



FJX1 Functions as an Oncogene in MGC-803 Cells and Correlated with Poor Prognosis of Gastric Cancer Patients

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Abstract

FJX1 (Four-Jointed Box kinase 1) plays an important role in the development of vertebrate systems. Emerging studies have reported that FJX1 was aberrantly expressed in several human malignant tumors. However, the function of FJX1 in Gastric Cancer (GC) has not been well characterized. In this study, we found that the expression of FJX1 was upregulated in 100 human GC tissues than that of the adjacent normal tissues by using IHC and qRT-PCR analysis. Moreover, survival analysis showed that the high FJX1 expression group had a poorer 5-year Overall Survival (OS) rate than that of the low FJX1 expression group, which was consistent with the TCGA database. *In vitro*, the function of FJX1 in diverse aspects of the MGC-803 human GC cells was systematically elucidated. The enforced introduction of FJX1 into the MGC-803 cells significantly promoted cell growth due to the increased cellular proliferation and decreased cellular apoptosis, which were detected by CCK-8, colony formation and TUNEL assays. Besides, the overexpression of FJX1 markedly promoted the migratory and invasive abilities of the MGC-803 cells, which were detected by wound healing and transwell invasion assays. At the same time, silencing FJX1 by RNA interference experiments in the MGC-803 cells showed the opposite results. Taken together, our data suggested that FJX1 might play an oncogenic role in GC progression, and the underlying mechanism is currently ongoing.

Keywords: Gastric cancer; Four-Jointed Box kinase 1 (FJX1); Oncogene; Overall survival

Introduction

Gastric Cancer (GC) is the fourth most common cancer types of cancer and the third leading cause of cancer-related mortality worldwide [1]. There are estimated 26,500 new cases of GC and 11,130 related deaths in the US in 2023 [2]. Because of the advancements in early detection of diagnosis and Multi-Disciplinary Treatment (MDT) for GC, the incidence and mortality of GC were obviously declining over the past 5 decades. However, a significant proportion of patients were firstly diagnosed at an advanced stage, and the 5-year survival rate of GC was still under 25% for stage III to IV disease [3,4]. GC is a complex, multistep and multifactorial process involving multiple genes mutations, signaling pathways abnormalities and biological changes. Therefore, it is crucial to identify new therapeutic targets for improving prognosis and efficient therapy for GC patients.

Four-Jointed Box kinase 1 (FJX1) was a Golgi secretory pathway kinase that was homologous to the *Drosophila* four-jointed (fj) kinase [5]. In *Drosophila*, fj, a transmembrane type I glycoprotein, was necessary for regulation of Planar Cell Polarity (PCP) [6]. The mutation of the *Drosophila* fj gene caused reduced and altered growth of the eye, leg and wing along the dorsal-ventral axis [7]. Recent studies had reported that FJX1 was abnormally expressed in a variety of diseases including cancer. FJX1 was overexpressed in eutopic endometrium from women with endometriosis and high levels of FJX1 proteins might play an important role in the pathogenesis of endometriosis [8]. FJX1 had also been reported to play important roles in several types of human malignancies such as nasopharyngeal carcinoma [9], melanoma [10], colorectal cancer [11], lung cancer [12] and head and neck cancer [13]. However, the potential functions of FJX1 in gastric cancer are still unclear.

In our study, firstly we assessed the FJX1 expression pattern between gastric tumor tissues and the adjacent normal tissues based on The Cancer Genome Atlas (TCGA) database. Besides, Overall Survival (OS) curves were also plotted according to the FJX1 expression using the Kaplan-Meier method. To verify the TCGA results, we collected 100 paired GC clinical samples and detected the

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expression of FJX1 between the gastric tumor tissues and adjacent normal tissues by qRT-PCR and IHC analysis, and analyzed its potential association with the clinicopathologic features of GC patients. Moreover, we successfully established FJX1-overexpressing and FJX1-silencing MGC-803 human gastric cancer cell lines, and the effects of FJX1 on diverse aspects of cancer-related biological behaviors, including cellular proliferation, apoptosis, migration and invasion, was systematically examined.

Materials and Methods

Bioinformatic analysis

For the RNA-Seq data, transcriptomic data of GC tissue samples and matched adjacent normal samples were downloaded from The Cancer Genome Atlas (TCGA) database (<https://tcga-data.nci.nih.gov/tcga/>). The mRNA expression level of FJX1 between GC tumor tissues and the adjacent normal tissues were analyzed and a box-and-whisker-plots was performed to describe the differences. To validate the prognosis value of FJX1 in GC patients, the patients in TCGA were classified into low- and high-expression groups based on the median value. Overall Survival (OS) curves were plotted on the basis of the Kaplan-Meier survival analysis which was performed for the patients in different groups.

Clinical samples

Tumor tissues and the corresponding adjacent normal tissues were obtained from the 100 GC patients at the Department of General Surgery, the First Affiliated Hospital of Soochow University, from 2015 to 2017. The pathologic classification and stage were verified by histological methods according to the World Health Organization (WHO) classification of gastric carcinoma [14] and the classification of Gastric Carcinoma (third edition) of the Japanese Gastric Cancer Association [15]. All patients had an accurate pathology diagnosis and underwent surgery without chemotherapy or radiotherapy before operation. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University.

