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Long non-coding RNA FTX promotes gastric cancer progression by targeting miR-215

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Abstract. – OBJECTIVE: Gastric cancer (GC) is one of the most common malignant tumors in the world, which is seriously harmful to people's health. The increasing number of studies have shown that long non-coding RNA (lncRNA) is related to the occurrence of gastric cancer. In this study, we aimed at investigating the role of lnc FTX in the occurrence of gastric cancer.

MATERIALS AND METHODS: The expression of FTX in gastric cancer patients and gastric cancer cell lines was detected by RT-qPCR. Univariate Kaplan-Meier method was used to analyze the relationship between FTX expression level, clinicopathological parameters and overall survival rate (OS). After transferring si-FTX and overexpression FTX plasmids into MGC-803 and SGC-7901, the expression of miR-215-3p was detected by RT-qPCR, and the changes of cell proliferation and cell cycle were detected by CCK-8 and flow cytometry. In addition, luciferase activity was used to detect whether miR-215-3p combined with FTX and SIVA1. Finally, Western blot (WB) was used to detect the change of SIVA1 protein expression by miR-215 mimic.

RESULTS: We found that the expression of FTX in tumor tissues of 71 GC patients was higher than that in paracancerous tissues, and the prognosis of patients with high FTX was poor. The expression of FTX in gastric cancer cells was higher than that in normal human gastric epithelial cells (GES-1). Transferring overexpression plasmid of FTX into gastric cancer cells (MGC-803 and SGC-7901) promoted cell proliferation and the ratio of cells in G0-G1 phase was decreased. Transferring si-FTX to MGC-803 and SGC-7901 led to opposite results. There was a negative correlation between the expression of mi215-3p and FTX in MGC-803 and SGC-7901 gastric cancer cells, and luciferase results showed that mi215-3p could directly bind to FTX and regulate cell growth and cell cycle changes. In addition, luciferase results showed that mi215-3p could bind directly to SIVA1. What's more, RT-qPCR and WB results showed that mi215 mimic could promote the expression of MGC-803, SGC-7901 SIVA1mRNA and protein.

CONCLUSIONS: According to these results, this study revealed that the previously neglected FTX-miR2153p-SIVA1 regulatory axis for the regulation of gastric cancer progression, which may be a potential target for the treatment of gastric cancer.

Key Words:

LncRNA FTX, MiR-215, SIVA1, Progression and metastasis, Gastric cancer.

Abbreviations

WB = Western Blot, BSA = bovine serum albumin, DMEM = Dulbecco's Modified Eagle's Medium, GC = gastric cancer, FBS = fetal bovine serum, RT-qPCR = reverse transcriptase-polymerase chain reaction, PBS = phosphate buffered saline, miRNA = MicroRNA, OS= overall survival.

Introduction

Gastric cancer (GC) is one of the most common, aggressive and malignant human tumors and has high mortality worldwide[1]. With the development of medical technology, the prognosis of GC patients has been improved; however, most GC patients have entered an advanced stage at the time of definite diagnosis, and their 5-year survival rate is significantly lower than that of early GC. At present, the treatment of patients with early gastric cancer is mainly a surgical treatment. However, chemotherapy is the primary choice for advanced patients, but the therapeutic effect is poor due to the influence of tumor metastasis and drug resistance. Therefore, it is very important to know the pathogenesis of GC at the genetic and molecular levels for preventing and treating GC[2,3].

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Long non-coding RNAs (lncRNAs), longer than 200 nucleotides, and microRNAs (miRNAs), non-coding RNA (19-25 nucleotides), have recently received great attention in revealing the complex mechanisms which can regulate many cells progresses, such as the proliferation, differentiation, and apoptosis[4-7].

FTX is a highly conserved lncRNA located at the X-chromosome inactivation (XCI) center[8]. In the past studies, it has been found that FTX is related to the occurrence of a variety of tumors, and the expression and function are different in different tumors[9,10]. Zhang et al[11] demonstrated that the expression of FTX increased in gliomas and could promote the growth of gliomas by inhibiting the expression of miR-342-3p and regulating the expression of AEG-1 (Astrocyte elevated-gene-1). Liu et al[12] found that the expression of FTX decreased in HCC tissues and FTX inhibited Wnt/ β-catenin signal to inhibit epithelial-stromal transformation and invasion. In addition, FTX combined with MCM2 inhibited the proliferation of HCC cells. He et al[13] observed that the expression of FTX was increased in renal cell carcinoma and sh-FTX could arrested the cell cycle of A498 and ACHIN in G0/G1 phase. Yang et al[14] detected that FTX is increased expression of FTX in colorectal cancer, which can be competitively combined with miR215 to promote the progression of colorectal cancer.

In this study, we aimed to examine the expression and function of FTX in the pathogenesis of GC, as well as to disclose molecular mechanisms. Firstly, we measured the expression of FTX in 71 GC patients' tissues. Then, proliferation ability was tested by CCK-8 and flow assay, through loss- and gain-of function approaches in MGC-803, SGC-7019. After that, the expression of SIVA1 was measured[15]. Our results revealed a critical function of lncRNA FTX in GC.

Patients and Methods

Patients

71 sample of GC tissue samples and their paracancerous tissue samples were collected by surgical resection at the First Hospital Affiliated to Army Medical University (Chongqing, China) from April 2012 to March 2013. The tissue samples were stored at liquid nitrogen. All of the patients were well informed and signed informed consent. The ethical consent of this study was granted from the Committees for Ethical Review of Research involving Human Subjects of our hospital.

