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Clinicopathological significance and molecular mechanism of AKT/GSK3β pathway-based fibrillin-1 expression in gastric cancer progression

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ABSTRACT

This research was developed to explore the significance of fibrillin-1 (FBN1) in the progression of gastric cancer and its relationship with the activation of the AKT/glycogen synthase kinase-3 beta (GSK3β) pathway. Immunohistochemical assays were adopted to detect FBN1 expression in chronic superficial gastritis, chronic atrophic gastritis, gastric cancer, and normal mucosa. The expression of FBN1 in gastric cancer and adjacent tissue samples was detected by reverse transcription-quantitative (RT-q) PCR and Western blot, and the relationship between FBN1 and the clinicopathological features of gastric cancer patients was analyzed. Lentivirus was utilized to construct SGC-7901 gastric cancer cell lines stably overexpressing and silencing FBN1, and the effects on cell proliferation, colony formation, and apoptosis were analyzed. AKT, GSK3β, and their phosphorylated proteins were detected by Western blot. Results showed that the positive expression rate of FBN1 increased successively in chronic superficial gastritis, chronic atrophic gastritis, and gastric cancer. FBN1 was up-regulated in gastric cancer tissues and correlated with the depth of tumor invasion. Overexpression of FBN1 promoted the proliferation and colony formation of gastric cancer cells, inhibited apoptosis, and promoted the phosphorylation of AKT and GSK3β. Silencing FBN1 expression inhibited the proliferation and clonal formation of gastric cancer cells, promoted apoptosis, and inhibited the phosphorylation of AKT and GSK3β. In conclusion, FBN1 was up-regulated in gastric cancer tissues and correlated with the depth of gastric tumor invasion. FBN1 silencing inhibited the progression of gastric cancer through the AKT/GSK3β pathway.

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Introduction

Gastric cancer is one of the most common malignant tumors of the digestive tract. Statistics have shown that gastric cancer ranks fourth in the incidence of malignant tumors in the world. In contrast, mortality ranks second, and the metastasis rate and recurrence rate of gastric cancer are very high (1). The incidence of gastric cancer shows remarkable sex differences, with gastric cancer in males being twice that in females (2). Moreover, the incidence of gastric cancer will increase with increasing age, and its incidence will increase rapidly and reach its peak in people aged 45-75 years (3). Since the lesions of early gastric cancer are very limited, most patients are already in the advanced stage of gastric cancer when they are diagnosed. The treatment process for patients with advanced gastric cancer is complex and long, and the prognosis of patients is extremely poor (4). Therefore, early screening and diagnosis of gastric cancer play a very important role in improving the treatment effect and prognosis of patients.

Current clinical screening methods for gastric cancer include X-ray barium meal, gastroscopy, gastric atrophy marker test, and Helicobacter pylori antibody test, among which gastroscopy is the gold standard for the diagnosis of gastric cancer (5). The main treatment methods for gastric cancer include surgical therapy, neoadjuvant chemotherapy, molecular targeted therapy, and immunotherapy. Surgical therapy is the main treatment method for gastric cancer (6). However, there is evidence that the long-term survival rate of gastric cancer patients after surgical treatment is low (7). As the etiology and pathogenesis of gastric cancer are still not completely clear and there is a lack of specific drugs to prevent and treat gastric cancer, in-depth exploration of molecular mechanisms in the process of gastric cancer will be of great significance for the early diagnosis and treatment of gastric cancer, as well as improving the prognosis and survival rate of patients.

In recent years, studies have confirmed that fibrin is involved in the occurrence and development of malignant tumor diseases (8). The fibrillin family mainly comprises fibrillin1 (FBN1), FBN2, FBN3, and FBN4. The FBN1 gene is involved in the process of various tumor diseases, such as colon cancer (9). Kerslake et al. (2021) verified the correlation between FBN1 and ovarian cancer by using cancer genome atlas data and experimental techniques (10). Yang et al. (2017) confirmed that miR-133b targets the FBN1 gene and participates in the regulation of proliferation and invasion of gastric cancer cells (11). However,

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no studies have confirmed the mechanism by which FBN1 expression regulates the progression of gastric cancer. To this end, this study aimed to first analyze the expression of FBN1 in different gastric disease tissues. Subsequently, FBN1-overexpressing/knockout gastric cancer cell lines were prepared to analyze the changes in cell proliferation, apoptosis, and AKT/GSK3β pathway status. The results of this study are expected to provide a reference for finding potential therapeutic targets and early diagnostic markers for gastric cancer.

