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Original Article

Fatty Acid Binding Protein 5 (FABP5) Promotes Aggressiveness of Gastric Cancer Through Modulation of Tumor Immunity

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ABSTRACT

Purpose: Gastric cancer (GC) is the second most lethal cancer globally and is associated with poor prognosis. Fatty acid-binding proteins (FABPs) can regulate biological properties of carcinoma cells. FABP5 is overexpressed in many types of cancers; however, the role and mechanisms of action of FABP5 in GC remain unclear. In this study, we aimed to evaluate the clinical and biological functions of FABP5 in GC.
Materials and Methods: We assessed FABP5 expression using immunohistochemical analysis in 79 patients with GC and evaluated its biological functions following in vitro and in vivo ectopic expression. FABP5 targets relevant to GC progression were determined using RNA sequencing (RNA-seq).
Results: Elevated FABP5 expression was closely associated with poor outcomes, and ectopic expression of FABP5 promoted proliferation, invasion, migration, and carcinogenicity of GC cells, thus suggesting its potential tumor-promoting role in GC. Additionally, RNA-seq analysis indicated that FABP5 activates immune-related pathways, including cytokine-cytokine receptor interaction pathways, interleukin-17 signaling, and tumor necrosis factor signaling, suggesting an important rationale for the possible development of therapies that combine FABP5-targeted drugs with immunotherapeutics.
Conclusions: These findings highlight the biological mechanisms and clinical implications of FABP5 in GC and suggest its potential as an adverse prognostic factor and/or therapeutic target.

Keywords: Fatty acid binding protein 5; Gastric cancer; Biomarker; Interleukin-17

INTRODUCTION

Gastric cancer (GC), a common malignancy of the digestive tract and the second most lethal cancer worldwide, exhibits poor prognosis [1]. In 2020, approximately 1,089,103 new GC cases and 768,793 GC-related deaths were reported [2]. Despite significant improvements

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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in chemotherapy, targeted therapy, and immunotherapy for GC in the previous decade, the prognosis remains very poor, with a 5-year survival rate of less than 30% [3,4]. Surgical resection remains the optimal choice for patients with resectable tumors; however, the proportion of recurrence after curative surgery ranges from 20% to 50% [4,5]. Over half of the patients exhibit local progression or metastatic GC upon initial diagnosis [1] and require systemic treatment. However, as GC is resistant to traditional chemotherapy, more effective genetic biomarkers and therapeutic approaches are urgently needed.

Members of the fatty acid-binding protein (FABP) family share a highly conserved homologous sequence that folds into a 14–15 kDa intracellular lipid-binding protein that binds hydrophobic ligands, including unsaturated and saturated long-chain fatty acids (LCFAs), and other lipids that are highly abundant in the cytosol [6,7]. FABPs not only participate in the absorption of fatty acids and intracellular storage, but also in mitochondrial lipid oxidation, signal transmission, transport, and membrane synthesis, as well as in modulation of gene expression and cell proliferation and differentiation [8]. Additionally, FABPs regulate the biological activities of cancer cells [9,10], with previous research showing that FABP expression in human glioma tissues regulates cell proliferation and respiration in glioblastoma cells [11].

Several studies have revealed that epidermal FABP (FABP5) is overexpressed in several malignancies, including kidney, cervical, and hepatic carcinomas [12-14]. FABP5 promotes tumor progression and metastasis via the epithelial-mesenchymal transition (EMT) pathway in hepatic carcinomas [14]. Additionally, upregulated FABP5 expression stimulates an increase in mitochondrial oxidative metabolism, leading to upregulation of CD137 expression in exhausted T cells in hepatocellular carcinoma, thereby representing an immune-metabolic marker [15]. Compared to adjacent tissues, cancer tissues show a higher proportion of infiltrating CD8+FABP5+ T cells that positively express programmed cell death protein-1 (PD-1) [15].

Immune-checkpoint inhibitors represent an advancement in cancer treatment, with promising results reported in several malignancies. In 2017, a PD-1-blockade agent was approved by Food and Drug Administration of the United States for treatment of patients with metastatic or recurrent locally advanced gastroesophageal junction adenocarcinoma and GC with tumor programmed cell death ligand-1 (PD-L1) expression of >1%. However, treatment with anti-PD-1/PD-L1 agents in patients with advanced gastric or gastroesophageal junction cancer yielded a response rate of 9.9% and disease control rate of 30.8%, both of which were lower than those for other tumors with a high mutation burden [16]. Moreover, treatment with a PD-L1 antagonist decreased *Fabp4* and *Fabp5* expression in gastric adenocarcinoma tumor cells and increased *Fabp4/5* expression in tissue-resident memory T cells by activating their lipid uptake, which prolonged their cell survival [17]. Furthermore, in vivo studies in these cells showed that, compared with responders to PD-L1 blockade, patient-derived GC xenograft mice in the non-responder group harbored fewer tissue-resident memory T cells [17]. These findings suggest that reprogramming fatty acid metabolism might represent a promising method for enhancing antitumor immunity in GC.

In the present study, we evaluated the function of *FABP5* in GC cell lines and identified it as a putative prognostic biomarker that can be used in patients with GC. Moreover, we revealed an association between *FABP5* and PD-L1, which provides a new perspective on the function of FABPs in antitumor immunology.

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MATERIALS AND METHODS

Tissue specimens and clinicopathological data

Tissues from 79 patients with GC and 25 non-tumor formalin-fixed paraffin-embedded (FFPE) tissue samples were acquired from 37–77-year-old patients who underwent radical surgical resection at the Department of Gastrointestinal Surgery at Zaozhuang Municipal Hospital (Zaozhuang, China) between January 2011 and December 2015. This study was approved by the Ethics Committee of Zaozhuang Municipal Hospital, and all the patients provided written informed consent. Patients who had received anti-malignant tumor therapy prior to surgery were excluded. We collected data related to clinical and pathological factors, including sex, age, tumor node metastasis (TNM) stage, tumor size, depth of tumor invasion, lymphatic metastasis, and tumor grading. Overall survival (OS) was defined as the time from initial surgery to death or final follow-up.

Immunohistochemical (IHC) analysis

Paraffin-embedded sections (4 μm thickness) were used for IHC analysis using standard procedures [18]. Antibodies to FABP5 (ab37267; Abcam, Cambridge, UK), PD-L1, tumor necrosis factor (TNF), IN-17 (22C3, Dako; Agilent Technologies, Santa Clara, CA, USA) were used for IHC analyses. Three experienced pathologists scored the slides independently. The immunostaining score was based on the degree of staining and staining area of the cells. Briefly, the percentage scores were defined as follows: 0 (0%), 1 (0%–30%), 2 (30%–60%), and 3 (>60%). The intensity score was defined as follows: 3, intense staining; 2, moderate staining; 1, weak staining; and 0, negative staining. Comprehensive staining intensity and percentage scores were presented as final scores ranging from 0 to 6, with the cut-off value being a staining intensity score of 3.