Cell Culture

Normal human gastric epithelial cells (GES-1) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human GC cell lines AGS, MGC-803, SGC-7019, and NCI-N87[16,17] cells were purchased from the Cell Resource Center, Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). AGS cells were cultured in F12 medium (Gibco Rockville, MD, USA); AGS, MGC-803, SGC-7019, and NCI-N87. All media were supplemented 10% fetal bovine serum (FBS; Gibco), penicillin (100 U/ml), and streptomycin (100 μg/ml), and all cells were incubated at 37°C in 5% appropriate incubator.

RNA Extraction and Real-time Quantitative PCR Assays

Total RNAs from the GC cells and clinical samples were extracted by using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol. Reverse transcription polymerase chain reaction (RT-qPCR) was performed using PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's protocol. The levels of mRNA expression were quantified by standard Real-time PCR protocol with SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan). Reactions and signal detection were measured using a real time PCR system (Bio-Rad, Hercules, CA, USA). GAPDH was used as a reference gene. The gene specific primers were as Table I.

Construction of Plasmid, siRNA and Cell Transfection

We insert the full length of human FTX cDNA into pcDNA3.1 (Invitrogen) vector to getting FTX-pcDNA3.1 for overexpression. For small interfering RNAs (siRNAs) analysis, three FTX siRNAs and negative control siRNA (si-NC) were provided by Invitrogen (Carlsbad, CA, USA), and the siRNA sequences targeting the sequence of FTX transcript were as follows[12]: FTX-siRNA1: 5'-GGAACAGGUAUCUCAGGAU3-', FTX-siRNA2: 5'-CCGAUCUGAAUGCCUUUAA-3'. We planted the cells into 6-well plate, when the confluence reached 40-60%, the cells were transfected with overexpression plasmid or siRNA by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocols. Puromycin (2 μg/

Table I. Primers for selected genes.

Gene name	Primers	
	Sense	Antisense
lnc FTX	GAATGTCCTTGTGAGGCAGTTG	TGGTCACTCACATGGATGATCTG
miRNA-215-3p	TGGATTTGGACGCATTGGTC	TTTGCACTGGTACGTGTTGATA
GAPDH	TGACGTGCCGCCTGGAAAC	CCGGGCATCGAAGGTGGAAGAG
SIVA1	CCTCCGAAGCTGACCCATCTG	ATGTCACTGCAGTCCACGAG
U6	CGCTTCGGCAGCACATATACT	CGCTTCACGAATTTGCGTGTC

mL) were used to select stably transfected cells. At indicated time point post the transfection, cells were harvested for further analysis.

Flow Cytometric Analysis and Cell Cycle

The treated MGC-803 and SGC-7019 cells were treated with trypsin and washed by PBS for twice. Next, precooled 75% ethanol was used to fixed at 4°C for more than 4 h. Then the pellets were washed by cool PBS for twice. The cell pellet was stained with FITC-Annexin V and Propidium Iodide (PI), and flow cytometry was conducted within 5 min. The images were obtained using a FACSCalibur system (BD Biosciences, Franklin Lakes, NJ, USA) equipped with a CellQuest software (BD Biosciences)[18].

CCK8 Assays

The cells were inoculated into 96-well plate with 2000 cells/well and cultured at 37°C with 5% CO_2 for 0, 24, 48, 72 h, and there were 3 repeated holes in each group. The determination of CCK8 was as follows: the 10 μl cell counting kit 8 (Dojindo, Molecular Technologies, Kumamoto, Japan) was added to 100 ul of the Dulbecco's Modified Eagle's Medium (DMEM) in each well and co-cultured in the darkness at 37°C for 2 hours. The 96-well plate was placed at an absorbance of 450 nm[19]. The whole experiment was repeated three times.

Luciferase Activity

Wt-FTX/mut-FTX sequences were inserted into the firefly luciferase in basic pGL3 control vector (Promega, Madison, WI, USA)[20]. MGC-803 and SGC-7019 cells were cultured overnight after being seeded into a 24-well plate, co-transfected with the Wt-FTX/mut-FTX reporter gene plasmid and miR-215-3p mimics or miR-NC. Renilla expression vector was transfected into each group to serve as a normalized control. After transfected for 48 h, Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to measure both firefly and renilla luciferase activities. Data were normalized against the activity of the renilla luciferase.

Western Blot

The total cell lysate was prepared by destroying cells with RIPA buffer containing protease and phosphatase inhibitor cocktail (Thermo-Fisher Scientific, Waltham, MA, USA) and centrifuged with 12000 rpm at 4°C for 10 minutes. The concentration of protein was determined by BCA protein analysis reagent (Thermo-Fisher Scientific). The same amount of protein samples was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). Anti-SIVA1 and anti-β-actin are purchased from Abcam (Cambridge, MA, USA, dilution at 1:1,000). HRP (horseradish peroxidase) coupled with secondary antibody (Abcam, dilution at 1:1,0000) was used for the detection of primary antibody. ECL chemiluminescence system (Thermo-Fisher Scientific) was used to visualize binding antibodies.

Statistical Analysis

Values were expressed as the mean ± SD. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) were used for statistical assay. The significance between groups was analyzed by Student's *t*-test. p value <0.05 was considered statistically significant.