Immunohistochemistry (IHC)

The tissue IHC was performed using a standard peroxidase-based staining method. Paraffin-embedded sections (4 µm thick) sections were deparaffinized with xylene and rehydrated with ethanol. Then antigen retrieval was performed in citrate buffer (pH 6.0) for 10 min at 90°C. To block endogenous peroxidase activities, the sections were treated with 3% hydrogen peroxide (H₂O₂) for 30 min. The slides were blocked with 5% bovine serum albumin (BSA; Boster Bioengineering, Wuhan, China) for 30 min at room temperature. Then the slides were incubated with rabbit anti-FJX1 antibody (cat no. 17417-1-AP, Proteintech, Shanghai, China, 1:300 dilution) at 4°C overnight. After washing with 1x PBS, the slides were incubated at 37°C for 30 min with secondary antibody (GK500710, Genetech, Shanghai, China). Finally, the slides were treated with 3,3-Diaminobenzidine (DAB) and counterstained with hematoxylin for microscopic examination. The results were observed by two independent investigators under an Olympus microscope BX 51 (Olympus, Japan) and scored as follows: 0, No staining; 1+, light; 2+, moderate; 3+, strong, according to the intensity of the staining. The percentage of positively stained cells was scored as follows: 0, No staining; 1, <25% staining; 2, 26% to 50% staining; 3, 51% to 75% staining; and 4, >75% staining. The product of the intensity and extent grades ≥ 4 of positive cells was considered high expression, and the score of 0-3 of positive cells was regarded as low expression.

Cell culture

The human GC cell lines AGS, SGC-7901, MGC-803, BGC-823 and MKN-28 were obtained from the Central Laboratory, the First Affiliated Hospital of Soochow University (Suzhou, Jiangsu, China). All the cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, USA) supplemented with 10% Fetal Bovine Serum (FBS, Serana, Germany) and 1% penicillin/streptomycin (Beyotime, China). All the cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C.

Transfection

The FJX1 overexpression vector was constructed on the vector (PCDH-EF1-MCS-CMV-copGFP-T2A-puro) containing Green Fluorescent Protein (GFP; from Dr. Jie Wu, Department of Oncology, The First Affiliated Hospital of Soochow University, Suzhou, China). The MSCV-GFP-FJX1 or MSCV-GFP control plasmid was transfected into the Phoenix A packaging cells using FuGENE HD transfection reagent (cat. no. 04709705001; Roche, Shanghai, China). *Lentivirus* supernatants were collected and used to infect the MGC-803 cells. For obtaining stably expressing cell lines, the cells were selected in the presence of 5 µg/ml puromycin (cat. no. J593; Amresco, Beijing, China). To knockdown the expression of FJX1 in the MGC-803 cells, the siRNA-FJX1 plasmid or the siRNA-control plasmid was transfected into the MGC-803 cells using Lipofectamine 2000 (Thermo Fisher Scientific, USA). The siRNA was purchased from the Sangon Biotech Company (Shanghai, China). The oligonucleotide encoding sequences are as follows: siRNA-FJX1 5'-GACCTAGTACAATGGACCGACTTAA-3' and siRNA-control 5'-GACGAATACTAGGCAGCCATTCTAA-3'.

Quantitative real-time PCR (qRT-PCR)

Equivalent amounts of RNA (2 µg) were reverse transcribed with SuperScript M-MLV (Promega, China) according to the manufacturer's instructions. All qRT-PCR reactions were performed with a LightCycler 480 System (Roche) in triplicate. Primers were designed using Primer-BLAST (PubMed) and synthesized from Sangon Biotech (China). cDNA was amplified using 2X LC480 SYBR Green Master Mix (Roche) according to the manufacturer's protocol. For data analysis, a target gene transcript was quantified in comparison to the house keeping gene (GAPDH) as a reference. The primer sequences were as follows: FJX1 5'-ATGTGGGACAAGTATAACGAGC-3'(F) and 5'-CTTGGCCTTACAGTGCAAAT-3'(R); GAPDH 5'-GGAGCGAGATCCCTCCAAAAT-3'(F) and 5'-GGCTGTTGTCATACTTCTCATGG-3'(R).

Western blot analysis

Total proteins were prepared with Radio Immunoprecipitation Assay (RIPA) buffer (Beyotime, China) according to standard procedures. Protein concentration was quantified using a BCA protein assay kit (Beyotime, China). Proteins (20 µg) were fractionated on an 10% SDS-PAGE gel and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% milk in PBST for 1 h at room temperature, then incubated with primary antibodies (Abs) overnight at 4°C. After washing, the membranes were incubated with secondary Abs. Finally, proteins were detected using the Odyssey system (LI-COR Biosciences, Lincoln, NE, USA). The band density was normalized to GAPDH and quantified by the ImageJ software. Ab against FJX1 (cat no.17417-1-AP, 1:400 dilution) was purchased from Proteintech (Shanghai, China). Ab against GAPDH (cat no.5174, 1:1000 dilution) was purchased from Abgent (Suzhou, China). Secondary Abs (1:10,000 dilution) against IRDye 680CW

(926-32222) and IRDye 800CW (926-32210) were obtained from LI-COR Biosciences (Lincoln, NE, USA).

Cell proliferation assay

Cell proliferation assay was performed using a Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Japan) according to the manufacturer's instructions. Cells were seeded into 96-well plates (5×10^3 cells/well) and the cell proliferation assay was performed at different time points (0, 24, 48, 72, and 96 h). Briefly, 20 μ L of CCK-8 solution was added to each well and incubated for 1 h at 37°C. The absorbance at a wavelength of 450 nm was measured using a microplate reader (ELx800; BioTek Instruments, USA). Each sample was evaluated in three replicates.

Colony formation assay

For colony formation assay, 3×10^3 cells were trypsinized to single-cell suspensions and plated in 6-well plates in complete culture medium containing 0.3% agar on the top of 0.6% agar in the same medium. The plates were incubated at 37°C in 5% CO₂ for 14 days, then the colonies were fixed and stained with 50 μ L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml). Colonies (≥ 50 cells per colony) were scored under the inverted phase-contrast fluorescence microscope IX71 (Olympus, Japan). Data were presented as the mean \pm standard deviation of five randomly scored fields.