Materials and Methods

The experimental materials

An FBN1 immunohistochemistry kit was purchased from Shanghai Qi Ming Biotechnology Co., Ltd. Fetal bovine serum, Roswell Park Memorial Institute (RPMI)-1640 medium, and trypsin were all purchased from Gibco, USA. The DKK-8 kit was purchased from Shanghai Enzyme-linked Biology. An Annexin V-FITC/propidium iodide (PI) staining kit was purchased from Merck Sigma–Aldrich, USA. The PrimeScript™ RT reagent Kit (Perfect Real Time) and TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) were purchased from Beijing Baoriyi Material Technology Co., LTD. BCA protein concentration detection kit and ECL chemiluminescence detection kit were purchased from Shanghai Beyotime Biotechnology Co., Ltd. Rabbit polyclonal antibody FBN1 (ab53076), rabbit polyclonal antibody FBN1 (ab231094), rabbit polyclonal antibody AKT (ab38449), rabbit monoclonal antibody GSK3β (ab32391), mouse monoclonal antibody β-actin (ab8226), and rabbit anti-human horseradish peroxidase-conjugated IgG secondary antibody (ab6759) were purchased from Abcam company, UK.

Source of experimental samples

Tissue samples from 15 patients with normal gastric mucosa, 21 patients with chronic superficial gastritis, 26 patients with chronic atrophic gastritis, and 30 patients with gastric cancer who were admitted to our hospital from April 2020 to December 2021 were collected. All 30 patients with gastric cancer underwent radical gastrectomy, and none of the patients had received chemotherapy or radiotherapy before surgery. During the operation, cancer tissue and gastric mucosa tissue 5 cm adjacent to the cancer were collected from gastric cancer patients. The collected tissue was preserved differently according to the experimental procedure. All subjects were aware of the trial procedure, agreed to collect samples for clinical study, and signed informed consent.

Immunohistochemical staining

The expression of FBN1 in normal gastric mucosa, chronic superficial gastritis, chronic atrophic gastritis, and gastric cancer was detected by immunohistochemical staining with an immunohistochemical kit. After the tissue was sealed with 3% H_2O_2 solution for 10 min, citric acid solution (pH=6.0) was added under heating conditions and incubated for 15 min for antigen repair. After cooling, 5% goat serum was used to seal the tissue for 30 min at 37°C. The FBN1 primary antibody was added and incubated overnight in a 4°C refrigerator. Phosphoric acid buffer (PBS) was used to wash the tissue, then the second antibody was added, and the cells were incubated at 37°C for 1 hour. After the tissue was washed with PBS, 3,3'-diaminobenzidine (DAB) solution was used for the chromogenic treatment of the staining results. After washing, a hematoxylin staining solution was used to restain the tissues. After the tissue was dehydrated with gradient alcohol, xylene was added to make the tissue transparent. Neutral gum was used to seal the film. The staining results were observed under a microscope, and the number of positively stained cells was counted in five fields randomly, after which the average value was taken.

Construction of gastric cancer cell lines with overexpression/knockout of FBN1

SGC-7901 gastric cancer cells were cultured in RPMI-1640 complete medium containing 10% fetal bovine serum in an incubator at 5% CO_2 and 37°C. After subculture, the cells were inoculated in a 24-well plate at a concentration of 2.5×10^5 cells per well. When the confluence of cells reached approximately 30%, the virus titer was determined to calculate the virus quantity, and the optimal concentration was selected for lentivirus infection of cells. Polybrene solution with a final concentration of 5 μg/mL was added to the cell culture medium, and the cell status was observed after 8 hours of culture. The original culture medium was removed and replaced with a fresh complete culture medium. After an additional 3 days of culture, a complete culture medium containing the appropriate concentration of puromycin was added for cell culture, and overexpression (OE) and knockout (KO) FBN1 stable infection cell lines were screened.