Results

Long Non-Coding RNA FTX Was Upregulated in Gastric Cancer and Predicted Poor Prognosis

Firstly, we detected the expression of FTX in tumor and paracancerous tissues of 71 gastric

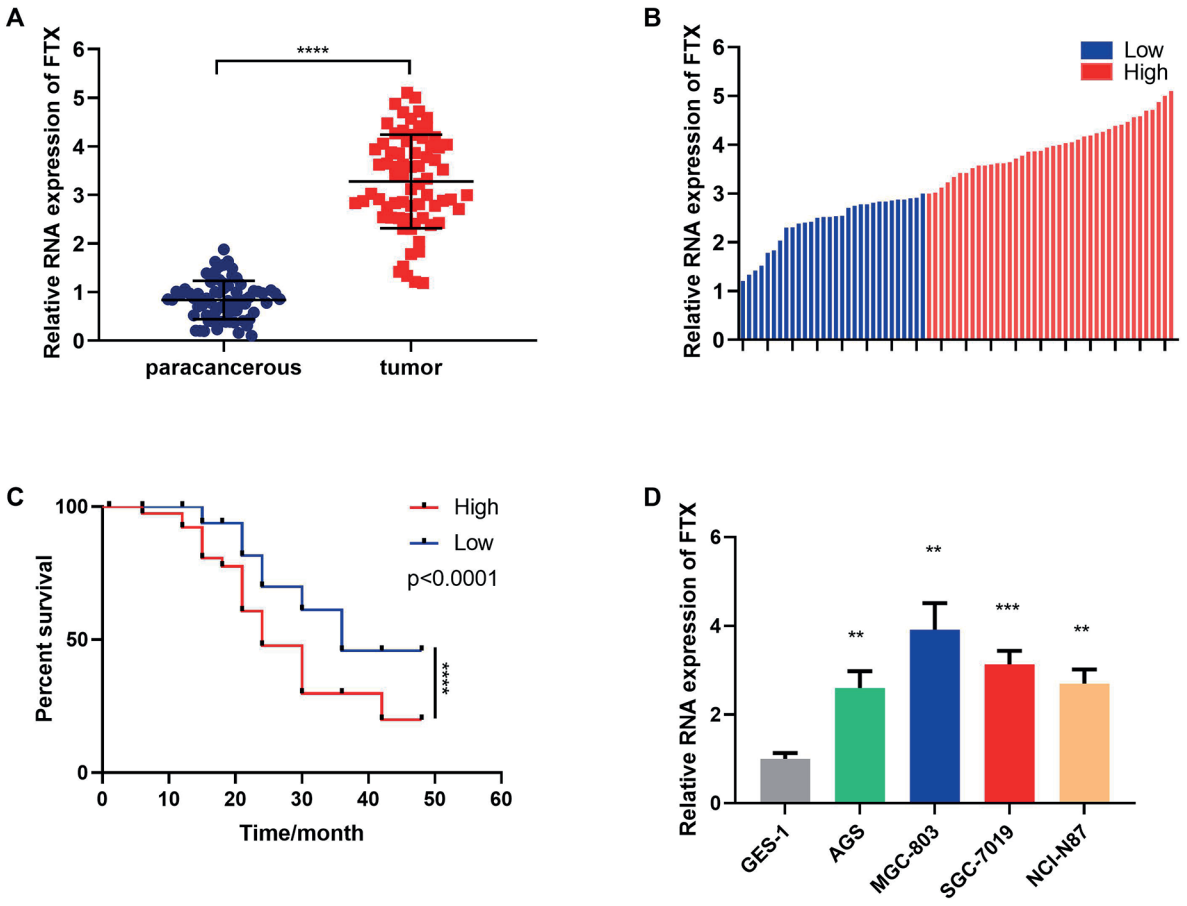

Figure 1. LncRNA-FTX is a poor prognostic indicator of GC. **(A)** The relative expression of lncRNA-FTX in human GC tissues (n = 71) and Para cancerous tissues (n = 71). The relative expression levels were normalized to the mean value of all patients. **** p<0.0001. **(B)** The relative expression of lncRNA-FTX in GC cancer tissues (n = 71). The relative expression of lncRNA-FTX was detected by RT-qPCR and normalized to GAPDH expression. **(C)** The differences survival curves between GC patients analyzed by Kaplan–Meier. Percent survival for patients expressing high and low levels of lncRNA-FTX. p-values were determined by the log-rank test. (**** p<0.0001). **(D)** The relative expression of lncRNA-FTX in different GC cell lines (AGS, MGC-803, SGC-7019, NCI-N87) detecting by RT-qPCR and a normal human gastric epithelial cell (GES-1) line (*** p<0.001, ** p<0.01 *vs.* GES-1 cell).

cancer patients by RT-qPCR. The expression of FTX in GC patients was significantly higher than that in paracancerous tissues (Figure 1A). According to the expression of FTX in tumor, the patients were divided into the group of low FTX (n = 32), using the mean expression level of FTX as the cut-off value, and the group of high FTX expression (n = 39). The difference of clinicopathological parameters between the high FTX expression group and low FTX expression group were statistically analyzed by Chi-square test (Table II). Clinicopathological analysis showed that FTX expression level was correlated with the progression of tumor invasion and TNM stage. Furthermore, the GC patients with high FTX expression had a poorer overall survival when compared with that in the group of low FTX expression (Figure 1C). In addition, we detected the expression of FTX in four common gastric cancer cells (AGS, MGC-803, SGC-7019, SNI-N87) and a kind of human gastric mucosal epithelial cells (GES-1) by RT-qPCR (Figure 1D), which suggested an increased expression of FTX in gastric cancer cells. These results suggest that the expression of FTX increases in GC tissues and cells.

Lnc FTX Regulated the Proliferation and Cell Cycle of GC Cells

To further research the role of FTX in gastric cancer, we studied the function of FTX in cell proliferation and cell cycle. The overexpression

plasmid was transfected into MGC-803, SGC-7019, both of which are common gastric cancer cell lines, and construct stable cell lines. Compared with the control group, the expression level of FTX in MGC-803, SGC-7019 transfected overexpression plasmid was significantly up-regulated (Figure 2A). When MGC-803, SGC-7019 transfected with si-FTX1 and siFTX-2, the expression level of FTX was decreased, and the efficacy of si-FTX1 was better, so in the next experiment we chose si-FTX1 as target (Figure 2B). We aimed to detect the proliferation and cell cycle changes of MGC-803, SGC-7019 cells by CCK8 which transfected with siRNA and overexpression plasmid. Compared with the control group, we found that siRNA could reduce the proliferation ability of cells, while the overexpression of FTX could increase the proliferation ability (Figure 2C, D). Then the cell cycle distribution of MGC-803, SGC-7019 cells was detected by flow cytometry. We found that compared with the control group, the expression of siRNA inhibited the growth of cells by preventing the cells from being in the G1-S phase, and the overexpression of FTX could reduce the distribution of cells in the G1-S phase (Figure 2E, F). When gastric cancer occurs, the increased expression of FTX can promote the proliferation of cells.