Cell culture and RNA interference

All GC cell lines were acquired from the Shanghai Institute of Cell Biology (Shanghai, China). The human gastric mucosal cell line GES-1 and the GC cell lines SGC-7901, HGC-27, AGS, BGC-823, and MGC-803 were cultured in RPMI-1640 medium (Gibco BRL, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA), 100 μg/mL streptomycin, and 100 U/mL penicillin. The culture medium containing the cells was placed in a humidified 5% CO_2 atmosphere at 37°C. Chemically modified small-interfering RNAs (siRNAs) targeting *FABP5* and control siRNAs were obtained from Genewiz (Suzhou, China). The sequences of the siRNAs are provided in the **Supplementary Table 1**. Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was used for siRNA transfection.

Establishment of FABP5-overexpressing cells

Lentiviral vectors overexpressing FABP5 (LV-FABP5) were purchased from Genewiz and used to transfect the GC cell lines according to the manufacturer's instructions. Stable transfected clones were screened using 2.5 μg/mL puromycin for the SGC-7901 cell line, whereas 1.0 μg/mL puromycin was used to screen stable clones of the BGC-823 cell line.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the GC cell lines using the TRIzol RNA Purification Kit (Invitrogen). Total RNA (up to 1 μg) was prepared, and first-strand cDNA was synthesized using the RevertAid First Strand cDNA synthesis kit (Toyobo, Osaka, Japan). cDNA was subsequently analyzed employing a Step One Plus real-time PCR system (Toyobo) using AceQ qPCR SYBR Green master mix (Toyobo). PCR primers were designed and synthesized by Genewiz, and their sequences are shown in the **Supplementary Table 2**.

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Western blot (WB)

Radioimmunoprecipitation lysis buffer and phenylmethylsulfonyl fluoride were used to extract total protein from the cells, and protein concentrations were measured using Bicinchoninic Acid Protein Assay Kit (Invitrogen). Next, 20 μg of protein was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The separated proteins were then transferred onto polyvinylidene fluoride membranes that were subsequently blocked with 5% skim milk for 2 hours at 26°C and later incubated with anti-FABP5 (1:1,000, ab37267; Abcam) and anti-glyceraldehyde 3-phosphate dehydrogenase (1:1,000, abs139055; Absin, Shanghai, China). Next, the membranes were incubated with secondary antibody (immunoglobulin G, 1:6,000, abs20040; Abcam) at room temperature (26°C) for 1 hour. Protein bands were visualized using an enhanced chemiluminescence reagent (Millipore, Bedford, MA, USA). Relative expression of the target proteins was calculated as the intensity of the target protein bands divided by gray value of internal reference protein bands.

Cell counting assay

Cells (3×10^3) were seeded into each well of a 96-well plate, followed by the addition of 10 μL of Cell Counting Kit (CCK)-8 solution (Absin) to each well and incubation for 2 hours at 37°C. Absorbance of each well was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony formation assay

Cells were seeded in 6 cm culture dishes (600 cells/dish), followed by the addition of cell culture medium (4 mL per dish). After 2 weeks, the cells were fixed with methanol for 10 minutes and stained with 0.1% crystal violet for 30 minutes. The number of viable colonies and rate of colony formation were then determined.

Cell cycle analysis

At the prescribed time, cells were collected, rinsed twice with phosphate-buffered saline (PBS), and fixed with 75% ethanol overnight at 4°C. After fixation, the cells were rinsed to remove the ethanol, and propidium iodide/RNase staining buffer (BD Pharmingen, East Rutherford, NJ, USA) was added to the test tube. After incubation at 26°C for 15 minutes, cell cycle progression was determined using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo software (FlowJo LLC, Ashland, OR, USA).

Wound healing assay

Cells (5×10^5 cells/well) were added in 6-well culture plates. Upon reaching a cell density of up to 90%, the culture medium was replaced with serum-free medium, and the plates were incubated for 24 hours. Upon reaching a cell density of 100%, a sterile 200 μL pipette tip was used to create an artificial wound, followed by washing with PBS and addition of serum-free medium. Images of cell migration in the scratched region were acquired using an inverted microscope (Olympus, Tokyo, Japan) at 0, 24, and 48 hours.

Cell invasion assay

Cell invasion assays were performed using 24-well Matrigel invasion chambers (8.0 μm pore size; Corning Inc., Corning, NY, USA) and polyester membrane inserts (8.0 μm pore size; Corning Inc.) according to the manufacturer's instructions. Briefly, 2×10^4 cells in FBS-free medium were seeded in the upper chambers, and medium containing 10% FBS was added to the lower chambers. After 48 hours, the cells in the upper chamber were gently cleared with a cotton swab, and Diff-Quik staining kit (Sysmex, Hyogo, Japan) was used to stain the membranes to allow cell counting.

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Tumor growth in xenografts

All animal experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of the Shandong Tumor Hospital. GC cells (1×10^6) were suspended in 50 μL PBS and 50 μL Matrigel (Corning Inc.) and injected subcutaneously into both the shoulders of 5-week-old male BALB/cSlc-nu/nu mice (Beijing HFK Bioscience Co., Ltd., Beijing, China). The experimental and control groups consisted of seven mice each. Every 3 days, the tumor size (length and width) was measured using a caliper, and the tumor volume (mm^3) was determined as (width [mm]$^2\times$length [mm])/2. Tumor growth was observed in all mice for 18 days.

RNA-seq analysis

Total RNA was collected using the TRIzol reagent (Invitrogen), and RNA integrity was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies). Next-generation sequencing was performed using a HiSeq instrument (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Sequences were analyzed and processed by Novogene (Beijing, China). Pathway analysis was used to identify significant pathways associated with differentially expressed genes according to Gene Ontology categories and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Statistical analysis

Statistical analysis was performed using SPSS software (v20.0; IBM Corp., Armonk, NY, USA), and a two-tailed $P<0.05$ was considered statistically significant. Continuous variables were compared using the Student's t-test or Mann-Whitney U test in cases of non-normal distribution. We used the χ^2 test (or Fisher's exact test, when appropriate) to compare categorical variables. The Kaplan-Meier method and log-rank test were used for survival analysis. Independent prognostic factors for survival were determined using the Cox proportional hazards model.

RESULTS

FABP5 is highly expressed in GC and correlated with poor prognosis

To explore FABP5-expression levels in GC, we performed IHC analysis on 79 GC samples and 25 normal gastric mucosal samples. The IHC results showed that FABP5 expression was significantly higher in GC samples than in control samples (**Fig. 1A**). FABP5 was strongly upregulated in 53.2% of all the GC samples relative to 16% in the control group. Additionally, qRT-PCR analysis of FABP5 expression across 5 GC cell lines (BGC-823, HGC27, AGS, MGC-803, and SGC-7901) relative to that in a normal gastric mucosal cell line (GES-1) revealed that FABP5 was upregulated in 3 GC cell lines (AGS, MGC-803, and SGC-7901) than in the control, whereas 2 GC cell lines (BGC-823 and HGC27) showed lower FABP5 expression relative to the control (**Fig. 1B**). WB analysis revealed that FABP5 was upregulated in SGC-7901 cell lines relative to the control, whereas the 4 GC cell lines (AGS, MGC-803, BGC-823, and HGC27) exhibited lower FABP5 expression relative to the control (**Fig. 1C**).