Cell proliferation detected by cholecystokinin octapeptide (CCK-8)

SGC-7901 cells were inoculated into 96-well plates at 5×10^4 cells/well. After cell adherence, 100 μL serum-free medium containing 10% CCK-8 reagent was added to each well. After shaking and mixing, the cells were cultured under dark conditions for 24 h. The absorbance of each well was measured at 450 nm using a multifunctional microplate reader. Three replicate wells were prepared for each group of cells, and the average value was taken.

The cloning ability of cells detected by plate cloning experiment

Cells in the logarithmic growth phase were selected, and SGC-7901 cells were inoculated into 6-well plates at a concentration of 1×10^6 cells/well. The culture was terminated when routine culture and cell cloning were visible to the naked eye. The original culture medium was discarded. The cells were washed twice with PBS and then fixed with 4% paraformaldehyde solution for 15 min. After the fixative was discarded, 1 ml crystal violet staining solution was added to stain the cells for 15 min, and the staining solution was slowly washed with running water. The cells were placed in a fume hood to dry, and the number of cloned cells was observed by the naked eye. The rate of cell clone formation was detected according to the equation: number of cloned cells/number of inoculated cells ×100%.

The apoptosis rate detected by flow cytometry

Cells in the logarithmic growth phase were selected, and SGC-7901 cells were inoculated into 6-well plates at a concentration of 1×10^6 cells/well. When the confluence of the cells reached approximately 70%, the original me-

Table 1. Primer information for RT–qPCR.

Gene	Primer sequences	Product length
FBN1	F: 5'-GCGGAAATCAGTGTATTGTCCC-3' R: 5'-CAGTGTTGTATGGATCTGGAGC-3'	130 bp
GAPDH	F: 5'-GGAGCGAGATCCCTCCAAAAT-3' R: 5'-GGCTGTTGTCATACTTCTCATGG-3'	197 bp

dium was discarded. After the cells were washed with PBS, 0.25% trypsin was used to digest the cells, and a complete culture medium was added to terminate digestion, followed by centrifugation at 1,000 rpm for 10 min. The apoptosis rate was determined according to the instructions of the Annexin V-fluorescein isothiocyanate/propidium iodide (V-FITC/PI) staining kit. After the cell precipitates were suspended in PBS, the cell concentration was adjusted, and the cells were centrifuged. Then, 200 μL Annexin V-FITC solution was added to suspend the cells, and the cells were incubated at room temperature without light for 15 min. After centrifugation at 1,000 rpm for 5 min, 190 μL Annexin V-FITC was added to the precipitate to suspend the cells, followed by 10 μL PI staining solution. After mixing, the cell apoptosis rate was detected by flow cytometry immediately under dark conditions. Annexin V-FITC staining showed green fluorescence and was able to label cells in apoptotic or necrotic states. PI staining showed red fluorescence, which could mark early and late apoptotic cells.

Real-time fluorescence quantitative PCR (RT–qPCR)

The cancer tissues and adjacent tissues of gastric cancer patients were cut into approximately 2 mm pieces and homogenized with 1,000 μL TRIzol reagent to form a granular tissue solution. Total RNA was extracted from tissues and overexpressed/silenced FBN1-stabilized SGC-7901 gastric cancer cells. The concentration, purity, and integrity of the extracted RNA were determined by spectrophotometry and agarose gel electrophoresis. Subsequently, reverse transcription of extracted RNA was carried out according to the instructions of the cDNA reverse transcription kit. The reaction system and reaction procedure were set according to the instructions of the RT–qPCR detection kit, and the expression levels of the target gene FBN1 and the reference gene GAPDH were detected. The primer information for quantitative gene expression detection is shown in Table 1. The relative expression level of FBN1 was calculated by the $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal reference gene.