Targeting Association between lnc FTX and miR-215-3p in GC Cells

In the past research, it has been found that miR215 is involved in the regulation of a variety of tumors, and the expression is different in distinct tumors[21-23]. We speculate whether the regulation of tumor by FTX is related to miR215, so we detected the expression of miR215-3p in tumor and paracancerous tissues of 71 gastric cancer patients by RT-qPCR. As shown in Figure 3A, the expression of miR-215-3p in GC patients was significantly lower than that in paracancerous tissues. A significant negative correlation was observed between the relative expression of FTX and miR-215-3p in GC tissues (Figure 3B). Bioinformatics analysis was performed to identify miR-215-3p targeted by FTX, and it was indicated that miR-215-3p and FTX had complementary binding sites (Figure 3C). The expression level of miR-215-3p in MGC-803 and SGC-7019, transfected with siRNAs or overexpression FTX plasmid, was significantly increased or decreased and detected by RT-qPCR (Figure 3D). To further investigate the association of miR-215-3p and FTX, a wt-FTX luciferase reporter vector (wt-FTX), and a mut-FTX 3'UTR luciferase reporter vector (mut-FTX) were constructed (Figure 3C). Then, we conducted a luciferase reporter assay. Compared with other

Table II. Association between FTX expression and clinicopathological characteristics of gastric cancer patients.

Parameter	FTX levels			*p*-value
	Cases (n=71)	Low (n=39)	High (n=32)	
Age(years)				
≤ 60	27	16	11	0.6285
> 60	44	23	21	
Sex				
Male	39	22	17	0.8145
Female	32	17	15	
Tumor size (cm)				
≤ 5	36	21	15	0.6365
> 5	35	18	17	
Tumor differentiation				
Well/moderate	41	28	13	0.0151*
Poor	30	11	19	
Clinical stage				
I-II	27	21	6	0.0032*
III-IV	44	18	26	
Node metastasis				
Presence	46	21	25	0.0461*
Absence	25	18	7	

The statistical significance of difference was measured by Pearson's χ^2 test. *p<0.05.