To evaluate the association between FABP5 levels, clinicopathological factors, and clinical outcomes, we divided the cohort into groups demonstrating high and low FABP5 expression according to IHC staining. Correlations between the expression levels of FABP5 and clinicopathological features are summarized in **Table 1**. The results showed that FABP5 levels in GC correlated with the tumor state and sites of lymph node metastasis, with FABP5 expression gradually increasing with progression in tumor stages (**Table 1**). Additionally,

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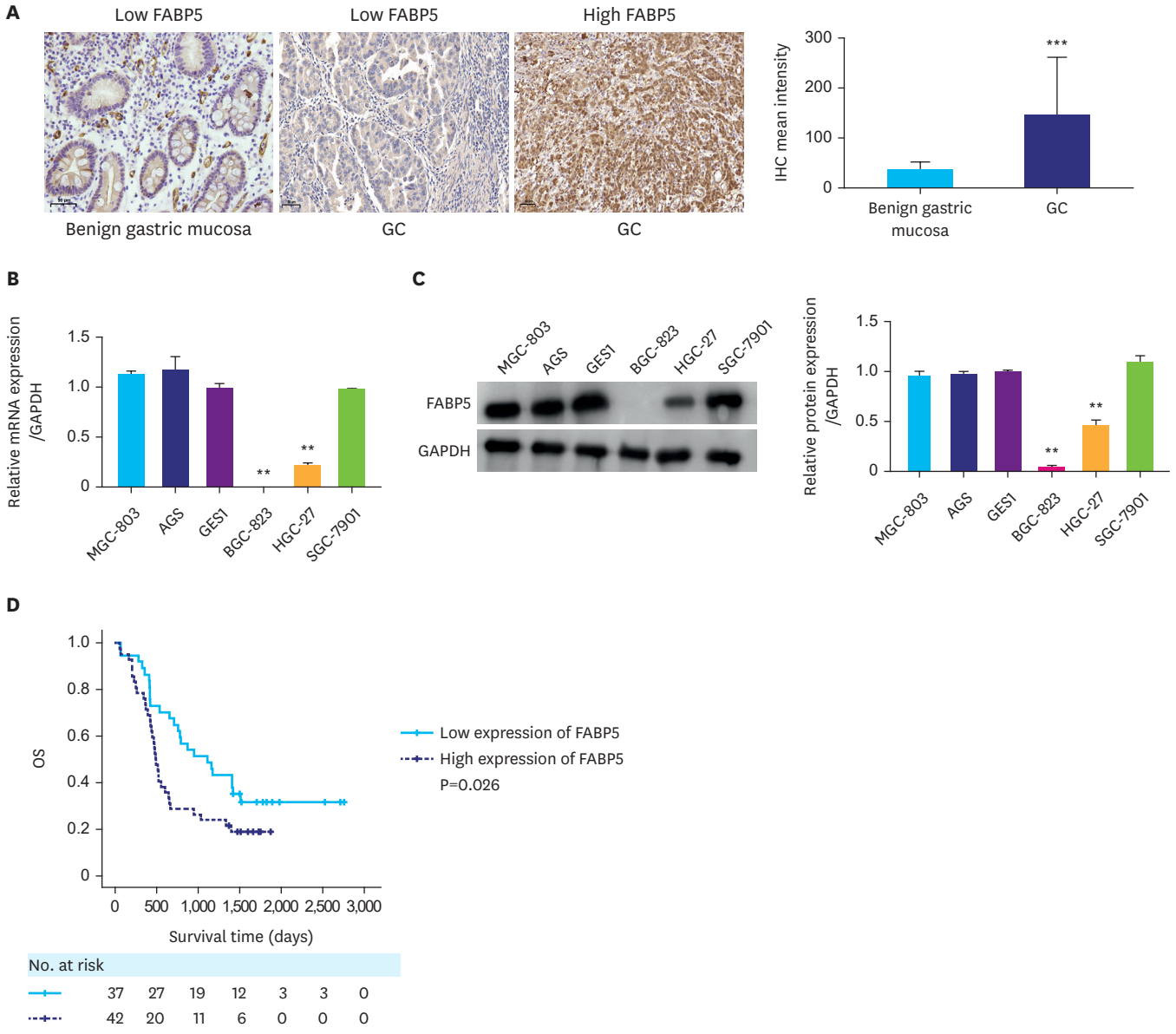

Fig. 1. Upregulated expression of FABP5 in GC cells correlates with poor prognosis. FABP5 expression was validated via IHC analysis of 79 GC FFPE samples and 25 normal gastric mucosal FFPE samples. (A) Representative images of FFPE tissue samples demonstrating IHC quantification of FABP5 expression. (B, C) Relative expression of FABP5 in 5 GC cell lines (BGC-823, HGC27, SGC-7901, MGC-803, and AGS) and a normal gastric mucosal cell line (GES-1) according to (B) qRT-PCR and (C) WB analyses. (D) Kaplan-Meier curve for OS in a cohort of 79 GC patients displaying correlation between FABP5 expression and OS.
FABP = fatty acid-binding protein; GC = gastric cancer; IHC = immunohistochemical; FFPE = formalin-fixed paraffin-embedded; qRT-PCR = quantitative reverse transcription polymerase chain reaction; WB = Western blot; OS = overall survival; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.
**P<0.01, ***P<0.001.

patients exhibiting T3–T4 tumor stages showed higher FABP5-expression levels than those with T1–T2 stages (62.3% vs. 22.2%, P=0.003), and FABP5 levels in the N2–N3 group were markedly higher than those in the N0–N1 group (61.4% vs. 31.8%, P=0.018).

Survival data were recorded for all the patients with GC (median follow-up period, 25.6 months; range, 2.1–90.2 months). Prognosis was worse in patients with high FABP5 expression than in those with low FABP5 expression (37.1 vs. 16.3 months, P=0.026) (**Fig. 1D**). According to univariate analysis of risk factors associated with OS, FABP5 expression

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Table 1. Correlation of *FABP5* expression and clinicopathological features of gastric cancer

Variable	Level of *FABP5* expression		P-value
	Low	High	
Sex			0.71
Male	25	30	
Female	12	12	
Age			0.434
>65 years	11	16	
≤65 years	26	26	
Depth of tumor invasion			**0.003**
T1–2	14	4	
T3–4	23	38	
Tumor size			0.879
>4 cm	27	30	
≤4 cm	10	12	
Tumor grade			0.244
Poor	9	11	
Moderate	12	20	
Well	16	11	
Lymph node metastases			**0.018**
N0–N1	15	7	
N2–N3	22	35	

The bold values refer to P<0.05.
FABP = fatty acid-binding protein.

combined with T stage, N stage, tumor diameter, and tumor differentiation suggested that FABP5 is a potentially important prognostic factor for patients with GC (**Table 2**). We then included factors found to be significant according to univariate analysis in multivariate Cox proportional hazards analysis, which confirmed high FABP5 expression as an independent biomarker of poor prognosis (hazard ratio, 1.89; 95% confidence interval, 1.66–2.19; P=0.043). These data indicate the importance of FABP5 expression in the occurrence and development of GC.