Terminal nucleotidyl transferase-mediated nick-end labeling (TUNEL) assay

TUNEL assay was performed according to the manufacturer's instructions of the TUNEL system kit (Roche). In brief, cells were cultured on cover slides overnight in a humidified incubator at 37°C and 5% CO₂. Firstly, the slides were fixed by the cold 4% paraformaldehyde for 30min and blocked by 3% H₂O₂ methanol solution for 10 min. Then the slides were treated with 1% Triton PBS solution for 2 min on ice. Avoiding the light, 50 μ L of TUNEL reaction solution were applied to incubate the cells on slides for 1 h at 37°C. Finally, the signals of TUNEL were converted using the Peroxidase (POD) for 30 min at 37°C, and the sections were treated with DAB for 3 min. Results were examined by the light System Microscope IX71 (Olympus, Japan).

Scratch wound healing assay

For the scratch healing assays, cells were treated with 10 mg/ml mitomycin C (Sigma, USA) for 3h. Subsequently, the cells were wounded using a pipette tip. Then the cells were washed with PBS for three times, and DMEM with 10% FBS was added. The wound closure was observed for 48 h using the light System Microscope IX71 (Olympus, Japan). The wound healing ability was calculated, compared to the width of wound closure for 0 h.

Transwell invasion assay

The transwell cell invasion assay was performed using transwell chambers (Corning, Lowell, MA, USA) according to the manufacturer's instructions. In brief, the insert members were coated with diluted Matrigel (BD Biosciences, San Jose, CA, USA). Then, 600 μ L of DMEM with 10% FBS was added to the lower chamber and 1×10^5 cells in 200 μ L FBS-free medium were added into the upper chamber. The cells were cultured for 24 h at 37°C and 5% CO₂, and the non-invading cells were removed. Finally, the insert membranes were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet (Beyotime, China), and photographed under the inverted Microscope IX71 (Olympus, Japan).

Statistical analysis

All the experiments were repeated at least 3 times. All quantitative data are presented as the means \pm SD and analyzed using the student's t-test. Data analysis was performed using the SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL). The association between FJX1 expression and the clinicopathological characteristics were evaluated by Pearson χ^2 test or Fisher's exact test based on the type of data. The overall survival curves were calculated using the Kaplan-Meier method and analyzed using the log-rank test. A $p < 0.05$ was considered to indicate a statistically significant difference.