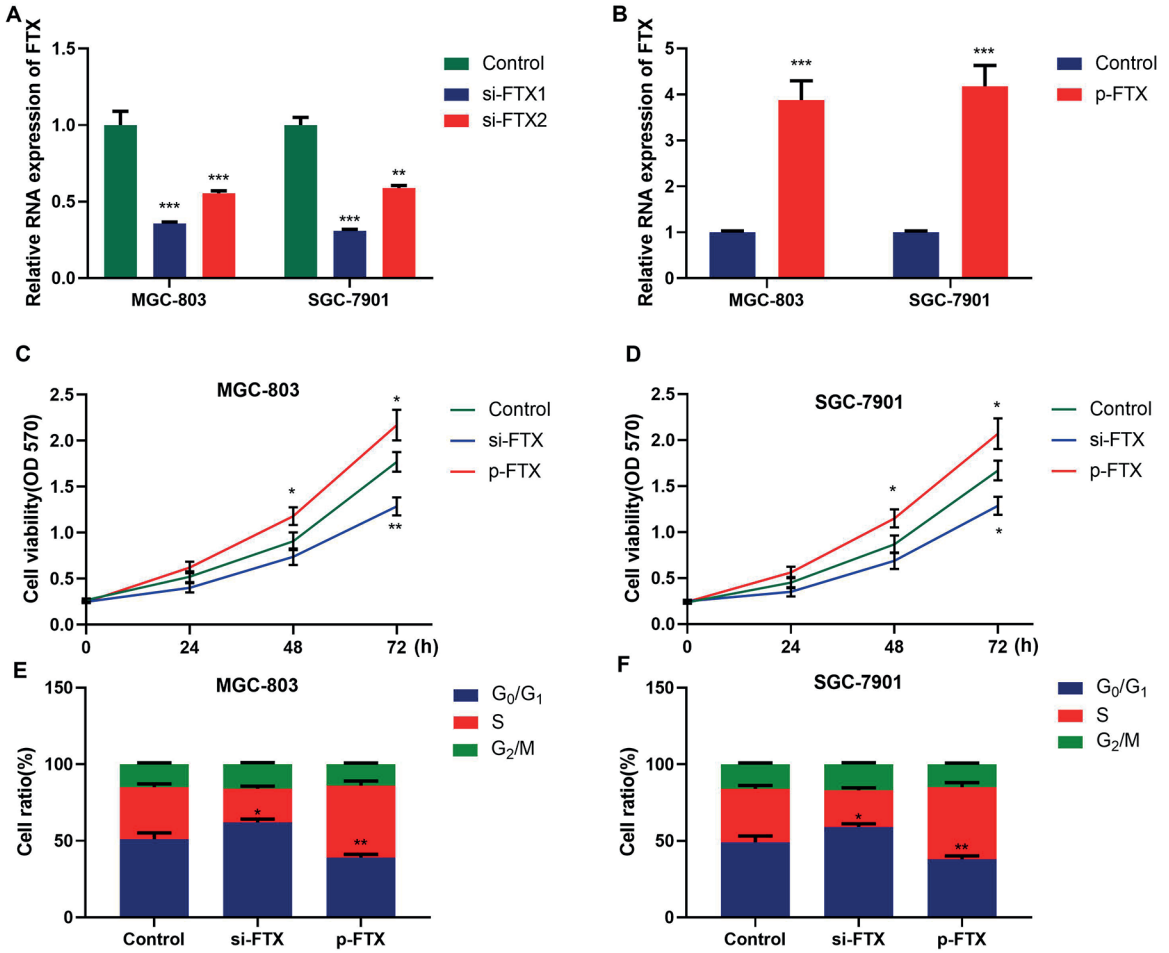

Figure 2. Silencing/overexpressing the expression of the lncRNA-FTX inhibits / promotes the progression of GC. **(A) (B)** The relative mRNA expression levels of lncRNA-FTX in MGC-803 and SGC-7901 cells transfected with siRNA and overexpression plasmids. ** $p<0.01$, *** $p<0.001$.**(C) (D)** The proliferation and viability of MGC-803 and SGC-7901 cells were detected by the Cell Counting Kit-8 (CCK-8) colorimetric assay after lncRNA-FTX knockdown or overexpressed, ** $p<0.01$, * $p<0.05$. **(E) (F)** The cycle distribution of the MGC-803 and SGC-7901 cells, treated with siRNA and overexpression plasmids, were assessed by flow cytometry. ** $p<0.01$, * $p<0.05$.

groups, co-transfection with miR-215-3p mimic and wt-FTX significantly decreased the luciferase activity of MGC-803 and SGC-7019 cells (Figure 3E and 3F). The data revealed that miR-215-3p could directly bind to FTX binding sites.

MiR-215 Regulated the Proliferation and Cell Cycle of GC Cells

To further study the role of miR-215-3p in gastric cancer cells, we studied the role of miR215-3p in cell proliferation and cell cycle. MGC-803 and SGC-7019 cells were co-transfected with NC siRNA and NC inhibitor (siNC + NC in group), NC siRNA and miR-215 inhibitor (siNC+miR-215 in group), FTX siRNA and NC inhibitor (siFTX+NC in group), or with FTX siRNA and miR-215 inhibitor (siFTX+miR-215 in group). The expression of miR215, cell reproduction and cell cycle in the above four groups were detected by RT-qPCR, CCK-8 and flow cytometry, respectively. As shown in Figure 4A, both in MGC-803 and SGC-7019 cells, the expression levels of miR-215-3p were markedly lower in the siNC+miR-215 in group, while they were higher in the siFTX+NC group, when compared with those in the siNC+NC in group. CKK-8 assay further implied that the proliferation of MGC-803 and SGC-7019 cells was significantly reduced in the siFTX+NC in group when compared with those in the siNC+NC in group, while these effects were rescued in the siFTX+miR-215-3p in group (Figure 4B and 4C). Then the cell cycle distribution of MGC-803, SGC-7019 cells were detected by flow cytom-

etry. We found that compared with the control group, the expression of siNC+miR215 in group inhibited the growth of cells by preventing the cells from being in the G1-S phase, and the overexpression of FTX could reduce the distribution of cells in the G1-S phase (Figure 4D and 4E). These results suggested that FTX regulate GC cell proliferation by binding miR-215-3p.

MiR215 Regulated Cell Proliferation and Cell Cycle by miR-215/SIVA1

To further explore the potential mechanism of how miR-215-3p inhibits the growth and cell cycle of gastric cancer cells, the online target prediction algorithm TargetScan predicted the potential targets of miR-215-3p. SIVA1 is a potential target for miR-215, which contains a hypothetical