FABP5 is correlated with GC cell proliferation

As elevated FABP5 levels are closely related to poor prognosis in GC, we explored whether FABP5 functions as a tumor promoter. To determine this, we performed gain-of-function assays in BGC-823 cells via transient transfection with a lentivirus that enabled FABP5 overexpression, which could be screened using puromycin and detected using immunofluorescence (**Supplementary Fig. 1**). We confirmed FABP5 overexpression using qRT-PCR and WB (**Supplementary Fig. 1**) and found that GC cell lines with elevated FABP5 expression exhibited short-term proliferative activities compared with controls (**Fig. 2A**). We then evaluated the influence of FABP5 overexpression on the long-term cell proliferation of

Table 2. Univariate and multivariate analyses of *FABP5* expression and clinicopathological factors associated with OS

Variable	OS					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (>65 years/≤65 years)	1.23	0.72–2.10	0.447			
Sex (female/male)	1.23	0.77–2.13	0.458			
FABP5 expression (yes/no)	1.56	1.33–1.94	0.028	1.89	1.66–2.19	0.043
Depth of tumor invasion	3.02	1.42–6.39	0.004	2.62	1.17–5.87	0.019
Lymph node metastases	2.37	1.25–4.50	0.008	1.79	0.92–3.51	0.088
Tumor grade	1.43	1.01–2.02	0.042	1.42	0.98–2.06	0.066
Tumor size	0.89	0.51–1.57	0.696			

The bold values refer to P<0.05.
FABP = fatty acid-binding protein; OS = overall survival; HR = hazard ratio; CI = confidence interval.

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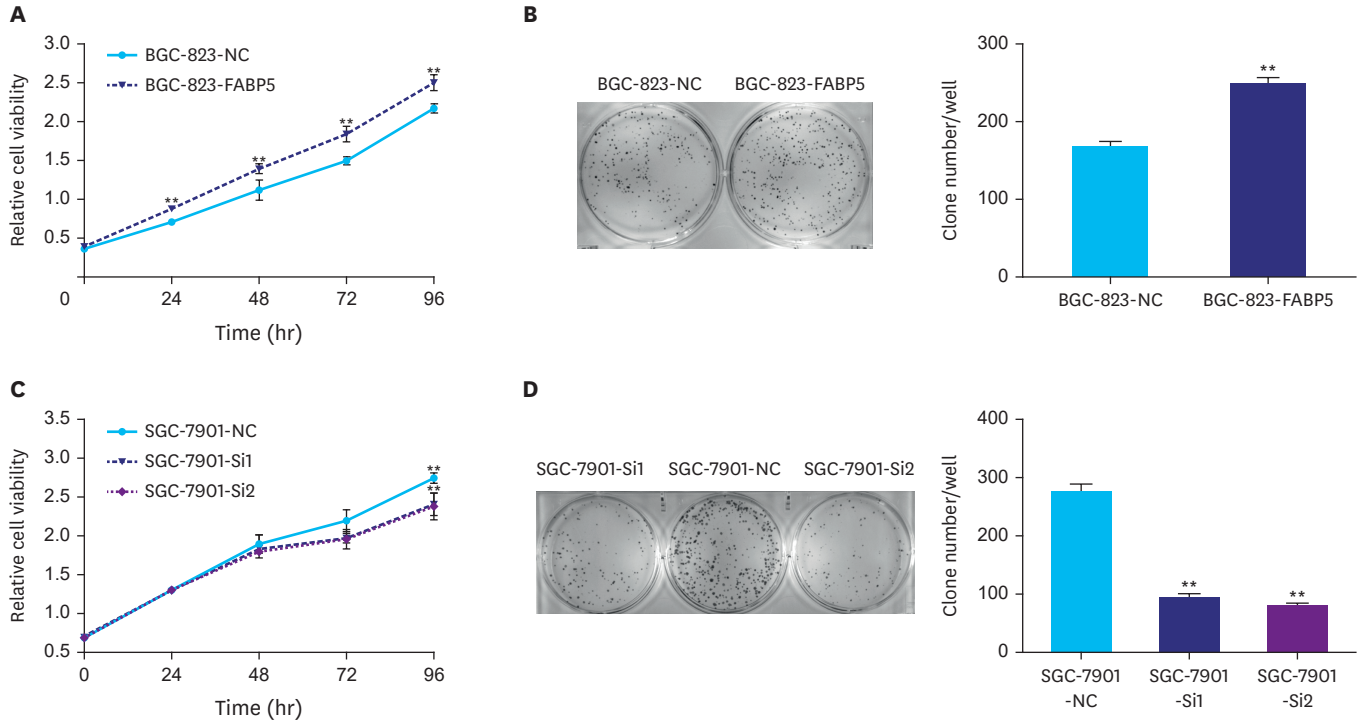

Fig. 2. FABP5 correlates with GC cell proliferation. (A) BGC-823 cells were transfected with FABP5 lentivirus (BGC-823-FABP5) or normal control (BGC-823-NC), and cell growth was evaluated using the CCK-8 assay at various time points post-transfection. (B) Photos of viable colonies of BGC-823 cells infected with the BGC-823-FABP5 or BGC-823-NC lentivirus. Data represent the mean ± standard deviation (n=3). (C) SGC-7901 cells were transfected with *FABP5* siRNA (SGC-7901-si1 and SGC-7901-si2) or normal control (SGC-7901-NC). Cell growth was evaluated using the CCK-8 assay at various time points post-transfection. (D) Photos of viable colonies of SGC-7901 cells transfected with *FABP5* siRNA or SGC-7901-NC. Data represent the mean ± standard deviation (n=3).
FABP = fatty acid-binding protein; GC = gastric cancer; CCK = Cell Counting Kit; siRNA = small-interfering RNA.
**P<0.01.

GC cell lines using colony formation assays; the results showed significantly increased colony numbers of the GC cells that overexpressed FABP5 than those of the control cells (**Fig. 2B**).

Next, we determined the effect of inhibited FABP5 expression on GC cell proliferation by siRNA-mediated knockdown of FABP5 in SGC-7901 cells. Following confirmation of FABP5 knockdown using qRT-PCR and WB (**Supplementary Fig. 2**), CCK-8 assays revealed that the knockdown exerted significant short-term anti-proliferative effects (**Fig. 2C**). Additionally, colony formation assays verified that stable knockdown of FABP5 decreased the number of GC cell colonies over the long term (**Fig. 2D**).

