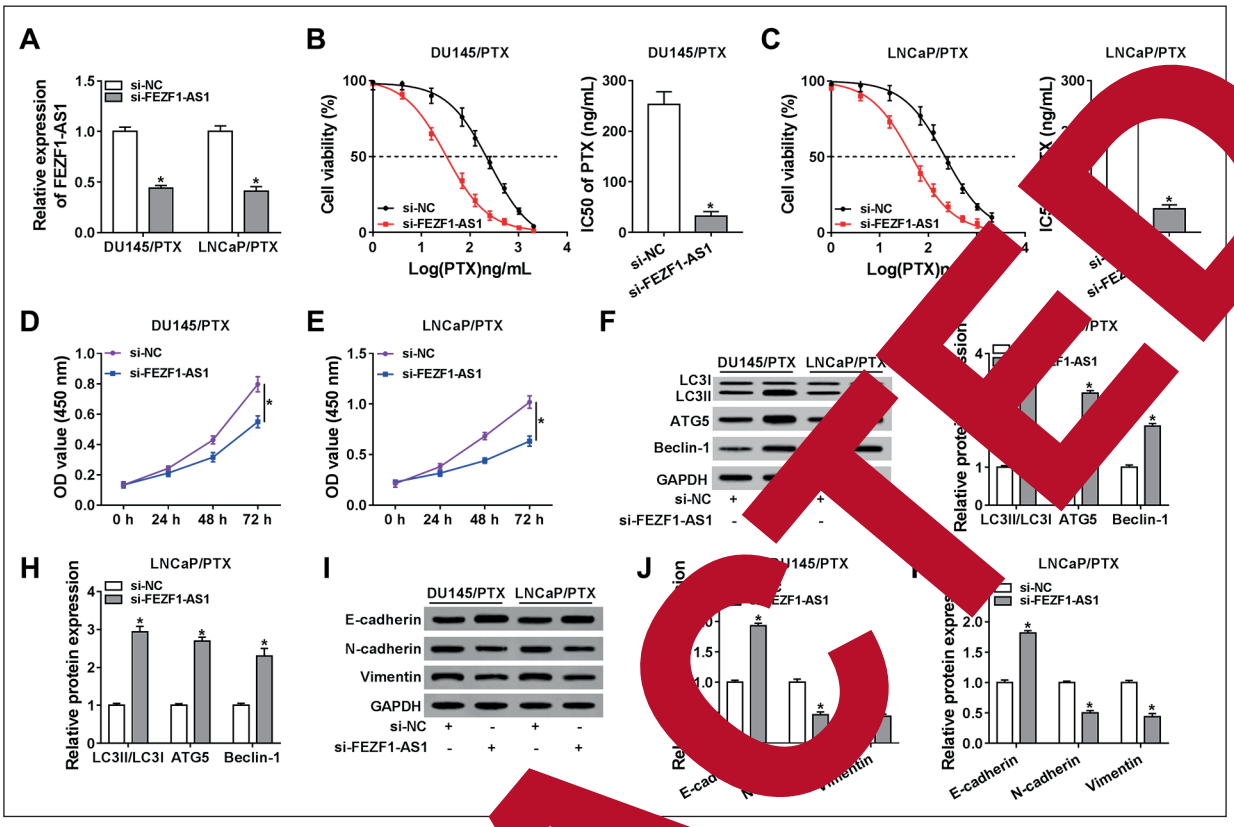

Figure 2. Knockdown of FEZF1-AS1 could inhibit cell viability and promoted cell autophagy in PC/PTX cells. **(A)** The expression of FEZF1-AS1 was detected in si-NC and si-FEZF1-AS1 groups in DU145/PTX and LNCaP/PTX cells with qRT-PCR. **(B** and **C)** IC50 values of DU145/PTX and LNCaP/PTX cells transfected with si-NC or si-FEZF1-AS1 treated with paclitaxel of different concentrations (0, 2, 4, 8, 16 ng/mL) were detected. **(D** and **E)** Cell proliferations were assessed in si-NC and si-FEZF1-AS1 groups of DU145/PTX and LNCaP/PTX cells with CCK-8 assay. (F to H) The protein expressions of LC3II/LC3I, ATG5 and Beclin-1 were measured in si-NC and si-FEZF1-AS1 groups of DU145/PTX and LNCaP/PTX cells with Western blot. **(I** to **K)** The protein expressions of E-cadherin, N-cadherin and Vimentin were measured in si-NC and si-FEZF-AS1 groups of DU145/PTX and LNCaP/PTX cells with Western blot. *$p < 0.05$.

was significantly higher in DU145/PTX and LNCaP/PTX cells than in DU145 and LNCaP cells (Figure 3F). The expression of miR-25-3p in si-FEZF1-AS1 group was significantly higher than that of si-NC group in DU145/PTX and LNCaP/PTX cells (Figure 3G and 3H). Not only that, the RIP assay showed FEZF1-AS1 was enriched for miR-25-3p in DU145/PTX and LNCaP/PTX cells (Figure 3I and 3J). These results reflected that miR-25-3p was target miRNA of FEZF1-AS1 and played a role in the paclitaxel resistance mechanism of PC.

FEZF1-AS Regulated Cell Viability, PTX and Autophagy by Inhibition of miR-25-3p in PC/PTX Cells

To further clarify the specific regulatory mechanisms of FEZF1-AS1, overexpressing or downregulating miR-25-3p of DU145/PTX and LNCaP/PTX cells was obtained by transfection of the vector of miR-25-3p or anti-miR-25-3p (Figure 4A and 4B). As shown in Figure 4C to 4F, overexpression of miR-25-3p remarkably reduced IC50 and cell proliferation whereas downexpression of miR-25-3p could significantly reverse the effect of inhibition of FEZF1-AS1 on DU145/PTX and LNCaP/PTX cells. A similar effect was observed in the protein expression of autophagy-related factors. miR-25-3p transfection contributed to the protein expression of LC3II/LC3I, ATG5 and Beclin-1, promoting autophagy in DU145/PTX and LNCaP/PTX cells. In addition, the effects of inhibition of FEZF1-AS1 were significantly inhibited by anti-miR-25-3p transfection in DU145/PTX and LNCaP/PTX cells (Figure 4C to 4I). Besides,