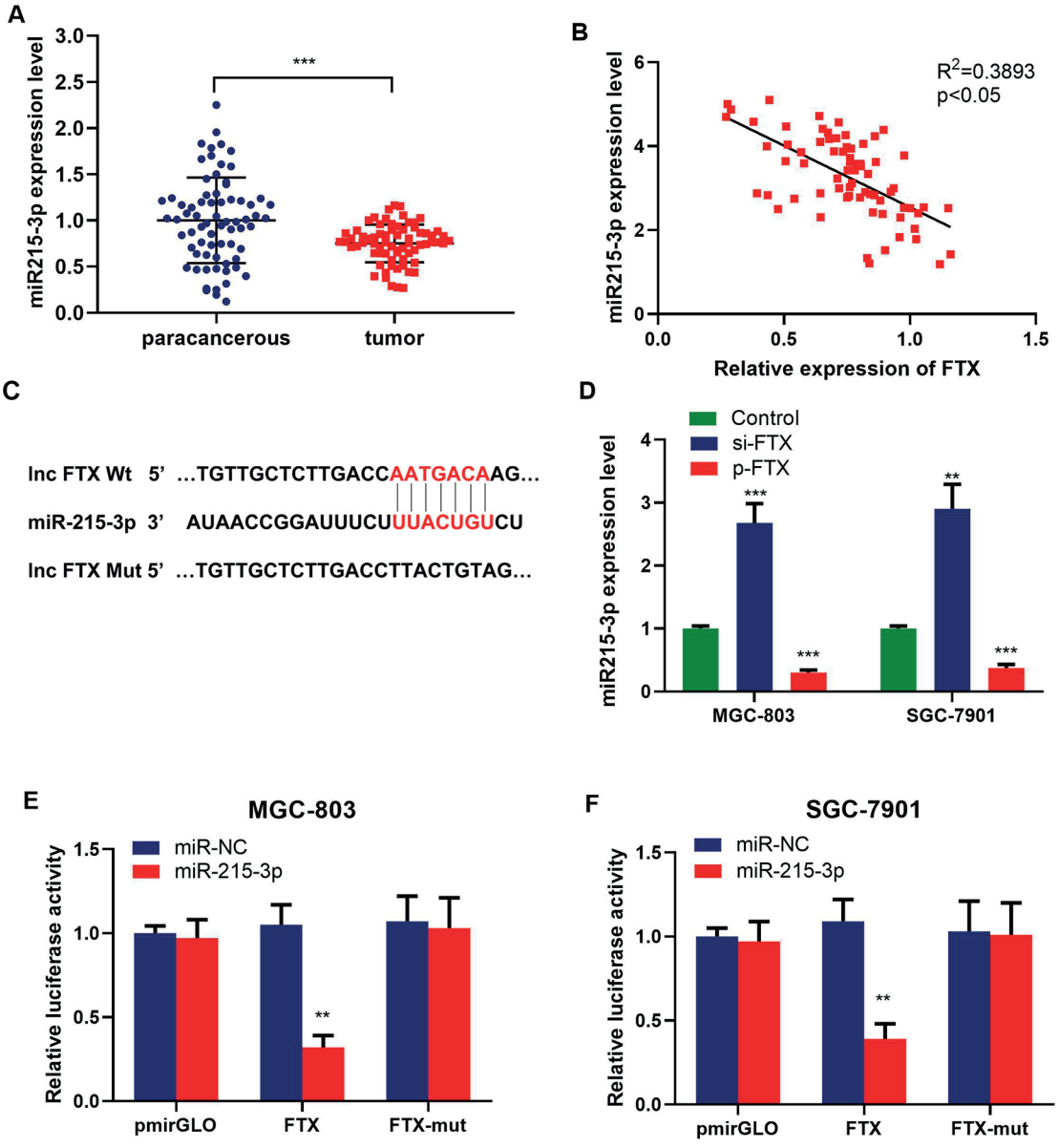

Figure 3. lncRNA-FTX can directly binding to miR-215-3p in GC cells. **(A)** The relative expression of miR215-3p in human GC tissues (n = 71) and Para cancerous tissues (n = 71). The relative expression levels were normalized to the mean value of all patients. *** $p<0.001$. **(B)** The negative correlation between the relative expression of miR-215-3p and the FTX. **(C)** The predicted binding sites between lncRNA-FTX and miRNA-215-3p. **(D)** The relative expression of lncRNA-FTX in the MGC-803 and SGC-7901 cells, treated with siRNA and overexpression plasmids, detected by RT-qPCR and normalized to U6 expression. ** $p<0.01$, *** $p<0.001$. **(E)(F)** The luciferase reporter assay. Co-transfection with miR-215-3p and FTX-Wt significantly increased the luciferase activity of MGC-803 and SGC-7901 cells compared with others group. * $p<0.05$.