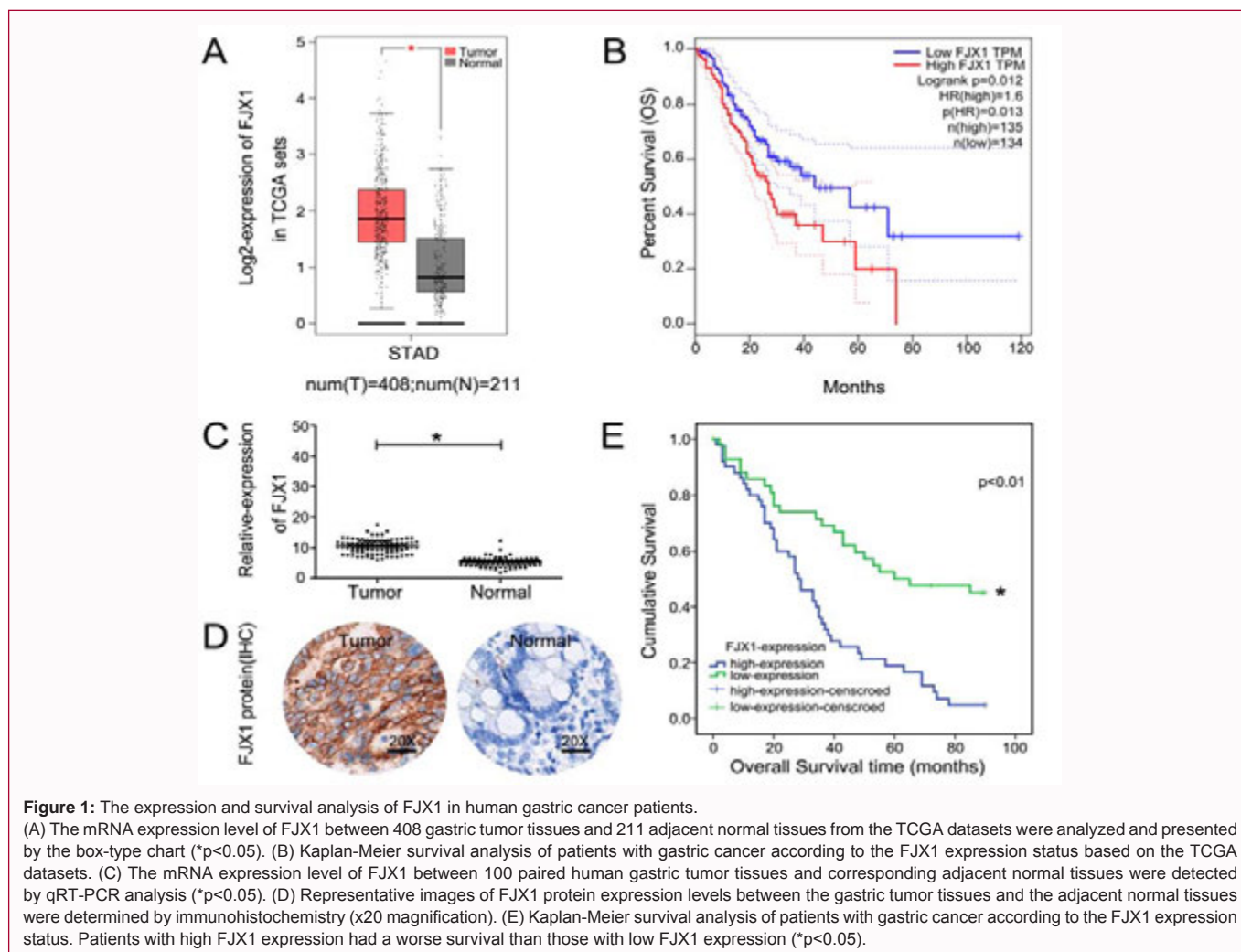
Results

The expression and survival analysis of FJX1 in human gastric cancer patients

In order to characterize the role of FJX1 in Gastric Cancer (GC), we used the data from TCGA to analyze the mRNA expression level of FJX1 in 408 gastric tumor tissues and 211 adjacent normal tissues. The expression of FJX1 in gastric tumor tissues was much higher than that of the adjacent normal tissues (Figure 1A). Moreover, Overall Survival (OS) curves were also plotted according to the FJX1 expression using the Kaplan-Meier method. Survival analysis showed that the high FJX1 expression group had a significantly shorter OS

Table 1: Clinical characteristics of gastric cancer patients.

Characteristics	No. of patients	FJX-1 expression		P- value
	(n=100)	High(n=57)	Low(n=43)	
Age (years)				
Median	66 (30-82)			
≤ 65	52	29	23	0.796
>65	48	28	20	
Gender				
Male	63	36	27	0.97
Female	37	21	16	
Tumor diameter				
≤ 5 cm	48	20	28	0.003
>5 cm	52	37	15	
Site of tumor				
Cardia	16	7	9	0.338
Body	29	17	12	
Antrum	49	28	21	
unknown	6	5	1	
Histological types				
Tubular adenocarcinoma	62	40	22	0.138
Mucinous adenocarcinoma	12	5	7	
Signet ring cell adenocarcinoma	13	7	6	
Undifferentiated carcinoma	13	5	8	
Tumor differentiation				
well	15	7	8	0.432
moderately	71	41	30	
poorly	14	9	5	
TNM Stage				
I-II	43	16	27	0.001
III-IV	57	41	16	



rate than that of the low FJX1 expression group in patients with GC (Figure 1B).

To conform this discovery, we detected the mRNA expression level of FJX1 between 100 paired human GC tumor tissues and corresponding adjacent normal tissues by using qRT-PCR analyses. As shown in Figure 1C, the expression of FJX1 was markedly higher in GC tumor tissues than that of adjacent normal tissues. Besides, the expression of FJX1 at the protein level of GC tumor tissues was also significantly upregulated than that of adjacent normal tissues, which was detected by IHC analyses (Figure 1D). The correlation between the expression of FJX1 and the clinical characteristics of enrolled GC patients was also investigated. As shown in Table 1, the expression of FJX1 in GC tissues was positively correlated with the tumor diameter and TNM stage of the GC patients, but was not correlated with the age, gender, histological types, tumor differentiation and tumor location. Furthermore, Kaplan-Meier plots were used to illustrate OS according to FJX1 expression status. Survival analysis indicated that the high FJX1 expression group had a significantly shorter OS rate than that of the low FJX1 expression group, and there was a statistically significance ($P < 0.01$) between the high FJX1 expression group and the low FJX1 expression group (Figure 1E). Taken together, these results indicated that FJX1 expression was frequently upregulated in GC tissues and the increased expression of FJX1 was an indicator of an unfavorable prognosis.

Establishment of FJX1-overexpressing and FJX1-silencing MGC-803 human gastric cancer cell lines

The expression of FJX1 was detected by qRT-PCR analysis of human gastric cancer cell lines including AGS, SGC-7901, MGC-803, BGC-823 and MKN-28. As shown in Figure 2A, the expression levels of FJX1 in AGS and SGC-7901 cells were relatively low compared with those of BGC-823 and MKN-28 cells. Then we chose the MGC-803 cell line for subsequent experiments because the FJX1 expression was moderate compared with other four cell lines (Figure 2A). For establishing a FJX1 overexpressing cell line, we transfected the reconstructed plasmid carrying both Green Fluorescent Protein (GFP) and human FJX1 cDNA into the MGC-803 cells. The percentages of GFP-positive cells between the two established cell lines were 94.04% and 95.71% detected by flow cytometry, indicating that the plasmids were successfully transfected into the MGC-803 cells (Figure 2B). The FJX1 expression at mRNA level in the MGC-803-FJX1 cells was significantly higher than that in the MGC-803 or MGC-803-control cells by qRT-PCR analyses (Figure 2C). Meanwhile, the expression of FJX1 at protein level was also markedly upregulated in the MGC-803-FJX1 cells compared to that in the MGC-803 or MGC-803-control cells by western blot analyses (Figure 2E). Besides, a FJX1-silencing cell line was also established for further studies. The MGC-803 cells were transfected with siRNA-FJX1 or siRNA-control plasmid, respectively. Both the mRNA and protein levels of FJX1

