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F-box protein 11 promotes the growth and metastasis of gastric cancer via PI3K/AKT pathway-mediated EMT

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ABSTRACT

F-box protein 11 (FBXO11) has both the E3 ubiquitin ligase activity and the methyltrasferase activity, and regulates metastasis, apoptosis and chemosensitivity in human cancer. However, the clinical significance and biological function of FBXO11 in gastric cancer (GC) are rarely known. Here, we demonstrated up-regulated expression of FBXO11 in GC tissues in comparison with that in tumor-adjacent tissues. Clinical analysis based on our specimens and the TCGA database revealed that FBXO11 overexpression was associated with large tumor size, lymph node metastasis and advanced TNM stage. Notably, GC patients with high FBXO11 expression showed a significant shorter overall survival. Cell proliferation and mobility were measured by CCK-8 and Transwell assays. FBXO11 silencing by transfection with two specific shRNAs attenuated proliferation, migration and invasion of MGC-803 cells. In accordance, FBXO11 overexpression promoted these cellular processes in SGC-7901 cells. Mechanistically, FBXO11 obviously facilitated epithelial-mesenchymal transition (EMT) process as suggested by immunoblotting and immunofluorescence data. Moreover, we found that FBXO11 promoted the activation of AKT pathway with increased phosphorylated AKT level in SGC-7901 cells. LY294002 and Wortmannin, phosphotidylinsitol-3-kinase (PI3K) inhibitors, blocked FBXO11 induced EMT, proliferation, migration and invasion of SGC-7901 cells. Phosphatase and tensin homolog (PTEN), which played a crucial role in regulating PI3K/AKT pathway, was negatively modulated by FBXO11 in GC cells. Taken together, our findings contribute to current understanding of the functions of FBXO11 and suggest a mechanism by which FBXO11 plays an oncogenic role in the development of GC possibly by inhibiting PTEN and subsequently promoting PI3K/AKT pathway activation.

1. Introduction

Gastric cancer (GC) is the fourth most frequent malignancy and contributes to the second leading cause of cancer mortality [1]. Although effective medical treatments such as surgery, chemotherapy and radiation have been improved, GC patients are usually diagnosed with advanced stage, resulting in a low five-year survival rate [2,3]. Currently, GC is still a globe health problem, which highlights the need for further studies of molecular mechanism of GC and identify effective therapeutic targets.

F-box protein 11 (FBXO11), also known as PRMT9, has both the E3 ubiquitin ligase activity and the methyltrasferase activity [4,5]. Further studies report that FBXO11 plays a critical role in the initiation and progression of colorectal cancer (CRC), lung cancer, diffuse large B-cell lymphoma (DLBCL), pancreatic cancer and breast cancer [5–9]. FBXO11 prohibits transcriptional activity of p53 by mediating its neddylation [5]. FBXO11, either deleted or mutated in DLBCL, regulates BCL6 abundance by promoting ubiquitylation and degradation [6]. FBXO11 is recognized as a candidate tumor suppressor in pancreatic cancer and prominently associates with poor patient survival [7]. Moreover, FBXO11 functions as a regulator of cell-cycle by promoting the degradation of substrates [10,11]. FBXO11 represses hypoxia-inducible factor-1α (HIF-1α) level under hypoxia condition by promoting unknown substrates, which controlling HIF-1α mRNA stability [12]. FBXO11 restrains epithelial-mesenchymal transition (EMT), tumor initiation and metastasis off breast cancer by targeting SNAIL for

Abbreviations: GC, gastric cancer; FBXO11, F-box protein 11; DLBCL, diffuse large B-cell lymphoma; CRC, colorectal cancer; HIF-1α, hypoxia-inducible factor-1α; EMT, epithelial-mesenchymal transition; PTEN, phosphatase and tensin homolog; PI3K, phosphotidylinsitol-3-kinase; UICC/AJCC, Union for International Cancer Control/American Joint Committee on Cancer; IHC, immunohistochemistry; qRT-PCR, quantitative real-time polymerase chain reaction; CCK-8, Cell Counting Kit-8; IF, Immunofluorescence

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ubiquitylation and degradation [8,9]. MicroRNAs have been reported to be upstream regulators of FBXO11. For instance, miR-21 promotes tumorigenesis by directly targeting FBXO11 [13]. miR-621 increases chemosensitivity to paclitaxel and carboplatin by inhibiting FBXO11 in breast cancer cells [14]. However, the characteristics and mechanisms of FBXO11 regulatory pathways and its association with patients' survival are still largely unknown for understanding the importance of the tumorigenesis of GC.

In the current study, we designed to assess expression of FBXO11 in both tumor tissues and cell lines of GC and to investigate the effects of FBXO11 on proliferation, invasion and migration in GC cell lines. Our studies demonstrated that upregulated expression of FBXO11 was found in tumor tissues of GC and promoted cell proliferation, migration and invasion in GC cell lines possibly by suppressing phosphatase and tensin homolog (PTEN) and activating phosphotidylinsitol-3-kinase (PI3K)/AKT pathway.