Western blot

The protein expression levels of FBN1 and β-actin in cancer tissues and adjacent tissues of gastric cancer patients were detected by Western blot, and the protein expression levels of FBN1, AKT, GSK3β, and β-actin in SGC-7901 gastric cancer cells stabilized by overexpression and silencing of FBN1 were detected. After tissue homogenization, total protein was extracted from tissue homogenates and cells by adding RIPA lysis buffer, and the concentration of extracted protein was quantitatively detected according to the bicinchoninic acid (BCA) kit instructions. According to the size of the target protein, the corresponding separation gel and concentrated gel were configured, and the protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE). At 4°C, the proteins in SDS–PAGE were transferred to polyvinylidene fluoride (PVDF) membranes at a constant current of 300 mA. After the film was washed with Tris-buffered saline Tween (TBST), block solution containing 5% skim milk powder was used for 2 h at room temperature. A diluted primary antibody was added and incubated overnight at 4°C. After the film was washed with TBST, a diluted secondary antibody was added and incubated at room temperature for 1 hour. After the membrane was washed with TBST, the target protein was developed and fixed according to the instructions of the ECL chemiluminescence detection kit. The bands of the target protein were photographed by a gel imaging system, and the relative expression level of the target protein was detected by ImageJ with β-actin as an internal reference.

Statistical treatment

SPSS 19.0 was used for statistical analysis of the test results. The count data were expressed as frequencies (percentages) and statistically analyzed using the chi-square test procedure. Measurement data are expressed as the mean ± SD and were statistically analyzed by univariate ANOVA. $P<0.05$ indicates a statistically significant difference, "*" and "#" indicate $P<0.05$, and "**" and "##" indicate $P<0.01$.

Results

Differences in FBN1 expression in different gastric tissues

Immunohistochemical staining was used to detect the expression of FBN1 in normal gastric mucosa, chronic superficial gastritis, chronic atrophic gastritis, and gastric cancer patients, and the results are shown in Figure 1. The positive numbers of FBN1 expression in normal gastric mucosa, chronic superficial gastritis, chronic atrophic gastritis, and gastric cancer patients were 2/15 (13.3%), 9/21 (42.9%), 16/26 (61.5%), and 27/30 (90.0%), respectively. The positive expression rate of FBN1 in different gastric mucosa tissues was significantly different ($P<0.05$).

Expression difference of FBN1 in gastric cancer and adjacent tissues

The expression differences of FBN1 in gastric cancer tissues and adjacent tissues were detected by RT–qPCR and Western blot, and the results are shown in Figure 2. Compared with paracancerous tissues, FBN1 mRNA and protein expression levels in gastric cancer tumor tissues were dramatically increased ($P<0.01$).

Differences in FBN1 expression in patients with gastric cancer with different clinical features

When the expression of FBN1 in gastric cancer tumor tissues was two times higher than that in adjacent tissues,

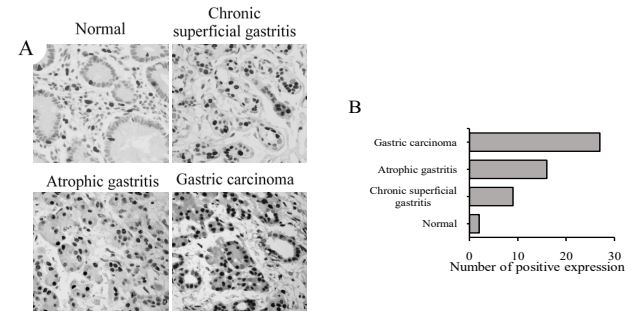

Figure 1. Differential expression of FBN1 in different gastric tissues. (A: immunohistochemical staining observation, ×400; B: the number of positive expressions).