RETRACTED

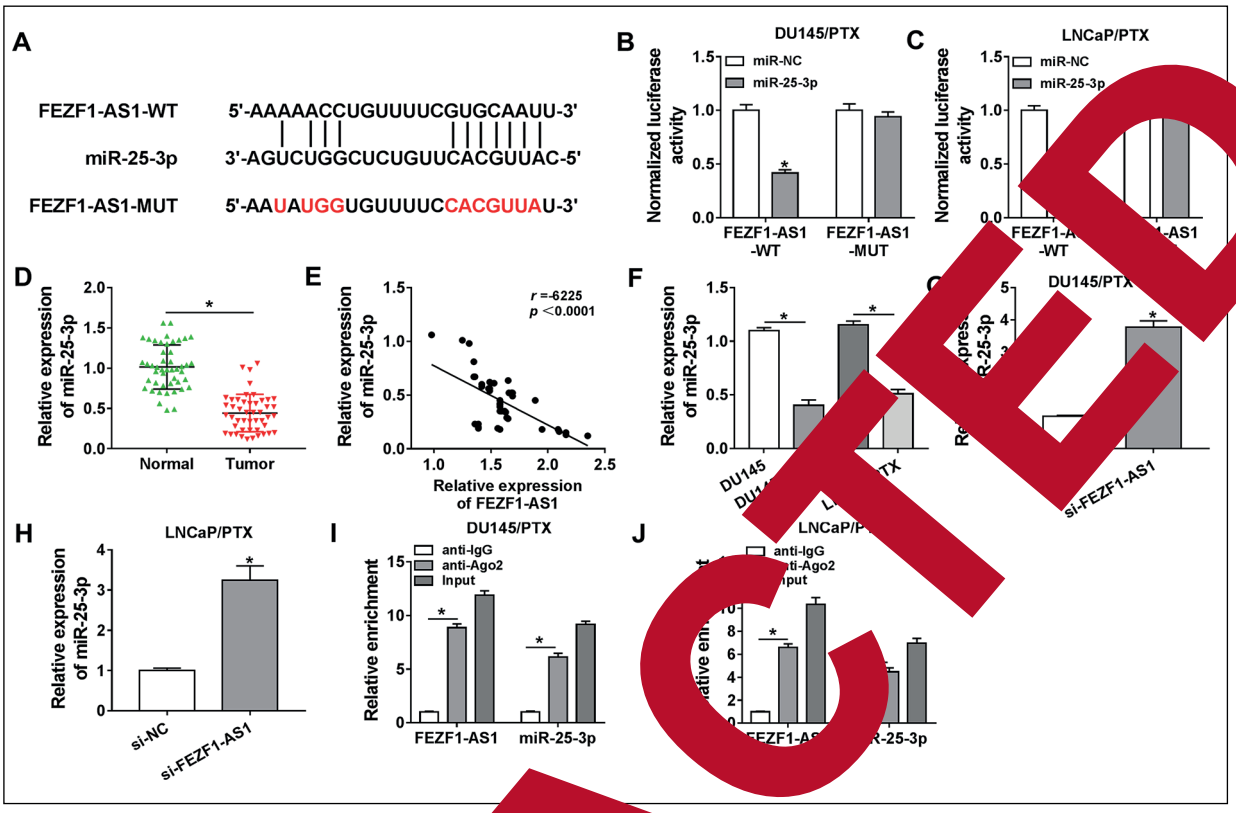

Figure 3. miR-25-3p was a target miRNA of FEZ S1. (binding sites for miR-25-3p on the FEZF-AS1. **(B** and **C)** The luciferase activities were measured in L /PTX PTX cells co-transfected with FEZF1-AS1-WT or FEZF1-AS1-MUT and miR-NC or miR-25-3p. **(D)** sion of miR-25-3p in normal tissues and PC tumor tissues was detected with qRT-PCR. **(E)** The correlation betwee 1-AS and miR-25-3p was measured in tumor tissues through Spearman correlation analysis. **(F)** The ion of miR in DU145/PTX, DU145, LNCaP/PTX and LNCaP cells was detected using qRT-PCR. (G and F) n of miR- was detected in si-NC and si-FEZF1-AS1 groups of DU145/PTX and LNCaP/PTX cells with CR. **(I J)** The enri nt of FEZF1-AS1 was detected in DU145/PTX and LNCaP/PTX cells using RIP assay. *$p <$

RETRACTED

miR-25-3p overex sion incre -cadherin protein expressi inhibited N- in and Vimentin pro ex on in DU145/PTX and LNCaP/PTX cells. The bition of miR-25-3p signif dy reversed t fects of DU145/PTX a NCaP/PTX cells t sfected with si-FEZ S1 (Fi e 4J to 4L). Taken together, FE S1 ated cell proliferation, and autopha MT in 145/PTX and LNCaP/ cel targ miR-25-3p, suggesting e reg echanism of FEZF1-AS1/mi -3p play important regulatory role to pac resistant of PC.

FEZF1-AS Regulated ITGB8 Expression Through Targeting miR-25-3p

W predicted the potential target gene of miR-25-3p using starBase 3.0. ITGB8 was a downstream target gene for miR-25-3p and highly expressed at protein expression in tumor tissues and DU145/PTX and LNCaP/PTX cells (Figure 5A, 5D and 5E). Luciferase reporter assay showed that, when miR-25-3p was combined with ITGB8-3UTR-WT, instead of ITGB8-3UTR-MUT, luciferase activities sharply reduced (Figure 5B and 5C). More than that, overexpression miR-25-3p significantly inhibited the protein expression of ITGB8 in DU145/PTX and LNCaP/PTX cells (Figure 5F). In addition, knocking out FEZF1-AS1 significantly suppressed the protein expression of ITGB8, which was reversed by downregulation of miR-25-3p (Figure 5G and 5H). These data indicate that ITGB8 is the target gene for miR-25-3p and FEZF1-AS1 regulated the protein expression of ITGB8 by targeting miR-25-3p in DU145/PTX and LNCaP/PTX cells.