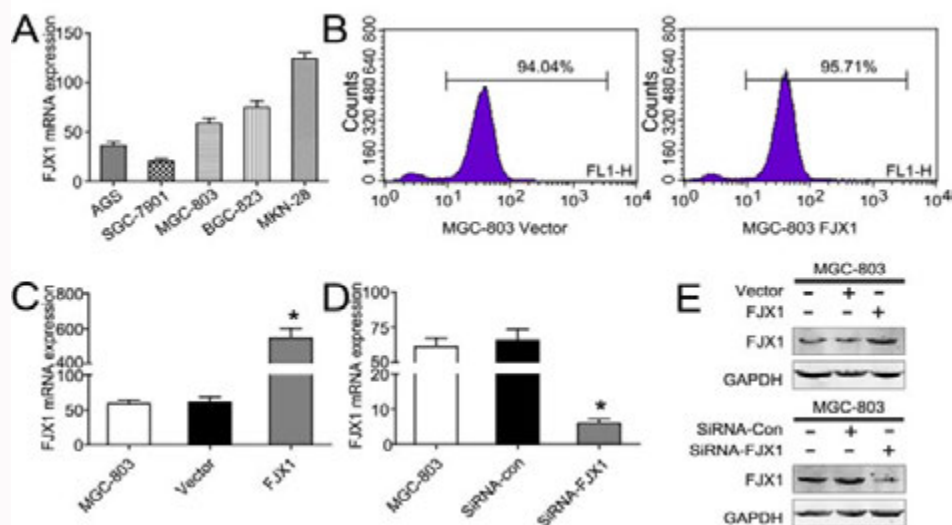


Figure 2: Establishment of FJX1-overexpressing and FJX1-silencing MGC-803 human gastric cancer cell lines.

(A) qRT-PCR analysis of the mRNA expression levels of FJX1 in AGS, SGC-7901, MGC-803, BGC-823 and MKN-28 human gastric cancer cell lines. (B) The GFP signals of the MGC-803-control and MGC-803-FJX1 cell lines were examined by flow cytometry. (C) The mRNA expression levels of FJX1 in the established MGC-803-control and MGC-803-FJX1 cell lines were detected by qRT-PCR analysis (* $p < 0.05$). (D) The mRNA expression levels of FJX1 between the siRNA-control and siRNA-FJX1 MGC-803 cells were measured by qRT-PCR analysis (* $p < 0.05$). (E) The protein expression levels of FJX1 among the established FJX1-overexpressing and FJX1-silencing MGC-803 cell lines were determined by western blot analysis.

were markedly lower in the siRNA-FJX1 MGC-803 cells as compared with the MGC-803 or siRNA-control MGC-803 cells, indicating a successful RNA interference (RNAi) of the FJX1 gene (Figure 2D, 2E). Taken together, these results indicated that FJX1 overexpressing and silencing MGC-803 human gastric cell lines were successfully established in our studies.

FJX1 promoted proliferation and reduced apoptosis of the MGC-803 cells

To investigate the role of FJX1 in the proliferative abilities of the MGC-803 cells, Cell Counting Kit-8 (CCK-8) assays were performed. As shown in Figure 3A, the OD450 values of the MGC-803-FJX1 cells were significantly higher than those of the MGC-803 or MGC-803-control cells at 48, 72 and 96 h, respectively. In contrast, FJX1 silencing showed the opposite results. To further characterize the effect of FJX1 on the proliferation of the MGC-803 cells, colony formation assays were performed. Representative images showed that the clone number of the MGC-803-FJX1 cells was significantly more than that of the MGC-803 or MGC-803-control cells, while FJX1 silencing showed the opposite results (Figure 3B, 3C).

To investigate whether FJX1 could affect the apoptotic capability of the MGC-803 cells, a TUNEL assay was performed. As shown in Figure 4A, 4B, the number of TUNEL positive cells of the MGC-803-FJX1 group was markedly reduced than that of the MGC-803 or MGC-803-control group. In contrast, the number of apoptotic cells of the siRNA-FJX1 group was significantly increased compared with that of the MGC-803 or siRNA-control group. Taken together, these results demonstrated that FJX1 increased the proliferative activity and decreased the apoptosis of the MGC-803 cells.

FJX1 enhanced the migratory and invasive abilities of the MGC-803 cells

Scratch would be healing assays were assessed to measure the role of FJX1 in the cellular migratory abilities of MGC-803 cells. As shown in Figure 5A, a scratched cell monolayer was formed and images

were captured at 48 h. It was shown that the MGC-803-FJX1 cells migrated from the edge towards the scratch center more rapidly than the MGC-803 or MGC-803-control cells, indicating an enhanced migratory ability (Figure 5B). While the siRNA-FJX1 MGC-803 cells showed the decreased migratory abilities compared to the MGC-803 or siRNA-control MGC-803 cells (Figure 5C, 5D), which further confirming that FJX1 might act as an oncogene in the progression of GC.

Besides, transwell invasion assay was performed to evaluate the role of FJX1 in the invasive abilities of the MGC-803 cells. As shown in Figure 6A, the number of cells that invaded the Matrigel layer from the MGC-803-FJX1 group was much more than that of the MGC-803 or MGC-803-control group. By the contrast, siRNA-FJX1 MGC-803 cells showed significantly reduced invasive abilities compared to the MGC-803 or siRNA-control MGC-803 cells (Figure 6B). Collectively, these results indicated that FJX1 enhanced the migratory and invasive abilities of the MGC-803 cells.