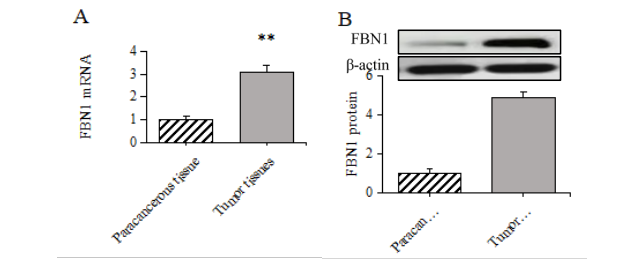

Figure 2. FBN1 mRNA and protein expression differences in gastric cancer tumors and adjacent tissues. (A: FBN1 mRNA expression; B: FBN1 protein expression).

it was defined as high expression. Subsequently, the expression levels of FBN1 in gastric cancer patients with different clinical characteristics were compared, and the results are shown in Table 2. There was no remarkable correlation between the FBN1 expression level and age, sex, tumor size, lymph node metastasis, or distal metastasis (P>0.05). The proportion of high FBN1 expression in T3 and T4 tumor infiltration patients was dramatically higher than in low FBN1 expression patients (P<0.05).

Validation of FBN1 expression in SGC-7901 cells after overexpression/knockout of FBN1

The mRNA and protein expression levels of FBN1 in SGC-7901 cells were detected by RT–qPCR and Western blot, and the results are shown in Figure 3. Compared with those in the control group, the mRNA and protein expression levels of FBN1 in the OE-FBN1 group were dramatically increased (P< 0.01), and the mRNA and protein expression levels of FBN1 in the KO-FBN1 group were dramatically decreased (P<0.01). Compared with those in the OE-FBN1 group, the mRNA and protein expression levels of FBN1 in the KO-FBN1 group were dramatically decreased (P< 0.01).

Effects of FBN1 overexpression/knockout on the proliferative activity of SGC-7901 cells

The CCK-8 method was used to detect the difference in proliferation activity of SGC-7901 cells in each group, and the results are shown in Figure 4. At 0 h, there was no remarkable difference in the proliferation activity of SGC-7901 cells among all groups (P>0.05). At 6 h, the proliferation activity of the OE-FBN1 group was dramatically higher than that of the control group and KO-FBN1 group (P<0.05). The proliferation activity of the

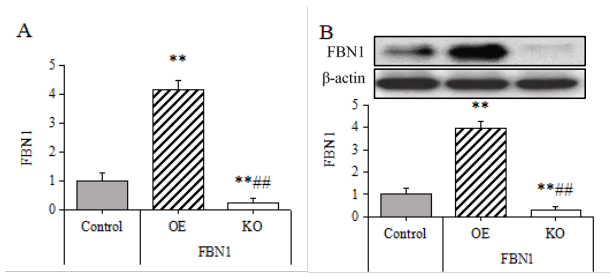

Figure 3. FBN1 mRNA and protein expression differences in SGC-7901 cells. (A: FBN1 mRNA expression; B: FBN1 protein expression).

Table 2. Correlation between FBN1 expression and clinical characteristics of gastric cancer patients.

Clinical features	Sample size (n/%)	FBN1 (n/%)		*P*
		High expression	Low expression	
Age (years old)				
＞60	18/60.0	11/61.1	7/38.9	0.719
≤60	12/40.0	7/58.3	5/41.7	
Sex				
Male	20/66.7	11/55.0	9/45.0	0.803
Female	10/33.3	6/60.0	4/40.0	
Tumor size (cm)				
＞3	14/46.7	9/64.3	5/35.7	0.667
≤3	16/53.3	10/62.5	6/37.5	
Infiltrating depth				
T1, T2	11/36.7	2/18.1	9/81.8	0.011
T3, T4	19/63.3	15/78.9	4/26.3	
Lymph node metastasis				
Yes	21/70.0	13/61.9	8/38.1	0.721
No	9/30.0	6/66.7	3/33.3	
The distal metastasis				
Yes	1/3.3	1/100.0	0/0.0	0.674
No	29/96.7	16/55.2	13/44.8	

KO-FBN1 group was dramatically lower than that of the control group (P<0.05). At 12 h, 24 h, 48 h, and 96 h, the proliferative activity of the OE-FBN1 group was dramatically increased compared with that of the control group (P<0.01), and the proliferative activity of the KO-FBN1 group was dramatically decreased (P<0.01). Compared with the OE-FBN1 group, the proliferation activity of the KO-FBN1 group was dramatically decreased (P<0.01).