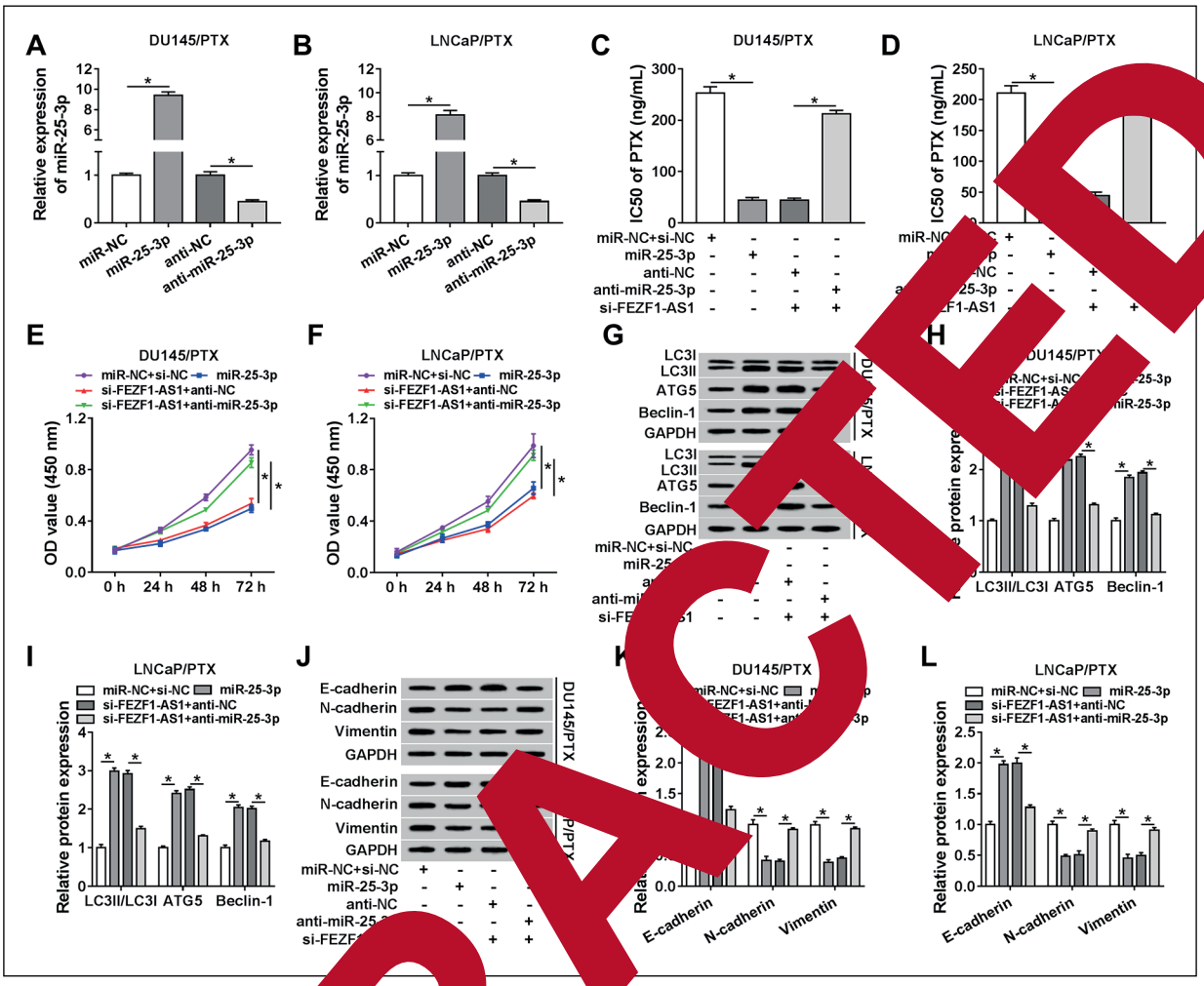

Figure 4. FEZF1-AS regulate[s] viability [and] and autophagy by inhibition of miR-25-3p in PC/PTX cells. **(A** and **B)** The expression of miR-25-3p was [measured] in mi[R-NC], anti-NC and anti-miR-25-3p groups of DU145/PTX and LNCaP/PTX cells with qRT-PCR. [(C** and **D)** Cell proliferations of] DU145/PTX and LNCaP/PTX cells were measured in miR-NC + si-NC, miR-25-3p, si-FEZF1-AS[1 +] anti-NC[, si-FEZF1-AS1 +] anti-miR-25-3p groups. **(E** and **F)** Cell proliferations of DU145/PTX and LNCaP/PTX cells [were] measured in [miR-NC + si-NC, miR-25-3p, si-FEZF1-AS1 + anti-NC, si-FEZF1-AS1 + anti-miR-25-3p groups with CCK-8 assay. **(G** to **I)** The protein expression of LC3II/LC3I, ATG5 and Beclin-1 was measured in miR-NC + si-NC, miR-25-3p, si-FEZF1-AS1 + anti-NC, si-FEZF1-AS1 + anti-miR-25-3p groups of DU145/PTX and LNCaP/PTX cells with Western blot. **(J** to **L)** The protein expression of E-cadherin, N-cadherin and Vimentin was measured in miR-NC + si-NC, miR-25-3p, si-FEZF1-AS1 + anti-NC, si-FEZF1-AS1 + anti-miR-25-3p groups of DU145/PTX and LNCaP/PTX cells with Western blot. $^{*}P < 0.05$.

The [Effect] of Low [Expression of] FEZF1-AS Expression on Cell [Viability], PTX and Autophagy Reversed by Promotion of ITGB8 in [DU145/]PTX Cells

[To further] clarify the role of ITGB8 in the regulatory mechanism of FEZF1-AS1, we obtained high- and low-expression ITGB8 of DU145/PTX and LNCaP/PTX cells to explore the function of ITGB8 (Figure 6A and 6B). The CCK-8 assay showed that the inhibition of ITGB8 significantly reduced IC50 and proliferative capacity, promoted protein expression of LC3II/LC3I, ATG5 and Beclin-1 in DU145/PTX and LNCaP/PTX cells, whereas increased expression of ITGB8 attenuated these effects of DU145/PTX and LNCaP/PTX cells transfected with si-FEZF1-AS1 (Figure 6C to 6I). Moreover, si-ITGB8 transfection enhanced E-cadherin protein expression and reduced N-cadherin and Vimentin protein expression. The effects of si-FEZF1-AS1 on E-cadherin, N-cadherin and Vimentin protein expression were reversed through promotion of ITGB8 in