Discussion

FJX1 was first reported as a *Drosophila* gene four jointed (fj) which expressed in the eye, leg, wing imaginal disks and in the optic lobe. The null-mutation of the *Drosophila* fj gene causes reduced and altered growth of the leg and wing along the proximal distal axis [7,16]. Then FJX1 was found to be expressed in the brain during embryogenesis and in the adult in mouse [17,18]. In recent years, FJX1 has been reported to be abnormally expressed in a variety of diseases. FJX1 was overexpressed in eutopic endometrium from women with endometriosis and high levels of FJX1 proteins might play an important role in the pathogenesis of endometriosis [8]. Previous studies had reported that the expression of FJX1 was upregulated in a variety of malignant tumors including melanoma [10], nasopharyngeal cancer [9,19], head and neck cancer [13,20], colorectal cancer [11,21], breast cancer [22], ovarian cancer [23], lung cancer [12] and oral squamous cell cancer [24]. However, the

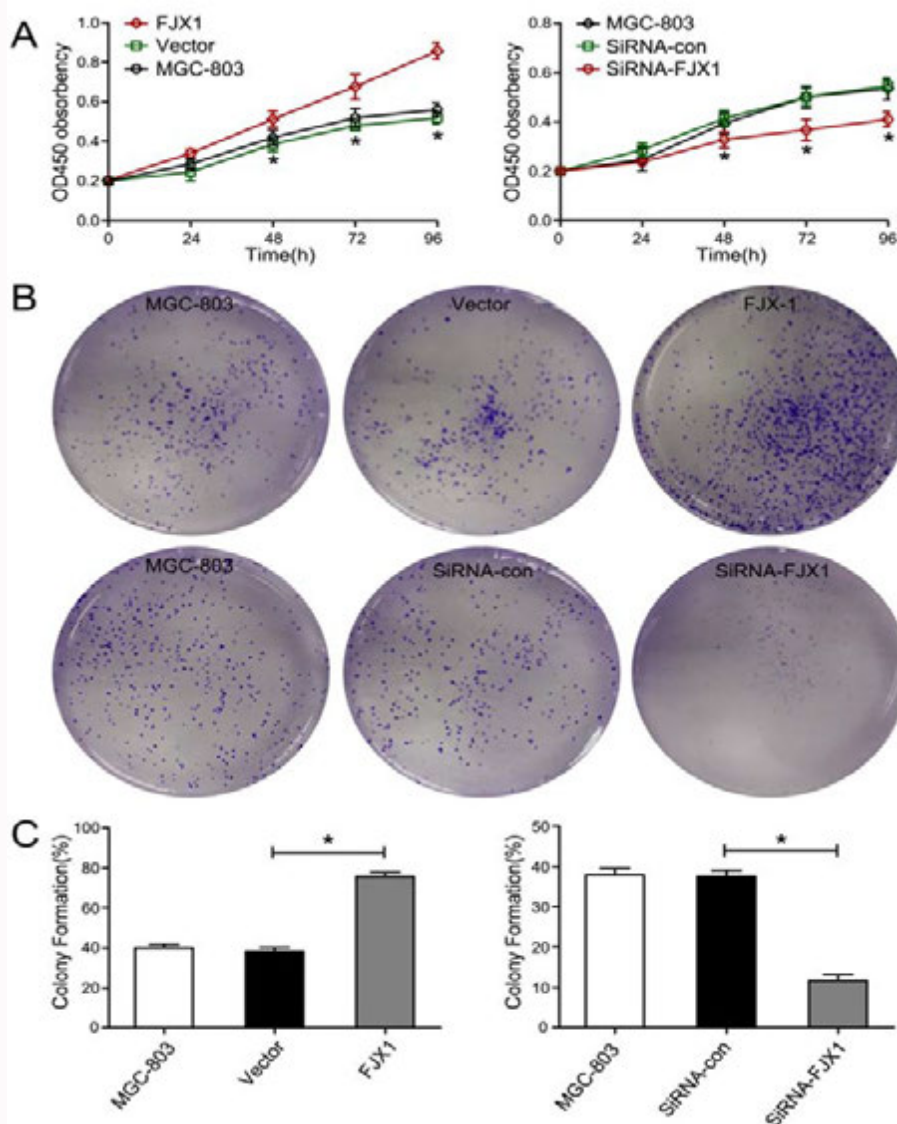


Figure 3: FJX1 promoted the proliferative ability of the MGC-803 cells.

(A) The proliferative abilities of the MGC-803 cells after FJX1 overexpressing or silencing were detected by the CCK-8 assay. Left, OD450 absorbency of the MGC-803, MGC-803-control and MGC-803-FJX1 cells at 24, 48, 72 and 96 h (* $p < 0.05$). Right, OD450 absorbency of the MGC-803, siRNA-control MGC-803 and siRNA-FJX1 MGC-803 cells at 24, 48, 72 and 96 h (* $p < 0.05$). (B) The colony formation assay was carried out to detect the proliferative abilities of MGC-803 cells after FJX1 overexpressing or silencing. Representative images of colonies among the MGC-803, MGC-803-control, MGC-803-FJX1, siRNA-control and siRNA-FJX1 MGC-803 cells were photographed. (C) The percentage of colony formation abilities were quantified and represented by the bar chart (* $p < 0.05$).

expression pattern of FJX1 in human GC tissue remains undefined. In this study, the FJX1 expression patterns of GC tissues were calculated due to the TCGA dataset. The results showed that the expression of FJX1 was significantly higher in GC tissues than that of adjacent normal tissues. To conform this discovery, we detected the expression of FJX1 between 100 paired human GC tumor tissues and corresponding adjacent normal tissues by using qRT-PCR and IHC analysis. Our data showed that FJX1 expressions were significantly up-regulated in GC tumor tissues compared with the adjacent normal tissues. To better understand the clinical relevance FJX1 expression in GC, we also compared the relationship between FJX1 and the clinicopathologic features. Clinicopathologic analyses revealed that high expression of FJX1 was positively correlated with the tumor diameter and TNM stage of the GC patients. But no significant differences were found in the age, gender, histological types, tumor differentiation and tumor location. To the best of our knowledge, for

the first time, we discovered the expression of FJX1 in human GC tissues.