2. Materials and methods

2.1. Patients

Eighty GC tissues and matched tumor-adjacent tissues were obtained from the China-Japan Union Hospital of Jilin University. Tissue specimens were conserved in liquid nitrogen and 10% formalin for further investigation. Patients who received immunotherapy, radiotherapy or chemotherapy before surgery were excluded. Tumor staging was based on the seventh Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) staging system [15]. We obtained written informed consent from every patient involved. Details of the clinicopathological data are shown in Table 1. The study was approved by the Research Ethics Committee of the China-Japan Union Hospital of Jilin University.

2.2. Cell culture and transfection

A normal human gastric epithelium cell line (GES-1) and GC cell lines (SGC-7901, MGC-803, MKN-28, and BGC-823) were obtained from the Cell Bank of Academia Sinica (Shanghai, China) and grown in DMEM medium with 10% heat-inactivated FBS (Gibco, Grand Island, NY, USA), and 1% Penicillin-Streptomycin Solution (100×; 100 U/ml Penicillin and 0.1 mg/ml Streptomycin for working; Sigma, St-Louis, MO, USA) separately in a humidified chamber with 5% CO2 and 95% air at 37 °C. FBXO11 shRNA, FBXO11 shRNA-1 non-targeting (NT) shRNA, FBXO11 expression plasmid (pcDNA3.1-ICT1) and empty vector (pcDNA3.1-NC) were obtained from GenePharma (Shanghai, China). All vectors were then transfected into GC cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following manufactures' protocol. LY294002 and Wortmannin were purchased from Selleck Chemicals (Houston, TX, USA). MG132 was obtained from Sigma.

Table 1
The correlation between FBXO11 and clinicopathologic features in gastric cancer (n = 80).

Characteristics		Total	FBXO11 expression		*P*
		High (n = 40)	Low (n = 40)		
Age (years)	< 65	35	18	17	.822
	≥65	45	22	23	
Sex	Male	62	29	33	.284
	Female	18	11	7	
Tumor differentiation	Well and moderate	32	13	19	.171
	Poor and other	48	27	21	
Size (cm)	< 5	37	14	23	.044[a]
	≥5	43	26	17	
Depth of invasion	mucosa to muscularis propria	10	3	7	.176
	adventitia to adjacent structure	70	37	33	
Lymph node metastasis	Absent	25	7	18	.008[a]
	Present	55	33	22	
Distant metastasis	Absent	69	32	37	.105
	Present	11	8	3	
TNM stage	I-II	33	10	23	.003[a]
	III-IV	47	30	17	

TNM: tumor-node-metastasis.
[a] Statistically significant.

2.3. Immunohistochemistry (IHC)

The antibody against FBXO11 (Abcam, Cambridge, MA, USA) was applied for IHC. The process of IHC and calculation of final IHC score were according to our previous study [16]. IHC score ≥ 3 was considered as positive expression of ICT1.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA from GC tissues or cell lines were extracted using Trizol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. PrimeScript Reverse Transcriptase kit (Takara, Dalian, China) was used for FBXO11 mRNA reverse transcription. The expression of FBXO11 was detected by ABI 7900HT Real-Time PCR System (Applied Biosystem, Foster City, CA, USA), using SYBR Green assays (TaKaRa Biotechnology, Dalian, China) and GAPDH was used as the internal control. The primers used for FBXO11 and GAPDH were designed and synthesized by Sangon Biotech (Shanghai, China).

2.5. Cell Counting Kit-8 (CCK-8) proliferation assay

Cell proliferation was evaluated by Cell Counting Kit (CCK-8; Beyotime, Shanghai, China) assay, which was carried out following standard procedure in 96 well plates. Briefly, cells with a density of 5×10^3 cells/well were seeded in a 96-well plate and grown to 80% confluence. Then, corresponding vectors were transfected into cells. After 0, 24, 48 and 72 h post-transfection, CCK-8 (10 µl per well) reagent was added and reaction system was incubated for 1 h under the same incubator conditions. The relative viability of cells transfected with different vectors was obtained at 490 nm absorbance.

2.6. Cell migration and invasion

The transwell assay was used to evaluate cell migration as previously described [16]. Cell invasion was assessed using BioCoat Matrigel Invasion Chamber (BD Biosciences Discovery Labware). Cell numbers for cell migration and invasion in three random fields were counted.

2.7. Immunofluorescence (IF) assay

Cells were fixed with 4% paraformaldehyde for 15 min at 4 °C on glass coverslips and permeabilized with 0.1% Triton-X 100 for 10 min at 4 °C. Then the cells were blocked with 1% bovine serum albumin for 1 h at 4 °C and incubated with primary antibodies including E-cadherin (Abcam) and Vimentin (Abcam) at 4 °C overnight. After rinsing with PBS, the cells were incubated with AlexaFluor 555-conjugated goat-anti-rabbit IgG for 1 h at room temperature. Subsequently, cell nuclei were stained with DAPI for 3 min. Finally, the cells were examined using a confocal laser-scanning microscope (Zeiss LSM710, German).