To investigate whether the proliferative effect of FABP5 was caused by cell cycle redistribution, we performed cell cycle analysis. The results showed that FABP5 overexpression significantly decreased the proportion of cells in the G0/G1 phase, whereas the proportion of cells in the S phase increased (**Fig. 3A**). By contrast, inhibition of FABP5 expression resulted in a significant increase in the number of GC cells in the G0/G1 phase and a decrease in the number of cells in the S phase (**Fig. 3B**). These findings indicate that FABP5 promotes the proliferation of GC cells by mediating cell cycle transition from the G0/G1 phase to S phase.

FABP5 expression is correlated with GC migration and invasion

Based on the results that suggested a relationship between FABP5 expression and the pathological stage of GC cells, we hypothesized that FABP5 expression is correlated with metastasis and aggressiveness of GC. To determine whether FABP5 can directly induce

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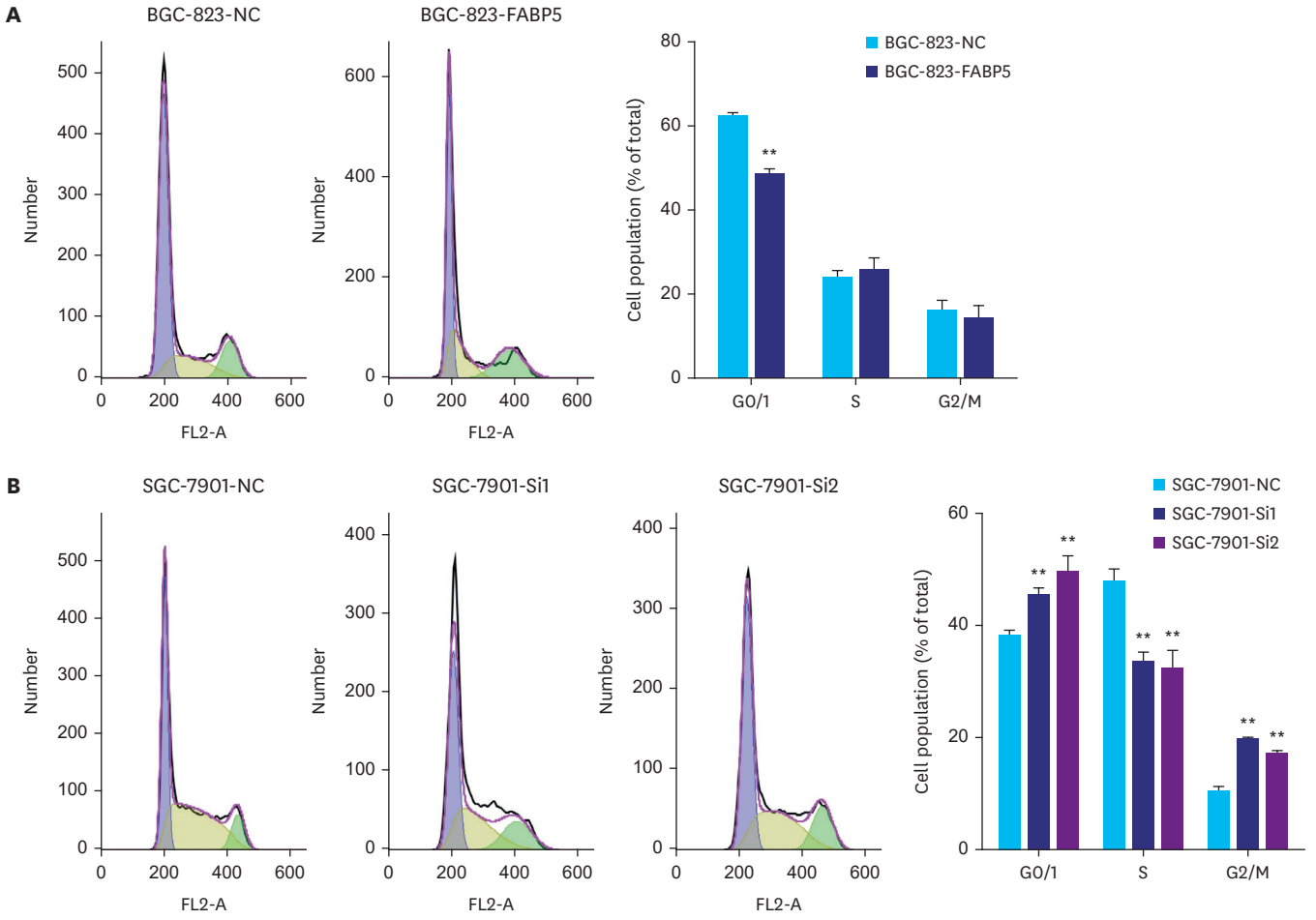

Fig. 3. FABP5 expression correlates with GC cell tumor growth. (A) BGC-823 cells were stained with propidium iodide at 24- and 48-hour post-transfection with FABP5 lentivirus (BGC-823-FABP5) or normal control (BGC-823-NC). Cell cycle distribution was analyzed using flow cytometry. (B) SGC-7901 cells were stained with propidium iodide at 24- and 48-hour post-transfection with *FABP5* siRNA (SGC-7901-si1 and SGC-7901-si2) or normal control (SGC-7901-NC). Cell cycle distribution was analyzed using flow cytometry.
FABP = fatty acid-binding protein; GC = gastric cancer; siRNA = small-interfering RNA.
**P<0.01.

the metastatic phenotype in GC, we performed cell invasion and wound healing assays to evaluate invasion and migration activities of GC cells. The results showed that FABP5 overexpression significantly promoted the invasion and migration of GC cells (**Fig. 4A and B**), whereas FABP5 knockdown reduced these activities (**Fig. 4C and D**).

FABP5 expression is correlated with GC tumorigenesis in vivo

To determine the influence of FABP5 overexpression on GC activity in vivo, we subcutaneously implanted BGC-823 cells overexpressing FABP5 into the shoulders of BALB/c mice. The results showed that the tumor size in the implanted mice was 1.5-fold larger than that in mice in the control group. This result suggests that mice carrying FABP5-overexpressing GC cells demonstrated increased tumor growth (**Fig. 5A**), thereby confirming the role of FABP5 in promoting GC tumorigenesis in vivo.