To evaluate the role of FJX1 in predicting prognosis in GC, OS curves were plotted on the basis of the Kaplan-Meier method. The results showed that the high FJX1 expression patients suffered a significantly poorer OS rate than that of the low FJX1 expression patients in TCGA dataset. In the same way, Kaplan-Meier plots were also used to illustrate OS according to the FJX1 expression level of 100 paired GC samples. Our clinical data showed that the high FJX1 expression group had a significantly shorter OS rate than that of the low FJX1 expression group. These findings illustrated that FJX1 might be an indicator of an unfavorable prognosis of GC patients, which was consistent with the previous studies. Huang et al. demonstrated that FJX1 was a new prognostic factor with a significant role in tumor immunity [25]. FJX1 was reported to be upregulated in human

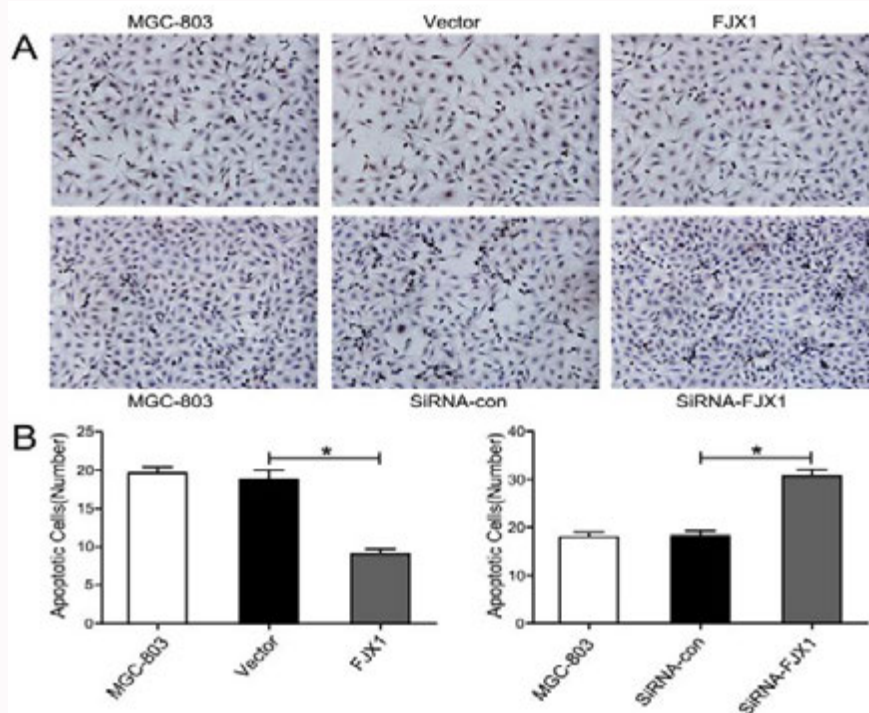


Figure 4: FJX1 reduced the apoptotic ability of the MGC-803 cells.

(A) The TUNEL assay was carried out to measure the apoptotic abilities of MGC-803 cells after FJX1 overexpressing or silencing. Representative images of TUNEL positive cells among the MGC-803, MGC-803-control, MGC-803-FJX1, siRNA-control and siRNA-FJX1 MGC-803 cells were photographed under an inverted microscope (x20 magnification). (B) The number of TUNEL positive cells were quantified and represented by the bar chart (*p<0.05).

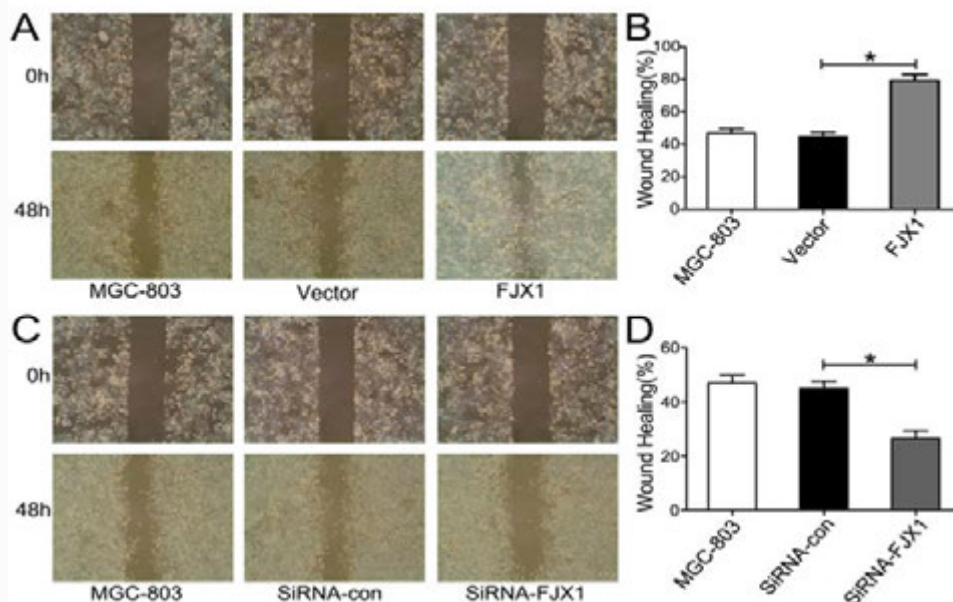


Figure 5: FJX1 enhanced the migratory ability of the MGC-803 cells.

(A) The migratory abilities among the MGC-803, MGC-803-control and MGC-803-FJX1 cells were detected by the scratch healing assay at 48 h. (B) The changes of percentages of wound healing after FJX1 overexpressing were quantified. (C) The migratory abilities among the MGC-803, siRNA-control MGC-803 and siRNA-FJX1 MGC-803 cells were detected by the scratch healing assay at 48 h. (D) The changes of percentages of wound healing after FJX1 silencing were quantified.

colorectal tumor epithelium as compared with normal epithelium and colorectal adenomas, and high expression of FJX1 was associated with poor patient prognosis [11]. Liu et al. also found that FJX1 was a candidate diagnostic and prognostic biomarker in CRC patients [21]. FJX1 was proved to be a target gene of miR-1249 and regarded as

independent predictor for Colon Adenocarcinoma (COAD), which was an indicator of poor OS for COAD patients [26].