Effects of FBN1 overexpression/knockout on the cloning ability of SGC-7901 cells

The difference in clone formation ability of SGC-7901 cells in each group was detected by plate clone formation time, and the results are shown in Figure 5. Compared with that in the control group, the number of cell clones formed in the OE-FBN1 group was dramatically increased (P<0.01), while the KO-FBN1 group had a dramatically reduced number of cell clones (P<0.01). Compared with the OE-FBN1 group, the number of cell clones formed in the KO-FBN1 group was dramatically reduced (P<0.01).

Effects of FBN1 overexpression/knockout on apoptosis of SGC-7901 cells

The difference in the apoptosis rate of SGC-7901 cells in each group was detected by flow cytometry, and the results are shown in Figure 6. Compared with the control group, the apoptosis rate of the OE-FBN1 group was dramatically decreased (P<0.01), and the apoptosis rate of the KO-FBN1 group was dramatically increased (P<0.01). Compared with the OE-FBN1 group, the apoptosis rate of the KO-FBN1 group was dramatically increased (P< 0.01).

Effects of FBN1 overexpression/knockout on the AKT/GSK3β pathway in SGC-7901 cells

Western blotting was used to detect the protein expression levels of AKT, p-AKT, GSK3β, and p-GSK3 β in SGC-7901 cells of each group (Figure 7). There was no remarkable difference in the expression levels of AKT and GSK3β in SGC-7901 cells among all groups (P>0.05). Compared with those in the control group, the expression levels of p-AKT and p-GSK3 β in the OE-FBN1 group were dramatically increased (P<0.01), and the protein expression levels of p-AKT and p-GSK3 β in the KO-FBN1 group were dramatically decreased (P<0.01). Compared with the OE-FBN1 group, the expression levels of p-AKT and p-GSK3 β in the KO-FBN1 group were dramatically decreased (P<0.01).

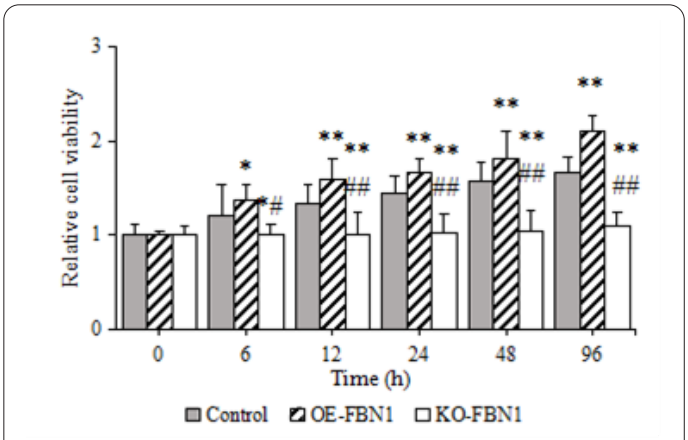

Figure 4. Comparison of the proliferation activity of SGC-7901 cells at different time points.

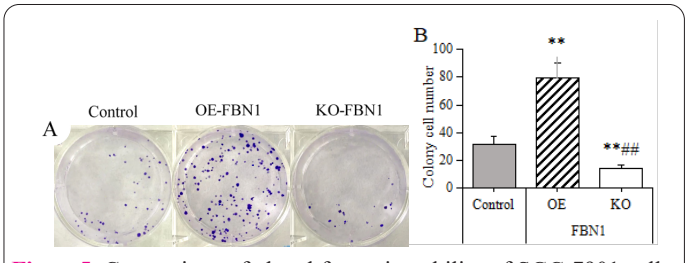

Figure5. Comparison of clonal formation ability of SGC-7901 cells. (A: the result of staining of cell clonal formation observed by the naked eye; B: the number of cell clones formed).