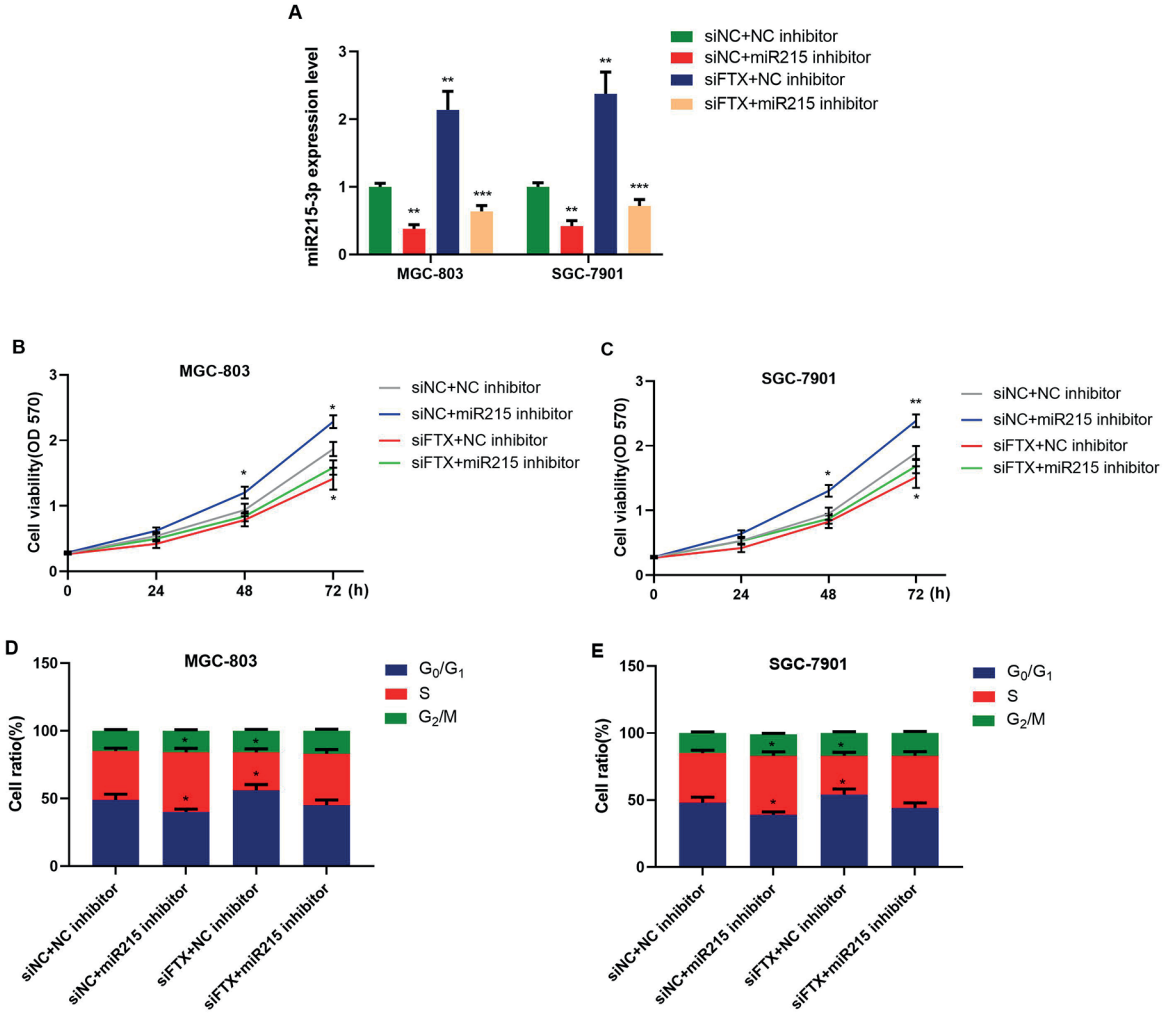

Figure 4. miR-215 can regulate the proliferation and cell cycle of GC cells. MGC-803 and SGC-7901 were co-transfected with NC siRNA and NC inhibitor (siNC+NC in), NC siRNA and miR-215 inhibitor (siNC+miR-215 in), FTX siRNA and NC inhibitor (siFTX+NC in), or with FTX siRNA and miR-215 inhibitor (siFTX+miR-215 in). (A) The relative expression of miR215-3p in treated MGC-803 and SGC-7901 cells post transfection. The relative expression levels were normalized to the mean value of all patients. ** $p<0.01$, *** $p<0.001$. (B) (C) The proliferation and viability of MGC-803 and SGC-7901 cells were detected by the Cell Counting Kit-8 (CCK-8) colorimetric assay, compared with the group of siNC+NC. ** $p<0.01$, * $p<0.05$. (D) (E) The cycle distribution of the MGC-803 and SGC-7901 cells were assessed by flow cytometry, compared with the group of siNC+NC. * $p<0.05$.

8bp binding site (Figure 5A). In the past, SIVA1 was considered to be a p53 target gene essential for p53-dependent apoptosis[24], although it may also promote cell proliferation by inhibiting p53 in some cases. Van Nostrand et al[25] found that the role of SIVA is independent from p53 but regulates tumor growth and metabolism through the mTOR pathway. Therefore, SIVA1 can promote tumorigenesis in a manner independent of p53.

In order to verify whether miR-215 binds directly to SIVA1, we cloned the wild type 3'UTR (wt-SIVA1) of SIVA1 and the mutant 3'UTR (mut-SIVA1) of SIVA1 into the reporter plasmid. Then Wt-SIVA1 vector (or Mut-SIVA1 vector) and miR-215 mimic (or miR-CTR) were co-transfected into MGC-803 and SGC-7019 cells. The results (Figure 5B) showed that the luciferase activity in wt-SIVA1+miR-215 group was significantly low-