To investigate the association between FABP5 and PD-L1 in GC, we performed IHC analysis to evaluate PD-L1 levels in 79 patients with GC. Among the 79 patients in the cohort, 40.5% demonstrated PD-L1-positivity (**Fig. 5B**). Interestingly, compared with the low FABP5

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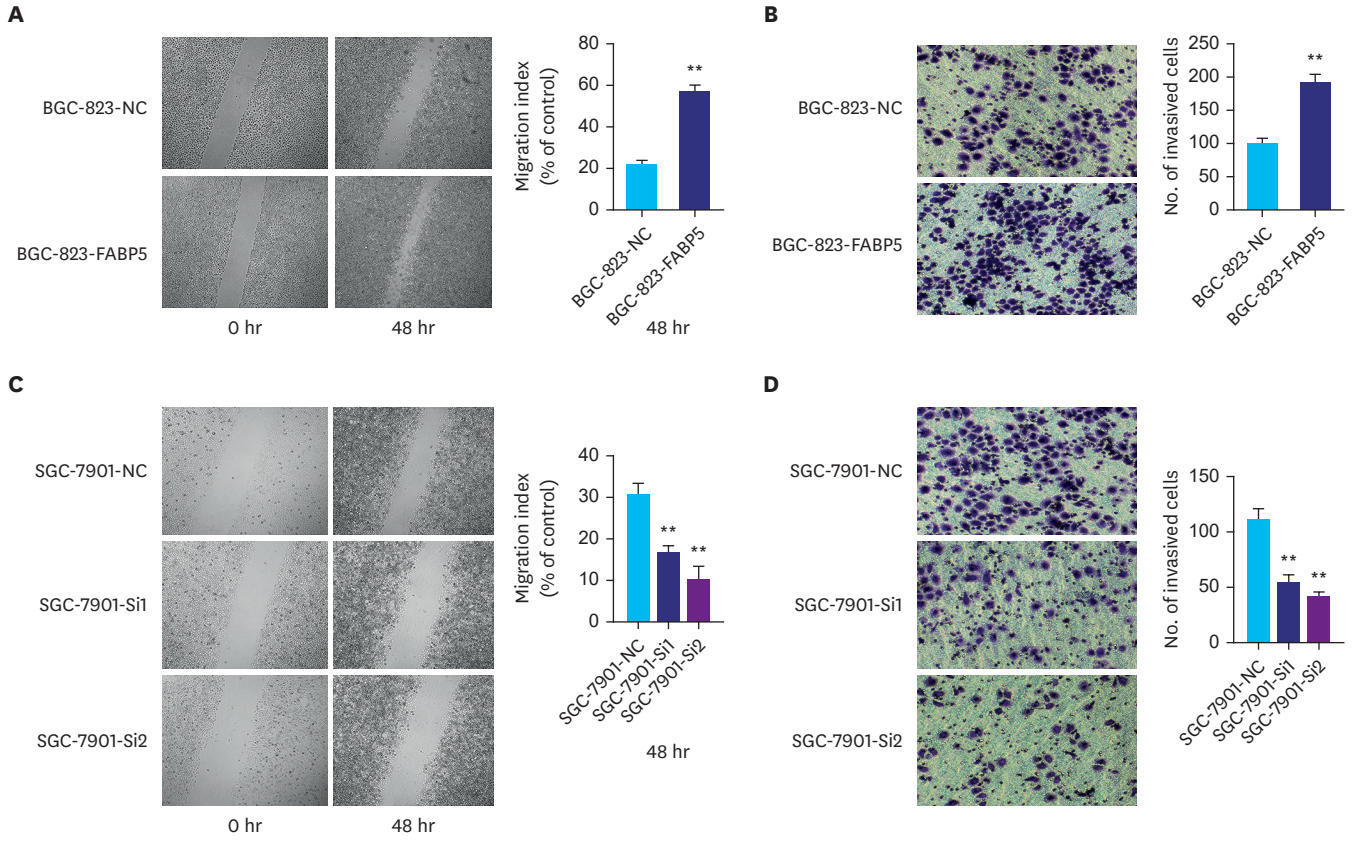

Fig. 4. FABP5 expression correlates with GC migration and invasion. (A) Wound healing assay of BGC-823 cells after transfection with FABP5 lentivirus (BGC-823-FABP5) or normal control (BGC-823-NC). Phase-contrast images were obtained at the indicated time points. Bar chart graphs represent data from 3 independent experiments. (B) Representative images of Transwell migration and invasion assays of BGC-823-FABP5 and BGC-823-NC cells. The numbers of invasive cells per field were calculated, and the results are presented as the mean ± standard deviation (n=3). (C) Wound healing assay of SGC-7901 cells after transfection with *FABP5* siRNA (SGC-7901-si1 and SGC-7901-si2) or normal control (SGC-7901-NC). Phase-contrast images were obtained at the indicated time points. Bar chart graphs represent data from 3 independent experiments. (D) Representative images of Transwell migration and invasion assays of SGC-7901-si1, SGC-7901-si2, and SGC-7901-NC cells. The numbers of invasive cells per field were calculated, and the results are presented as the mean ± standard deviation (n=3).
FABP = fatty acid-binding protein; GC = gastric cancer.
**P<0.01.

expression group, the group with high FABP5 expression showed a higher PD-L1 positivity rate (59.5% vs. 18.9%, P<0.05). To verify these results, we determined whether an association exists between FABP5 and PD-L1 in xenograft murine models using IHC analysis. The results confirmed that mice with elevated FABP5 expression showed a significantly higher PD-L1 positivity rate in vivo (21.4% vs. 64.3%, P<0.05; **Fig. 5C**).

Furthermore, to identify the targets of FABP5 relevant to GC progression, we performed RNA-seq analysis using tissues obtained from the murine xenograft model. According to KEGG pathway enrichment analysis, FABP5 enhanced immune-associated gene expression, including genes related to the TNF signaling pathway, cytokine-cytokine receptor interaction pathway, and interleukin (IL)-17 signaling pathway (**Fig. 5D**). IHC results showed that mice with elevated FABP5 expression showed significantly higher TNF (**Fig. 5E**) and IL-17 (**Fig. 5F**) positivity rates in vivo (P<0.05). In addition, we found that the expression of PD-L1, TNF, and IL-17 in GC cell lines positively correlated with that of FABP5 (P<0.05; **Fig. 5G**).