Furthermore, the role of FJX1 in GC progression was systematically examined. Overexpression of FJX1 significantly promoted cell growth of the MGC-803 cells due to increased cellular

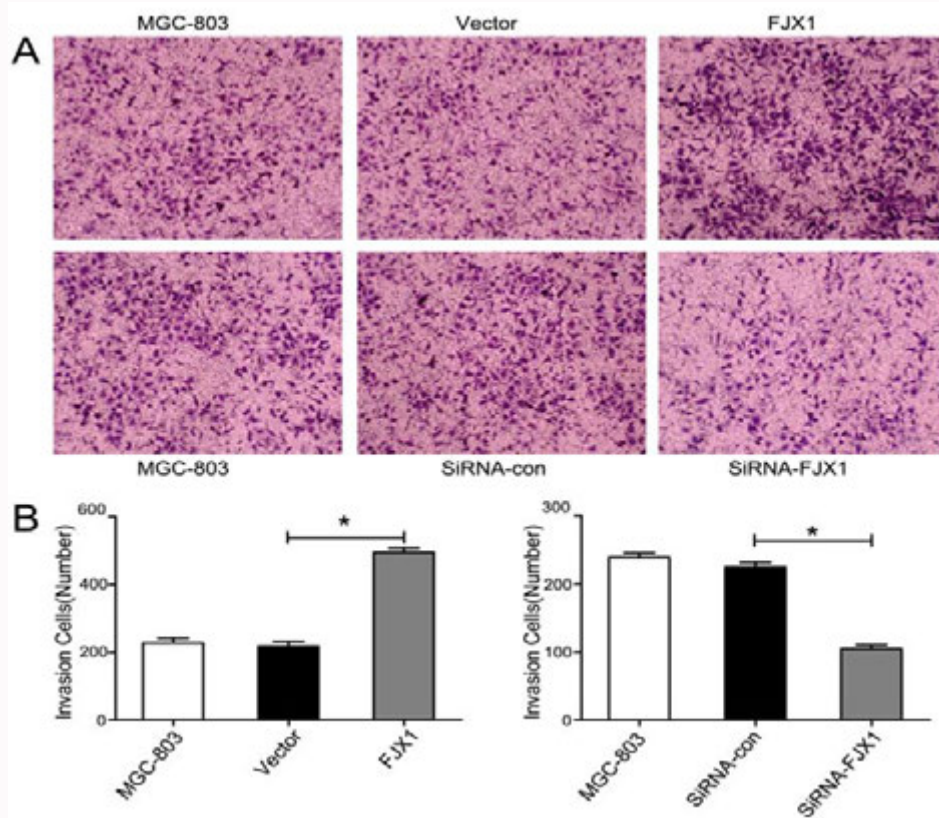


Figure 6: FJX1 enhanced the invasive ability of the MGC-803 cells.

(A) The invasive abilities of the MGC-803 cells after FJX1 overexpressing or silencing were detected by transwell invasion assay. Representative images of invaded cells among the MGC-803, MGC-803-control, MGC-803-FJX1, siRNA-control and siRNA-FJX1 MGC-803 cells were photographed under an inverted microscope (x20 magnification). (B) The number of invaded cells were quantified and represented by the bar chart (* $p < 0.05$).

proliferation and decreased cellular apoptosis. The migratory and invasive abilities of the MGC-803 cells were also promoted by the overexpression of FJX1. However, silencing FJX1 showed the opposite results. These findings suggested that FJX1 might function as an oncogene in the GC progression, and the underlying mechanism is currently ongoing. Although the exact role of FJX1 in human had not been completely understood, data from the developmental studies suggested that the function of FJX1 in cancer development could be linked to its role in promoting growth and cell movement. Except for PCP signaling, Hippo signaling pathway might be involved in the oncogenic effects of FJX1 in human gastroenterology malignancies. Hippo signaling pathway, firstly identified in *Drosophila*, was highly conserved and commonly regarded as a regulator of organ size and a tumor suppressor in human [27]. In *Drosophila*, it was the upstream regulatory factors of Hippo signaling pathway through its downstream target *ft* [28], while Yki was the key downstream transcription co-activator [29]. What was interesting to note was that *fj* was one of Yki target genes, suggesting *fj* form a feedback loop where *fj* overexpression further regulated Yki activation [30]. Similarly, a feedback loop containing FJX1 and YAP (the homologous of Yki) was observed in mammalian [31]. The oncogenic role that FJX1 played in Hippo signaling pathway required more work to elucidate. The alteration of key protein expression in Hippo signaling pathway will be determined and other related works will be further studied in the next step.

In conclusion, our findings of this study provide a key tumor-promoting role of the FJX1 in the development and progression of

GC. FJX1 plays an important role in cellular biological functions, including cell growth, proliferation, apoptosis, migration and invasion of the MGC-803 cells. To the best of our knowledge, for the first time, we provide evidence that FJX1 is overexpressed in GC tissues and high expression of FJX1 is associated with a shorter OS rate of GC patients. FJX1 may thus be a novel target for GC therapy and may be further considered as a potential prognostic factor in the future.

Conclusion

In conclusion, this study provided evidence that FJX1 was overexpressed in human gastric tumor tissues and correlated with poor prognosis of gastric cancer patients. FJX1 plays an oncogenic role in the development and progression of gastric cancer. However, further study is needed to elucidate the underlying mechanisms.

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