RETRACTED

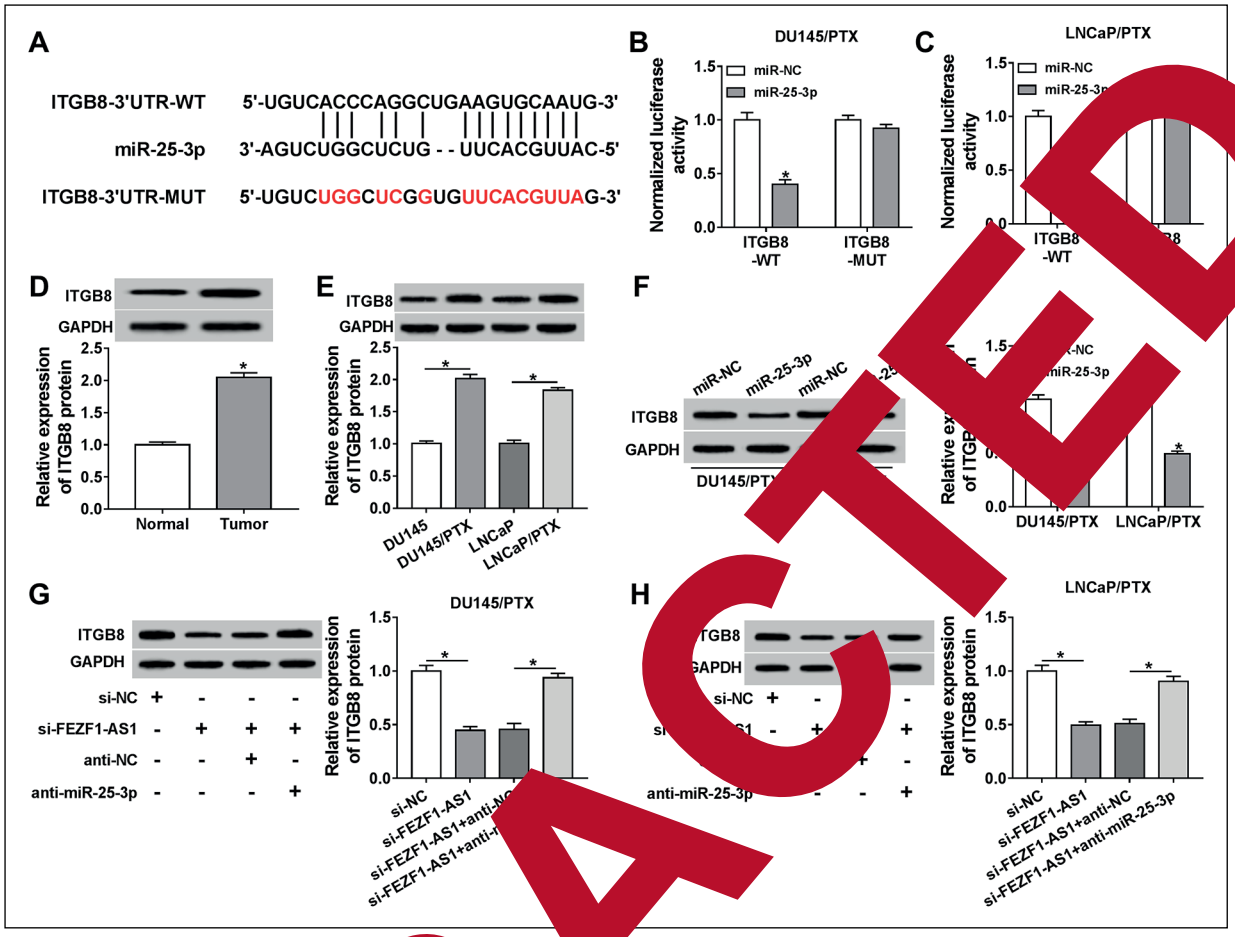

Figure 5. FEZF1-AS regulated ITGB[illegible]n through[illegible]ting miR-25-3p. **(A)** Predicted binding sites for miR-25-3p on the ITGB8 transcript. **(B** and **C)** Th[illegible]iera[illegible]ivities we[illegible]sured in DU145/PTX and LNCaP/PTX cells co-transfected with ITGB8-WT or ITGB8-MUT[illegible]miR-NC[illegible]miR-25-3p. **(D)** The protein expression of ITGB8 in normal tissues and PC tumor tissues was detected wit[illegible]tern blot[illegible]otein expression of ITGB8 in DU145/PTX, DU145, LNCaP/PTX and LNCaP cells was detected using[illegible]CR.[illegible]otein expression of ITGB8 was detected in si-NC, si-FEZF1-AS1, si-FEZF1-AS1 + anti-NC[illegible]-miR-25-3p groups of DU145/PTX and LNCaP/PTX cells with Western blot. *$p < 0.05$.

DU145/PTX a[illegible]LNCa[illegible]cells (Figure 6J to 6L). Thes[illegible]ults indicated[illegible]e effects of low FEZF1[illegible]expression on cell[illegible]bility, EMT and auto[illegible]y were[illegible]ersed by promotion of ITGB8 in[illegible]TX c[illegible]

[illegible]ckda[illegible]of F[illegible]1-AS1 Suppressed [illegible]r Gr[illegible]d Decreased PTX Se[illegible]ivities [illegible]C Cells in Vivo

[illegible]verified the regulatory mechanism [illegible]in mice. The results showed that [illegible]ucing the expression of FEZF1-AS1 in DU145/[illegible] and LNCaP/PTX cells significantly inhibited [illegible]umor volume and reduced tumor weight (Figu[illegible]e 7A and 7B). In addition, the expression of FEZF1-AS1 and the mRNA and protein expression of ITGB8 in sh-FEZF1-AS1 group were higher than that in sh-NC group (Figure 6C and 6D). The expression of miR-25-3p in sh-FEZF1-AS1 group was higher than that in sh-NC group (Figure 6C). The results showed that low expression of FEZF1-AS1 could reduce PTX sensitivities and inhibit tumor growth via regulating miR-25-3p/ITGB8 axis.