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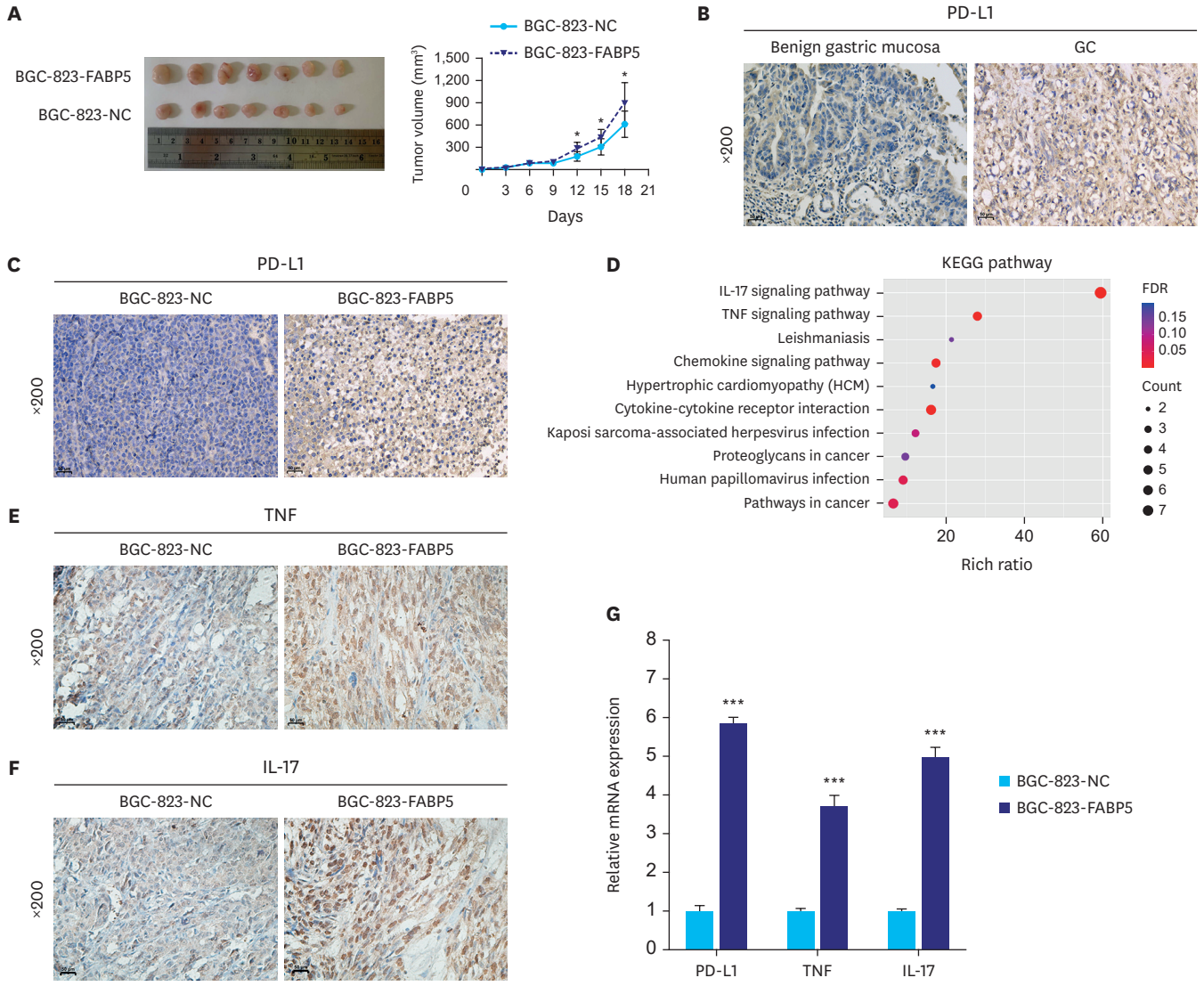

Fig. 5. FABP5 expression correlates with GC tumorigenesis in vivo. (A) BGC-823 cells stably overexpressing FABP5 (or control cells were injected subcutaneously in BALB/c mice (n=5/group); tumor volumes were monitored every 3 days. (B, C) The mice were sacrificed 18 days after injection. Representative images of FFPE tissue samples with PD-L1 expression in (B) GC patients and (C) xenograft murine model. (D) KEGG analysis reveals a significantly enriched pathway in BGC-823 cells stably overexpressing FABP5. (E, F) Representative images of FFPE tissue samples with (E) TNF and (F) IL-17 expression in the xenograft murine model. (G) Expression of PD-L1, TNF, IL-17 and FABP5 in the gastric cancer cell line.
FABP = fatty acid-binding protein; GC = gastric cancer; FFPE = formalin-fixed paraffin-embedded; PD-L1 = programmed cell death ligand-1; KEGG = Kyoto Encyclopedia of Genes and Genomes; TNF = tumor necrosis factor; IL = interleukin.
*P<0.05, ***P<0.001.

DISCUSSION

FABPs are small, highly conserved proteins that bind to LCFAs [19]. As a transporter of LCFAs, FABPs also participate in the growth, metabolism, migration, and progression of cancer cells [20]. FABP5 is upregulated in various cancers, and a previous study demonstrated its ability to mediate the progression of renal clear cell carcinoma via the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway [12]. Additionally, FABP5 is overexpressed in breast cancer, and the FABP5/peroxisome proliferator-activated receptor δ axis plays a crucial role in tumorigenesis and metastasis of breast cancer cells by enhancing stability of the epidermal growth factor receptor [21,22].

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The results of the present study demonstrate overexpression of FABP5 in GC cells and tissues compared to that in normal control cells, along with a significant upregulation of FABP5 levels in advanced GC. This suggests that FABP5 may be closely associated with tumorigenesis and progression of GC. Additionally, we found that patient survival rate was negatively correlated with FABP5 expression, and univariate and multivariate analyses identified FABP5 expression as a predictor of poor prognosis of GC. Furthermore, proliferation, invasion, migration, and tumorigenicity of GC cells were promoted because of ectopic expression of FABP5. These findings are consistent with those of a previous study showing that FABP5 reprograms fatty acid metabolism and induces lymphatic metastasis in cervical cancer [23].

PD-L1 is an important immune-checkpoint protein capable of binding to PD-1 and CD80 present on the surface of T cells, thereby protecting cancer cells by inhibiting T effector functions [24]. Additionally, PD-L1 is important in controlling gastric adenocarcinoma cell metabolism and is expressed in all the stages of gastric adenocarcinoma, both in tumor cells and the immune stroma [25]. In this study, we found that PD-L1 expression in GC cells is related to lipid metabolism. In human patients, PD-L1 expression is positively associated with FABP5 levels, as confirmed in murine models. These findings suggest that reprogramming the metabolism of lipids might represent a promising therapeutic approach, in combination with PD-1 blockade, for GC treatment.

RNA-seq analysis, performed to investigate the relevant pathways affected by FABP5 overexpression, revealed differential expression of molecules involved in immune-related signaling pathways, including IL-17 and TNF-α. Wang et al. [26] showed that FABP5 expression mediates EMT via TNF-α-dependent nuclear factor-kappa B (NF-κB) signaling in low-grade gliomas. Additionally, Liu et al. [15] showed the potential of FABP5 as an immune-metabolic marker of hepatocellular carcinoma and that FABP5 is enriched in mitochondrial oxidative metabolism of exhausted T cells. Moreover, FABP5 regulates the inflammatory function of dendritic cells and macrophages and counterbalances the levels of T helper (Th) 17 and regulatory T cells via the IL-21/retinoic acid receptor-related orphan receptor/IL-17 pathway [27]. Lee et al. [28] showed that FABP5 may directly induce Th17 responses in patients with atopic dermatitis with an atopic march by significantly increasing Th17-inducing cytokines in keratinocytes and IL-17A in T cells. Furthermore, Senga et al. [29] reported that FABP5 promotes cytokine production via NF-κB signaling, which is activated by reactive oxygen species and protein kinase C in prostate and breast cancers.