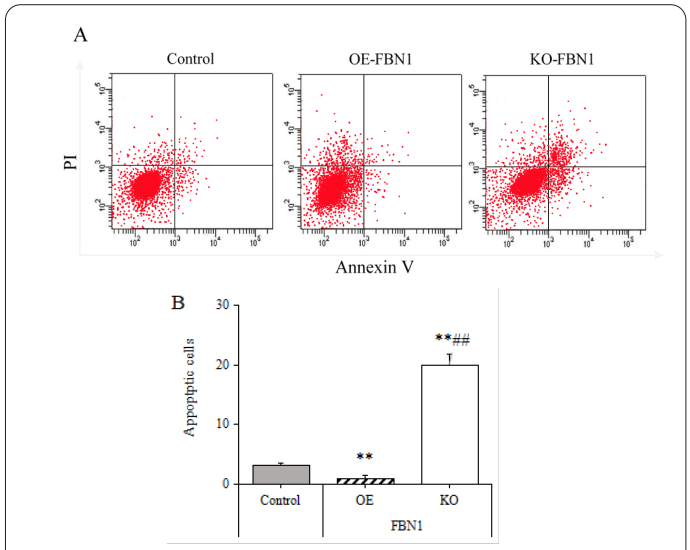

Figure 6. Comparison of apoptosis rates of SGC-7901 cells. (A: flow cytometry; B: detection of apoptosis rate).

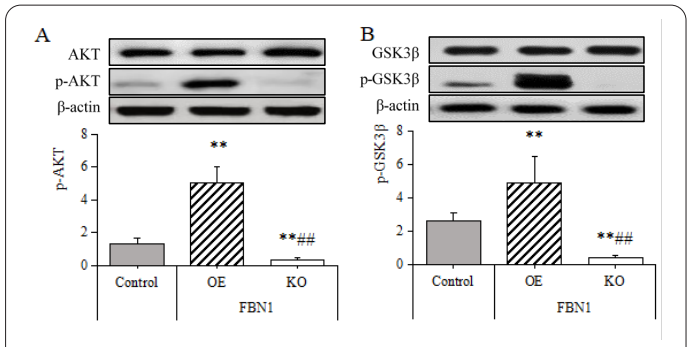

Figure 7. Comparison of AKT/GSK3β pathway-related protein expression levels in SGC-7901 cells. (A: AKT protein expression and phosphorylation level; B: GSK3β protein expression and phosphorylation level).

Discussion

Gastric cancer is a very common malignant tumor, and the annual new cases of gastric cancer in China account for approximately 40% of the global total (12). The progression of gastric cancer is a multifactor, multistage, and multigene-coregulated process. Studies have confirmed that the dysregulation of FBN1 gene expression may be closely related to the occurrence and development of tumors, including ovarian cancer, thyroid cancer, and colon cancer (13-15). These results suggest that FBN1 may be a potentially important gene promoting the development and development of cancer. However, the function and mechanism of the FBN1 gene's involvement in gastric cancer still need to be further explored. In this work, the

expression difference of FBN1 in different gastric diseases and its regulatory effect on the proliferation and apoptosis of gastric cancer cells were investigated in depth.