Discussion

With the continuous development of molecular biology and bioinformatics technology, more and more molecular mechanisms of cancer have been discovered. Accumulating

RETRACTED

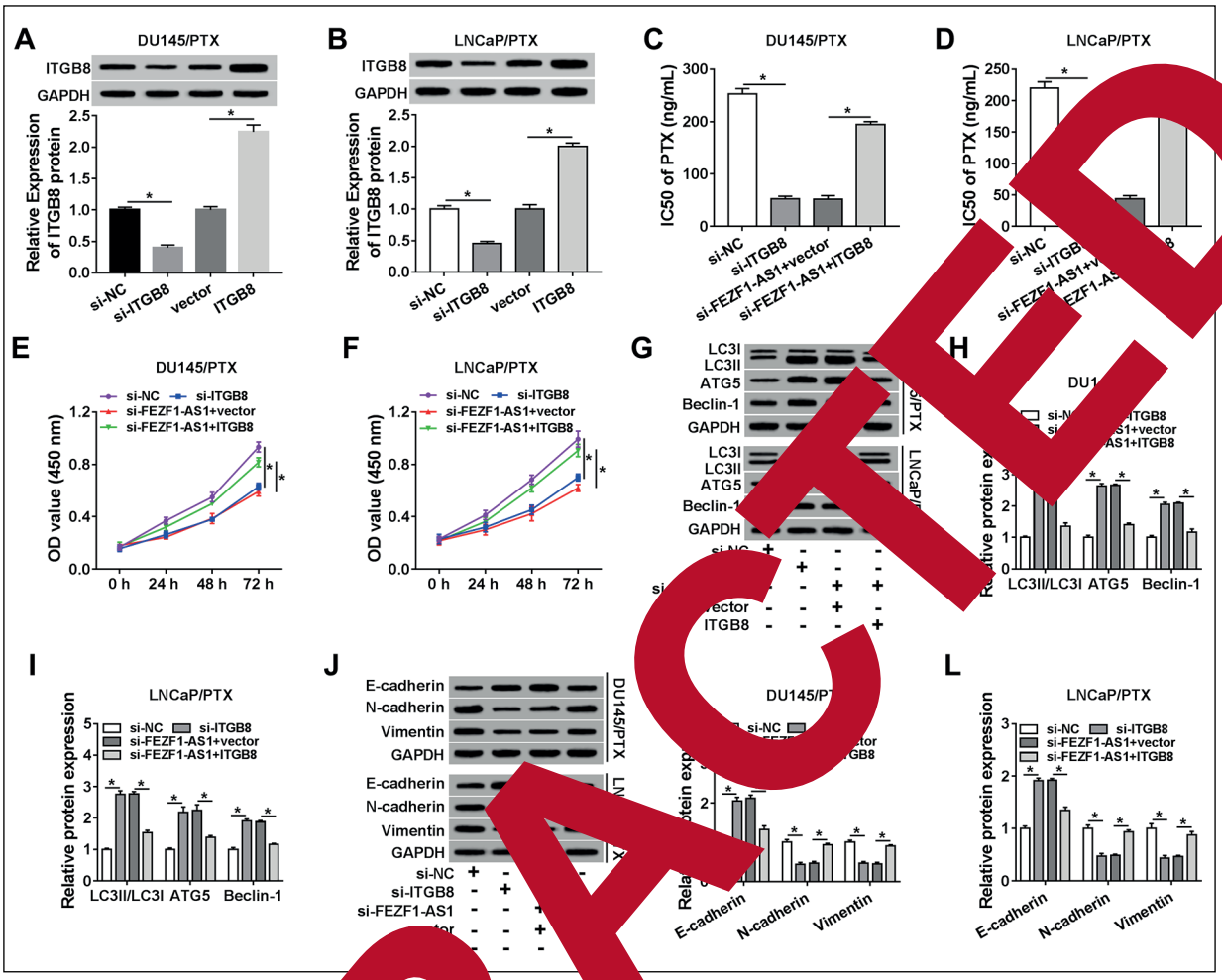

Figure 6. The effects of low l-AS ex ion on cell ability, EMT and autophagy were reversed by promotion of ITGB8 in PC/PTX cells. **(A** The pr of ITGB8 was detected in si-NC, si-ITGB8, vector and ITGB8 groups of DU145/PTX and L blot. **(C** and **D)** IC50 values of DU145/PTX and LNCaP/PTX cells were measured in si-NC, GB8, -AS1 + vector, si-FEZF1-AS1 + si-ITGB8 groups. **(E** and **F)** Cell proliferations of DU145/PTX and LN TX cells easured in si-NC, si-ITGB8, si-FEZF1-AS1 + vector, si-FEZF1-AS1 + si-ITGB8 groups with CCK-8 a G to **I)** The pro ression of LC3II/LC3I, ATG5 and Beclin-1 was measured in si-NC, si-ITGB8, si-FEZF1-AS1 + v ZF1-AS1 + si- 8 groups of DU145/PTX and LNCaP/PTX cells with Western blot. **(J** to **L)** The protein exp of rin, N-cadherin and Vimentin was measured in si-NC, si-ITGB8, si-FEZF1-AS1 + vector, si-FEZF1-AS1 + si- GB8 grou U145/PTX and LNCaP/PTX cells with Western blot. *$p < 0.05$.

RETRACTED

evi sug ed tha many lncRNAs are abnorm ssed i ncer cells, implying a relati h th aracteristics of PC cells, ing and metastasis[32-34]. Drug resi ce is a portant issue in the current tre of PC[35,36]. More than that, lncRNA i involved in the chemoresistance chanisms of various cancers, including col- al cancer, gastric cancer, cervical cancer, b cancer and prostate cancer[37-40]. Interstingly, increased MALAT1 expression could contribute to chemoresistance in gastric cancer, lung cancer, and prostate cancer [41-43]. Autophagy and EMT are important mechanisms of tumor cell development. Autophagy can improve the adaptability of cells, and abnormal autophagy mechanism will lead to the appearance of cancer cells[44]. EMT is an important biological process for tumor cells to acquire the ability to migrate and invade. Moreover, lncRNA plays an important role in the regulation of autophagy and EMT[45]. It has been observed that lncRNA CPS1-IT1 inhibited EMT and metastasis *via* suppressing hypoxia-induced autophagy by