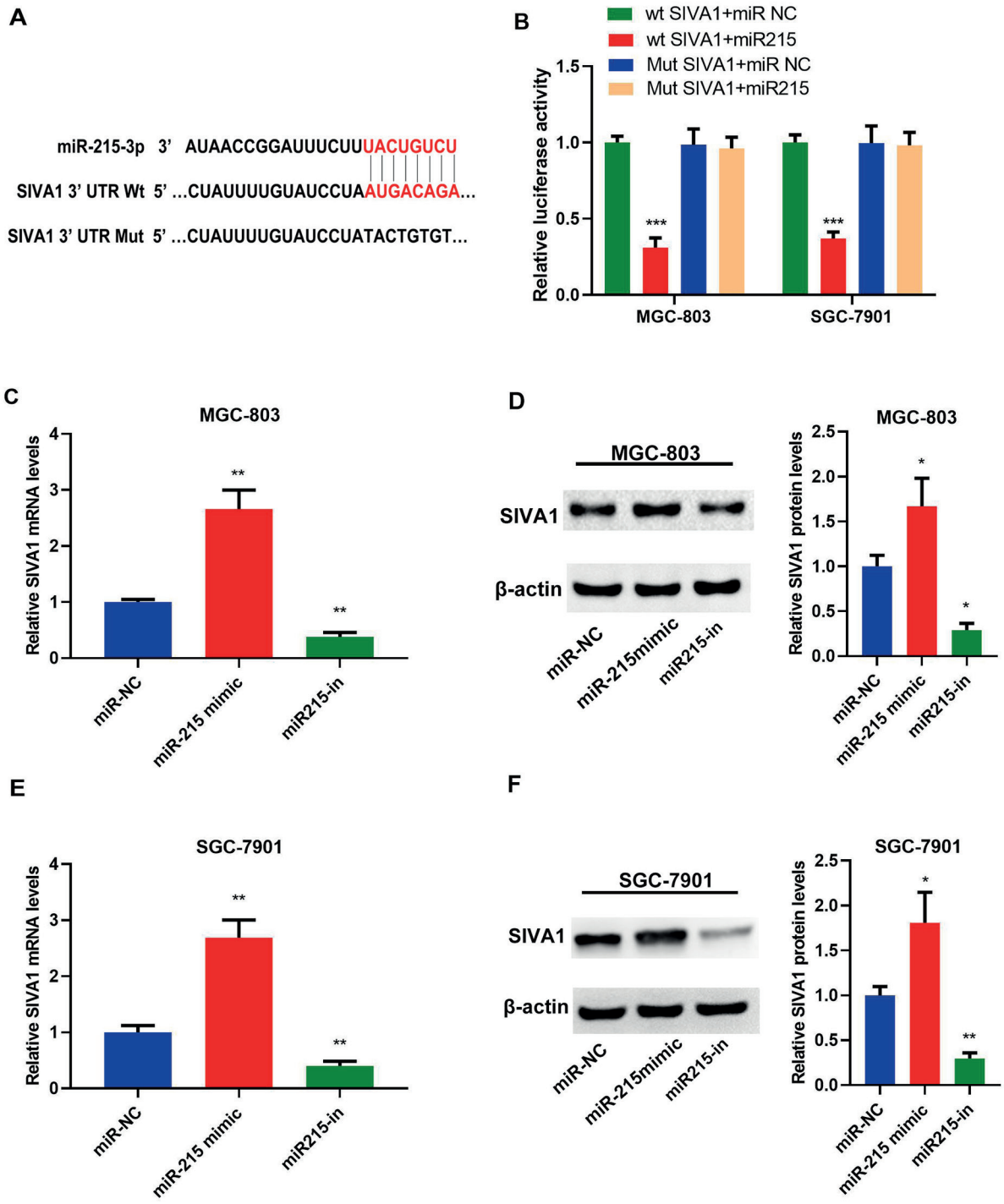

Figure 5. miR-215-3p can directly binding to SIVA1 in GC cells. **(A)** The 3'UTR of SIVA1 possessing wild type or mutant binding site of miR-215. **(B)** The luciferase activity of vectors with wild type or mutant SIVA1 3'UTR was measured after transfection with miR-215 mimic or miR-NC in MGC-803 and SGC-7901 cells. *** $p<0.001$. **(C)** The relative expression of SIVA1 mRNA was significantly increased by miR-215 inhibitor and decreased by miR-215 mimic in MGC-803 cells. ** $p<0.01$. **(D)** The expression of SIVA1 was measured by WB and the expression level of SIVA1 protein was significantly increased by miR-215 inhibitor and decreased by miR-215 mimic in MGC-803 cells. ** $p<0.01$. **(E)** The relative expression of SIVA1 mRNA was significantly increased by miR-215 inhibitor and decreased by miR-215 mimic in SGC-7901 cells. ** $p<0.01$. **(F)** The expression of SIVA1 was measured by WB and the expression level of SIVA1 protein was significantly increased by miR-215 inhibitor and decreased by miR-215 mimic in SGC-7901 cells. ** $p<0.01$.

er than that in the control group ($p < 0.0001$), but the inhibitory effect of miR-215 mimic was eliminated by Mut- SIVA1 vector (Figure 5B). Furthermore, we further demonstrated the effect of miR-215 on SIVA1 in human gastric cancer cells. miR-215 mimic simulation could significantly decrease the expression of mRNA of SIVA1 and protein of SIVA1 cells and miR-215-inhibitor (miR215-in) caused reversed affection in MGC-803 (Figure 5C and 5D) and SGC-7901 (Figure 5E and 5F). These results show that miR-215 can bind directly to SIVA1 and regulate the expression of SIVA1.

Discussion

LncRNA is more than 200 nucleotides in length, does not express proteins, and is expressed at specific stages of disease, tissue, or development. These ncRNAs disorders may also affect the regulation of eukaryotic genomes and provide cell growth advantages, leading to progressive and uncontrolled tumor growth. Therefore, lncRNA may provide a new direction for cancer research.

In this paper, we detected the expression of FTX in gastric cancer specimens and surrounding non-tumor tissues. We also determined the function of FTX in gastric cancer cells by using the method of acquisition and loss of FTX expression. The results showed that the expression of FTX in gastric cancer tissues was higher than that in adjacent normal gastric tissues, and the prognosis of GC patients with high expression of FTX was poor. When the overexpression plasmid of FTX and si-FTX was transfected into MGC-803, SGC-7019 cells, the result showed that FTX could promote the growth of gastric cancer cells, and si-FTX could capture gastric cancer cells in G0-G1 phase.

In order to further study the mechanism of FTX in gastric cancer cells, we predicted the possible target binding of FTX, in which miR215-3p is one of the potential targets. It has been found that miR215 is involved in the metabolism of many kinds of tumors[26,27]. Liu et al[28] found that mi215-3p inhibits the growth and metastasis of cervical cancer cells by targeting SOX9. Others found that lncRNA-CDC6 competitive binding to miR215 promoted breast cancer progression[29]; Yang et al[14] found that lncRNA FTX sponges miR-215 and inhibits vimentin phosphorylation promoting the progression of colorectal cancer. In this paper, we found that the expression of FTX in gastric cancer cell line MGC-803, SGC-7019 was negatively correlated with miR215-3p, and miR215-3p could bind directly to FTX. After MGC-803, SGC-7019 cells were treated with miR215-mimic, the growth of the two kinds of cells was inhibited. In addition, we predict the potential target of miR215-3p, and find that SIVA1 is the potential target. SIVA1 can promote tumorigenesis in a manner independent of p53[25]. We confirmed that miR215-3p could bind directly to SIVA1 by double luciferase report, and the expression of SIVA1[30] increased after MGC-803, SGC-7019 was treated with miR215- inhibitor. These data also indicate that there might be FTX/miR215-3p/SIVA1 axis in MGC-803, SGC-7019. The FTX served as ceRNA via sponging miR-215-3p, and thereby upregulating the expression of SIVA1 to promote the progression in GC.

So far, we have found that FTX can reduce the expression of SIVA1 and promote the proliferation of gastric cancer cells by competitive binding to miR215-3p during the occurrence of gastric cancer[31].

Conclusions

These results suggested that the expression of lncRNA FTX was increased in patients with gastric cancer and FTX was an important prognostic factors of GC patients. What's more, our findings firstly revealed that lncRNA FTX could promote the progression and metastasis of gastric cancer through miR-215-3p. The present data elucidate a potential mechanism underlying the tumor-oncogenic role of FTX in GC and indicate that lncRNA FTX might be used as a promising prognostic marker and a potential target.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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