In this study, FBN1 gene expression was first detected and showed a gradually increasing trend in normal gastric mucosa, chronic superficial gastritis, chronic atrophic gastritis, and gastric cancer tissues and showed strong expression in gastric cancer tissues. Chronic superficial gastritis and chronic atrophic gastritis are precancerous lesions of gastric cancer (16,17). The results suggested that FBN1 can be used as a marker to determine precancerous lesions of gastric cancer, which may be closely related to the progression of gastric cancer and the prognosis of patients. Subsequently, the difference in FBN1 expression levels between gastric cancer tissues and adjacent tissues was further detected, and it was found that the expression level of FBN1 in gastric cancer tissues was dramatically higher than that in adjacent tissues. It was also found that the expression level of FBN1 was closely related to the degree of tumor invasion of gastric cancer, and the higher the expression level of FBN1 was, the deeper the degree of tumor invasion in patients. Wang et al. (2022) showed that FBN1 could mediate the phosphorylation of vascular endothelial growth factor receptor 2, activate the downstream adherent plaque kinase/protein kinase B pathway, promote the metastasis of ovarian cancer and regulate the resistance and sensitivity of ovarian cancer to the chemotherapy drug cisplatin (18). These results indicated that FBN1 could be involved in the genesis and development of tumors as an oncogenic factor. Subsequently, in this study, the gastric cancer SGC-7901 cell line with FBN1 overexpression and knockout was prepared by lentivirus transfection technology, and the effect of overexpression and knockout was verified by RT–qPCR and Western blot. Subsequently, the effect of FBN1 expression regulation on the proliferation activity of gastric cancer cell SGC-7901 was detected by CKK-8 assay (19), and it was found that overexpression of FBN1 could promote the proliferation of gastric cancer cell SGC-7901, while deletion of FBN1 could inhibit cell proliferation. Plate clonogenesis assays (20) and flow cytometry (21) were used to detect the effects of FBN1 expression regulation on the clonogenesis and apoptosis of SGC-7901 gastric cancer cells, and it was found that overexpression of FBN1 could promote the clonogenesis and inhibit apoptosis of SGC-7901 gastric cancer cells. FBN1 knockout inhibited cell clonogenesis and promoted cell apoptosis. These results confirmed that FBN1 could play a role in the progression of gastric cancer as an oncogenic gene.

Many studies have confirmed that signal pathway transduction is closely related to the progression of gastric cancer (22,23). AKT/GSK3β is an important intracellular signal transduction pathway that regulates the activation of a variety of downstream effector molecules, participates in the biological processes of cell proliferation and apoptosis, and participates in the progression of tumors (24). AKT is a Ser/Thr protein. When phosphorylated and activated at T308 and S473, AKT can activate GSK3β, Caspase-9, and BAD and participate in the regulation of tumor progression and the inflammatory response (25). GSK3β is a serine/threonine kinase and is widely distributed in eukaryotic cells. Studies have confirmed that GSK3β regulates cell proliferation and apoptosis, embryonic development, and immune responses (26). An increasing number of studies have confirmed that changes in the expression levels of key proteins in the AKT/GSK3β signaling pathway are involved in the occurrence and development of gastric cancer (27,28). Sukawa et al. (2012) showed that the hyperphosphorylation of AKT is closely related to the poor prognosis of gastric cancer patients (29). To investigate whether the AKT/GSK3β pathway is involved in gastric cancer when FBN1 expression is abnormal, the expression and phosphorylation levels of AKT and GSK3β proteins in SGC-7901 gastric cancer cells after FBN1 overexpression and knockout were detected. The results showed that FBN1 overexpression promoted the phosphorylation of AKT and GSK3β in SGC-7901 cells, while FBN1 knockout inhibited the phosphorylation of AKT and GSK3β in SGC-7901 cells. These results suggested that the abnormally high expression of FBN1 can activate the AKT/GSK3β signaling pathway to play a role in promoting the progression of gastric cancer.

This study aimed to explore the mechanism by which FBN1 expression regulates the progression of gastric cancer. The results revealed that FBN1 was strongly expressed in gastric cancer tissues. FBN1 knockout can inhibit the proliferation and clonal formation of gastric cancer cells and promote apoptosis by inhibiting the activation of the AKT/GSK3β signaling pathway. Nevertheless, the limitation of this study is that only gastric cancer cells were used to verify the effects of FBN1 expression regulation on cell proliferation, clonal formation, and apoptosis. To verify the effect of FBN1 expression regulation on gastric cancer tumor growth and survival rate, animal tumor-bearing models should be prepared for further exploration. In conclusion, this study can provide experimental materials for finding new biomarkers and therapeutic targets for the early diagnosis of gastric cancer.

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