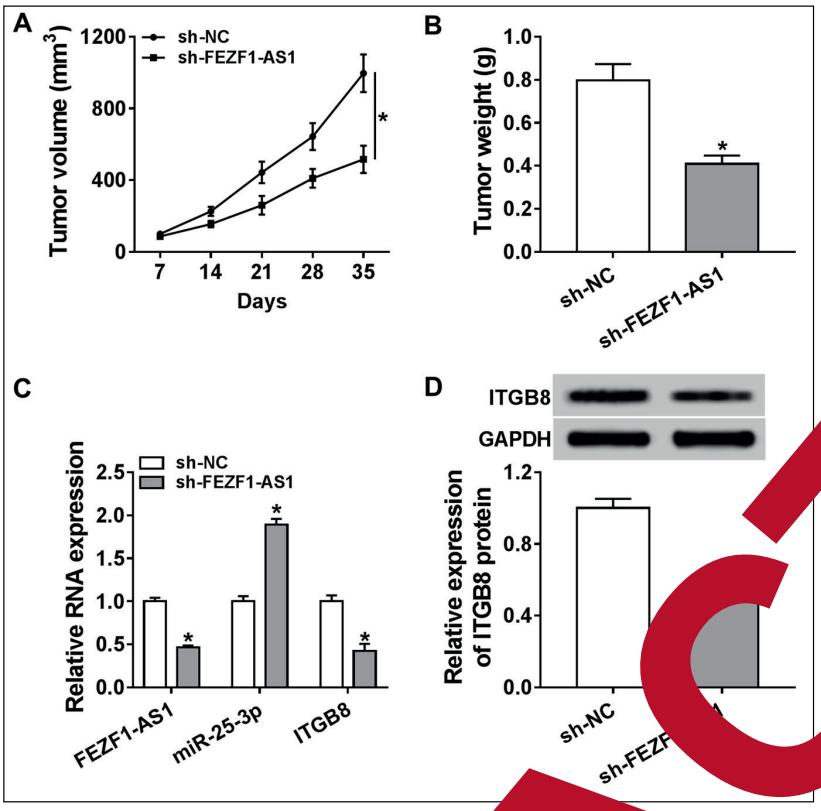

Figure 7. Knockdown of FEZF1-AS1 suppressed tumor growth and decreased PTX sensitivities of PC cells *in vi*. **(A)** The tumor growth curves of s. FEZF1-AS1 groups *in vivo*. **(B)** The mean tumor we. as measure in sh-NC and sh-FE. S1 groups *in vivo*. **(C)** The expre. FEZF1-AS1, miR-25-3p and ITGB8 . ted in sh-NC and sh-FE. -AS1 g. h qRT-PCR. **(D)** Th. in expressio. P in sh-NC . -FEZF1-AS1 gro. measure. g Weste. ot. *$p < 0.$

RETRACTED

inactivation of HIF-1αin colorectal cance. In the present research we fou. at FEZF. AS1 expression was increa. tissues and PC/PTX cells, imply. that F. F1-AS1 was closely related to . taxel . in PC. Importantly, redu. ZE. sion inhibited proli. tion . MT as well as promoted aut. gy in PC. cells, revealing that t. expressio. EZE1-AS1 contribu. to p. axel sensiti. e of PC/PTX cells de. reasing . hemoresistance of PC cells. RNAs have . been shown to play a. important role in . ncer drug resista. Trans. tome analysis revealed that ma. iRN. were differentially expressed in ca. and i. ved in the metastasis, . lifera. and . g sensitivity of cancer . o dru. paclitaxel[47-49]. Therefore, mi. a signi. tly reduced the resistance of l. e-refractory PC cells to paclitaxel in P. er, we demonstrated that FEZF1-. 1 directly targeted miR-25-3p, and that re. ng miR-25-3p significantly suppressed the r. ce of PC/PTX cells transfected with si-F. F1-AS1 and reduced the sensitivity of PC/PTX cells to paclitaxel. Thus, FEZF1-AS1 decreased the paclitaxel resistance of PC cells through downregulating miR-25-3p. Next, we found that the ITGB8 is the target gene of miR-25-3p. We thought that ITGB8 was involved in the regulatory mechanism of FEZF1-AS1/miR-25-3p. Many studies[50-52] demonstrated that ITGB8 also participated in chemoresistance of various cancers. Wang et al[53] showed that silencing ITGB8 reversed gefitinib resistance for hepatic cancer. In addition, ITGB8 also regulated cell progression in lung cancer and colorectal cancer[51, 54]. In this paper, we found that the inhibition of ITGB8 expression significantly reduced drug-resistant cell proliferation, EMT and promoted autophagy and increased paclitaxel sensitivity in PC cells. Moreover, increasing the expression of ITGB8 reversed the inhibitory effect of si-FEZF1-AS1 on PC/PTX cells and reduced the resistance of PC cells.

LncRNAs have been reported to act as competing endogenous RNAs (ceRNAs) to modulate mRNA *via* sponging miRNA in the regulation mechanism of cell growth in cancers. Besides, in hepatocellular carcinoma, lncRNA CCAT1 promoted autophagy through regulating ATG7 expression by competitively binding miR-181[55].

Otherwise, lncRNA APP, acts as a competing endogenous RNA, contributing to prostate cancer progression *via* binding miR-228 to regulate ZEB2/CDH2 expression[56]. Therefore, we could infer that lncRNA FEZF1-AS1 promoted chemoresistance, autophagy and EMT through regulating ITGB8 expression *via* competitively binding miR-25-3p in PC.

Conclusions

In this study, we first found and verified that lncRNA FEZF1-AS1 promoted chemoresistance, autophagy and EMT through regulation of miR-25-3p/ITGB8 axis in PC, providing a new regulatory mechanism of paclitaxel-resistance of PC and a novel therapeutic target.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Partic

The present study was approved by the Ethical ew Committee of West China Hospital, Sichuan Univers

References

1) Siegel RL, Miller KD, Jema Canc tatistics, 2017. CA Cancer J Clin ; 67: 7-3
2) Al-Batran SE, Jäger E, S 4. Ch advanced gastric c cer. ol 2007; : 729.
3) Safavy A, Rais Khazaeli M sbaum DJ, Bonner JA. P derivatives fo ed therapy of can to e development of smart taxanes. J ed Chem ; 42: 4919-4924.
4) Weav How Taxol/pa kills cancer cells. Mol Cell 2014; 25: 267 1.
5) A ong DL ndy B, Wenzel L, Huang HQ, Baer-R, Lele opeland LJ, Walker JL, Burger RA; l Oncolo oup. Intraperitoneal cisplatin, paclitaxel, but not intravenous cisplatin, paclitaxel, and ovarian cancer. N Engl J Med 354: .
6) zi C, Cuccuru G, Supino R, Zuco V, lini C, Sc G, Zunino F. Cell cycle checkpoint efficiency and cellular response to paclitaxel in breast cancer cells. Prostate 2001; 48: 254-264.
7) Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Ve- B, Regev A, Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Gene Dev 2011; 25: 1915-1927.
8) Chakravarty D, Sboner A, Nair SS, Giannopoulou E, Li R, Hennig S, Mosquera JM, Pauwels J, Park K, Kossai M, MacDonald TY, Fontugne J, Erho N, IA, Ghadessi M, Davicioni E, Jenkins RB N, Chen Z, Nakagawa S, Hirose T, B NH, tran H, Fox AH, Elemento O, Rubin The oestrogen receptor alpha-regulated l NEAT1 is a critical modulator of prostate ca t Commu 2014; 5: 5383.
9) Li J, Meng H, Bai Y, Wang egulation N and its role in canc etastasis. Onc 2016; 23: 205-217.
10) Liu JH, Chen G, YW, J, Luo Z. Expression and prog cance cRNA MALAT1 in p eatic tissue an Pac J Cancer P 2014; 15: 2 27
11) Ye S, Ya o X, Song W W, Zheng S. Bioinf atic hod to predic two regulation mech sm: TF A-mRNA and lncRNA-miRNA-mRNA in pan cancer. Cell Biochem 2014; 70: 18 8.
12) ang P, Xu J, Wang Y, Cao X. An interferon-independent lncRNA promotes viral replication by modulating ar metabolism. Science 2017; 358: 1051-105
13) ng P, Cao hou R, Yang X, Wu M. The lncRNA s activation of inflammasomes in ma s. Nat Commun 2019; 10: 1495.
14) Chen H, Meng T, Liu X, Sun M, Tong C, Liu J, Wang Du J. Long non-coding RNA MALAT-1 is downated in preeclampsia and regulates proliferation, apoptosis, migration and invasion of JEG-3 trophoblast cells. Int J Clin Exp Pathol 2015; 8: 12718-12727.
15) Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, Wang Y, Huang J, Xu M, Yan J, Yu J. lncRNA H19/miR-675 axis represses prostate cancer metastasis by targeting TGFBI. FEBS J 2014; 281: 3766-3675.
16) Prensner JR, Chen W, Han S, Iyer MK, Cao Q, Kothari V, Evans JR, Knudsen KE, Paulsen MT, Ljungman M, Lawrence TS, Chinnaiyan AM, Feng FY. The long non-coding RNA PCAT-1 promotes prostate cancer cell proliferation through cMyc. Neoplasia 2014; 16: 900-908.
17) Chen N, Guo D, Xu Q, Yang M, Wang D, Peng M, Ding Y, Wang S, Zhou J. Long non-coding RNAFEZF1-AS1 facilitates cell proliferation and migration in colorectal carcinoma. Oncotarget 2016; 7: 11271-11283.
18) Liu YW, Xia R, Lu K, Xie M, Yang F, Sun M, De W, Wang C, Ji G. LincRNA FEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-mediated H3K4me2 demethylation. Mol Cancer 2017; 16: 39.
19) Zhou C, Xu J, Lin J, Lin R, Chen K, Kong J, Shui X. Long non-coding RNA FEZF1-AS1 promotes osteosarcoma progression by regulating miR-4443/NUPR1 axis. Oncol Res 2018 Feb 22. doi: 10.3727/096504018X15188367859402. [Epub ahead of print]

RETRACTED

20) Zhu LF, Song LD, Xu Q, Zhan JF. Highly expressed long non-coding RNA FEZF1-AS1 promotes cells proliferation and metastasis through Notch signaling in prostate cancer. Eur Rev Med Pharmacol Sci 2019; 23: 5122-5132.

21) Reddy KB. MicroRNA (miRNA) in cancer. Cancer Cell Int 2015; 15: 38.

22) Hong L, Yang Z, Ma J, Fan D. Function of miRNA in controlling drug resistance of human cancers. Curr Drug Target 2013; 14: 1118-1127.

23) Ivanovic RF, Viana NI, Morais DR, Silva IA, Leite KR, Pontes-Junior J, Inoue G, Nahas WC, Srougi M, Reis ST. miR-29b enhances prostate cancer cell invasion independently of MMP-2 expression. Cancer Cell Int 2018; 18: 18.

24) Ru P, Steele R, Newhall P, Phillips NJ, Toth K, Ray RB. miRNA-29b suppresses prostate cancer metastasis by regulating epithelial-mesenchymal transition signaling. Mol Cancer Ther 2012; 11: 1166-1173.

25) Schaefer A, Jung M, Miller K, Lein M, Kristiansen G, Erbersdobler A, Jung K. Suitable reference genes for relative quantification of miRNA expression in prostate cancer. Exp Mol Med 2010; 42: 749-758.

26) Cochetti G, Poli G, Guelfi G, Boni A, Egidi MG, Mearini E. Different levels of serum microRNAs in prostate cancer and benign prostatic hyperplasia: evaluation of potential diagnostic and prog tic role. Onco Targets Ther 2016; 9: 7545-75

27) Wu X, Zhou H, Yue B, Li M, Liu F, Qiu C, Che Ma X. Upregulation of microRNA-25-3p inhibits f- eration, migration and invasion of osteosarc cells in vitro by directly targeting SOX4. Mol M Rep 2017; 16: 4293-300.

28) Wu JB, Ye XH, Xian SX, Don ssions of SERCA2a and miR-25-3 in my dium of rats with heart failure herapeu ffects of Xiefei Lishui recipe. Zh o Yin Li Xue Za Zhi 2017 : 1-

29) Lapointe VL, Verp A, Steve . The changing integrin ex ion and a ro tegrin ß8 in the chond ifferentiation senchymal stem PL e 2013; 8: e8 035.

30) Huang J Cai JL, Hua Kang L, Huang MJ, Wang ang JP. miR19b motes the growth and tastasis of colorect ncer via directly t ng ITG Am J Cancer Res 2017; 7: 1996-

31) W ian D, W T, Lu T, Huang X, Zhang P, iR-199 enhances cisplatin sensitivity ian er cells by targeting ITGB8. col R : 1649-1657.

32) ik R, Zha , Cieslik M, Niknafs YS, Pitchiaya S, o Y. Integrative analysis of androgen recep- long non-coding RNA in prostate cancer. ancer Res 2016; 76: 983.

Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, ang Y, Huang J, Xu M, Yan J, Yu J. lncRNA H19/ -675 axis represses prostate cancer metastas s by targeting TGFBI. FEBS J 2014; 281: 3766-3775.

34) Li L, Dang Q, Xie H, Yang Z, He D, Liang L, Song W, Yeh S, Chang C. Infiltrating mast cells enhance prostate cancer invasion via alteri n- cRNA-HOTAIR/PRC2-androgen rec nhibitor MMP9 signals and increased stem/ population. Oncotarget 2015; 6: 1 -14190.

35) Ni J, Cozzi P, Hao J, Duan W, G P, Kearsley J, Li Y. Cancer stem cells in prostat chemoresistance. Curr Cancer g Targ 4; 14: 225-240.

36) Aoyama Y, Sobue S, M N, In e C, Ka Y, Nishizawa Y, Ichih , Kyog M, Suzuk Nozawa Y, Murat odula of the sphingolipid rheostat i inv itaxel tance of the huma state cell li C3-PR. Biochem B ys Res C a 2 486: 551-557.

37) Han P w BM, Lv JC, M, Gu XY, Yu ZW, H, Bai Liu YL, Cui BB. The lncRNA CRNDE promotes ctal cancer cell proliferd chemoresi via miR-181a-5p-mediated regulation of Wn catenin signaling. Mol Cancer 2017; 16: 9.

Jia J, Zhan D, Li Z, Li H, Qian J. The contrary functions of RNA HOTAIR/miR-17-5p/PTEN axis and She uzheng injection on chemosen- of c cancer cells. J Cell Mol Med 669.

39) Shen CJ, Cheng YM, Wang CL. LncRNA PVT1 epigenetically silences miR-195 and modulates EMT hemoresistance in cervical cancer cells. J Target 2017; 25: 637-644.

40) Si X, Zang R, Zhang E, Liu Y, Shi X, Zhang E, Shao L, Li A, Yang N, Han X, Pan B, Zhang Z, Sun L, Sun Y. LncRNA H19 confers chemoresistance in ERα-positive breast cancer through epigenetic silencing of the pro-apoptotic gene BIK. Oncotarget 2016; 7: 81452-81462.

41) YiRen H, YingCong Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, Ende C, XiZhou L, Yanfan C. Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance via miR-23b-3p sequestration in gastric cancer. Mol Cancer 2017; 16: 174.

42) Wang H, Wang L, Zhang G, Lu C, Chu H, Yang R, Zhao G. MALAT1/miR-101-3p/MCL1 axis mediates cisplatin resistance in lung cancer. Oncotarget 2017; 9: 7501-7512.

43) Xue D, Lu H, Xu HA, Zhou CI, He XH. Long noncodingRNA MALAT1 enhances the docetaxel resistance of prostate cancer cells via miR-145-5p-mediated regulation ofAKAP12. J Cell Mol Med 2018; 22: 3223-3237.

44) Goldsmith J, Levine B, Debnath J. Autophagy and cancer metabolism. Methods Enzymol 2014; 542: 25-57.

45) Gibson NW. Therapeutic opportunities associated with EMT and cancer prevention. Cancer Epidemiol Biomarkers Prev 2006; 15: ED01-ED03.

46) Zhang W, Yuan W, Song J, Wang S, Gu X. LncRNA CPS1-IT1 suppresses EMT and metastasis of

RETRACTED

colorectal cancer by inhibiting hypoxia-induced autophagy through inactivation of HIF-1α. Biochimie 2018; 144: 21-27.

47) Muluhngwi P, Klinge CM. Roles for miRNAs in endocrine resistance in breast cancer. Endocr Relat Cancer 2015; 22: R279-300.

48) García-Becerra R, Santos N, Díaz L, Camacho J. Mechanisms of resistance to endocrine therapy in breast cancer: focus on signaling pathways, miRNAs and genetically based resistance. Int J Mol Sci 2012; 14: 108-145.

49) Wang Z, Li Y, Ahmad A, Azmi AS, Kong D, Banerjee S, Sarkar FH. Targeting miRNAs involved in cancer stem cell and EMT regulation: An emerging concept in overcoming drug resistance. Drug Resist Updat 2010; 13: 109-118.

50) Mertens-Walker I, Fernandini BC, Maharaj MS, Rockstroh A, Nelson CC, Herington AC, Stephenson SA. The tumour-promoting receptor tyrosine kinase, EphB4, regulates expression of Integrin-β8 in prostate cancer cells. BMC Cancer 2015; 15: 164.

51) Xu Z, Wu R. Alteration in metastasis potential and gene expression in human lung cancer cell lines by ITGB8 silencing. Anat Rec (Hoboken) 2012; 295: 1446-1454.

52) Kumar V, Soni UK, Maurya VK, Singh K, Jha RK. Integrin beta8 (ITGB8) activates VAV-RAC1 signaling via FAK in the acquisition of endometrial epithelial cell receptivity for blastocyst i... Sci Rep 2017; 7: 1885.

53) Wang WW, Wang YB, Wang DQ... Z, Sun RJ. Integrin beta-8 (ITGB8) silen... verses gefitinib resistance of human hepa... cer Hep-G2/G cell line. Int J Cl... Exp M... 15; 8: 3063-3071.

54) Huang L, Cai JL, Hu... Z, Ka... L, Huan... Wang L, Wang JP. m... p-3p pr... es the gro... n and metastasis ... recta... cer via directly targeting ITGB... An... es 201... 1996-2008.

55) Guo J, Ma... ng X, Jin H, ... NA CCAT1 promote... hagy via re... g ATG7 by spong... ...in hepatocellu... carcinoma. J Cell B... hem 2... 0: 17975-17983.

56) Su... Zhang W, Nia... X, Li Y, Liu F, Wang F, ...hao L, Zhu Y, ... Sun Y. The previously uncharacterized lncRNA APP promotes prostate cancer progression by acting as a competing endogen... RNA. Int J Cancer 2020; 146: 475-486.

RETRACTED