In summary, we demonstrated that FABP5 is overexpressed in GC cells and identified its potential role in tumorigenesis. Previous studies have shown that FABP5 plays a key role in cancer cell proliferation and metastasis. The present study supports such findings in GC and suggests that FABP5 can be used as a prognostic biomarker for GC. Additionally, the results indicate the potential efficacy of targeting FABP5 as a strategy for therapeutic use in GC. Furthermore, our data showing that FABP5 overexpression regulated PD-L1/TNF/IL-17 signaling offer a rationale for possible development of combination therapies that include FABP5-targeted drugs, PD-1-blockade agents, and other immunotherapies.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Sequences of siRNAs targeting *FABP5* and control siRNA

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Supplementary Table 2

Primer sequences used in this study

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Supplementary Fig. 1

Transfection of BGC-823 cells with the *FABP5* lentivirus. (A) Immunofluorescence of infected BGC-823 cells screened using puromycin. (B, C) *FABP5* expression according to qRT-PCR (B) and WB (C).

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Supplementary Fig. 2

Transfection of SGC-7901 cells with *FABP5* siRNA. (A) Confirmation of decreased *FABP5* mRNA levels by qRT-PCR and (B) *FABP5* knockdown by WB.

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REFERENCES

1. Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018;10:239-248.
PUBMED | CROSSREF
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-249.
PUBMED | CROSSREF
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
PUBMED | CROSSREF
4. Kim Y, Spolverato G, Ejaz A, Squires MH, Poultsides G, Fields RC, et al. A nomogram to predict overall survival and disease-free survival after curative resection of gastric adenocarcinoma. Ann Surg Oncol 2015;22:1828-1835.
PUBMED | CROSSREF
5. Marrelli D, De Stefano A, de Manzoni G, Morgagni P, Di Leo A, Roviello F. Prediction of recurrence after radical surgery for gastric cancer: a scoring system obtained from a prospective multicenter study. Ann Surg 2005;241:247-255.
PUBMED | CROSSREF
6. Thumser AE, Moore JB, Plant NJ. Fatty acid binding proteins: tissue-specific functions in health and disease. Curr Opin Clin Nutr Metab Care 2014;17:124-129.
PUBMED | CROSSREF
7. Storch J, Thumser AE. Tissue-specific functions in the fatty acid-binding protein family. J Biol Chem 2010;285:32679-32683.
PUBMED | CROSSREF
8. Glatz JF, Storch J. Unravelling the significance of cellular fatty acid-binding proteins. Curr Opin Lipidol 2001;12:267-274.
PUBMED | CROSSREF

9. Cataltepe O, Arikan MC, Ghelfi E, Karaaslan C, Ozsurekci Y, Dresser K, et al. Fatty acid binding protein 4 is expressed in distinct endothelial and non-endothelial cell populations in glioblastoma. Neuropathol Appl Neurobiol 2012;38:400-410.
PUBMED | CROSSREF
10. Lee D, Wada K, Taniguchi Y, Al-Shareef H, Masuda T, Usami Y, et al. Expression of fatty acid binding protein 4 is involved in the cell growth of oral squamous cell carcinoma. Oncol Rep 2014;31:1116-1120.
PUBMED | CROSSREF
11. Lin H, Patel S, Affleck VS, Wilson I, Turnbull DM, Joshi AR, et al. Fatty acid oxidation is required for the respiration and proliferation of malignant glioma cells. Neuro-oncol 2017;19:43-54.
PUBMED | CROSSREF
12. Lv Q, Wang G, Zhang Y, Han X, Li H, Le W, et al. FABP5 regulates the proliferation of clear cell renal cell carcinoma cells via the PI3K/AKT signaling pathway. Int J Oncol 2019;54:1221-1232.
PUBMED | CROSSREF
13. Wang W, Jia HL, Huang JM, Liang YC, Tan H, Geng HZ, et al. Identification of biomarkers for lymph node metastasis in early-stage cervical cancer by tissue-based proteomics. Br J Cancer 2014;110:1748-1758.
PUBMED | CROSSREF
14. Ohata T, Yokoo H, Kamiyama T, Fukai M, Aiyama T, Hatanaka Y, et al. Fatty acid-binding protein 5 function in hepatocellular carcinoma through induction of epithelial-mesenchymal transition. Cancer Med 2017;6:1049-1061.
PUBMED | CROSSREF
15. Liu F, Liu W, Zhou S, Yang C, Tian M, Jia G, et al. Identification of FABP5 as an immunometabolic marker in human hepatocellular carcinoma. J Immunother Cancer 2020;8:e000501.
PUBMED | CROSSREF
16. Wang BC, Zhang ZJ, Fu C, Wang C. Efficacy and safety of anti-PD-1/PD-L1 agents vs chemotherapy in patients with gastric or gastroesophageal junction cancer: a systematic review and meta-analysis. Medicine (Baltimore) 2019;98:e18054.
PUBMED | CROSSREF
17. Lin R, Zhang H, Yuan Y, He Q, Zhou J, Li S, et al. Fatty acid oxidation controls CD8(+) tissue-resident memory T-cell survival in gastric adenocarcinoma. Cancer Immunol Res 2020;8:479-492.
PUBMED | CROSSREF
18. Fan Y, Zhou Y, Lou M, Gao Z, Li X, Yuan K. *SLC6A8* is a potential biomarker for poor prognosis in lung adenocarcinoma. Front Genet 2022;13:845373.
PUBMED | CROSSREF
19. Zhang W, Chen R, Yang T, Xu N, Chen J, Gao Y, et al. Fatty acid transporting proteins: roles in brain development, aging, and stroke. Prostaglandins Leukot Essent Fatty Acids 2018;136:35-45.
PUBMED | CROSSREF
20. Amiri M, Yousefnia S, Seyed Forootan F, Peymani M, Ghaedi K, Nasr Esfahani MH. Diverse roles of fatty acid binding proteins (FABPs) in development and pathogenesis of cancers. Gene 2018;676:171-183.
PUBMED | CROSSREF
21. Levi L, Lobo G, Doud MK, von Lintig J, Seachrist D, Tochtrop GP, et al. Genetic ablation of the fatty acid-binding protein FABP5 suppresses HER2-induced mammary tumorigenesis. Cancer Res 2013;73:4770-4780.
PUBMED | CROSSREF
22. Powell CA, Nasser MW, Zhao H, Wochna JC, Zhang X, Shapiro C, et al. Fatty acid binding protein 5 promotes metastatic potential of triple negative breast cancer cells through enhancing epidermal growth factor receptor stability. Oncotarget 2015;6:6373-6385.
PUBMED | CROSSREF
23. Zhang C, Liao Y, Liu P, Du Q, Liang Y, Ooi S, et al. FABP5 promotes lymph node metastasis in cervical cancer by reprogramming fatty acid metabolism. Theranostics 2020;10:6561-6580.
PUBMED | CROSSREF
24. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol 2012;24:207-212.
PUBMED | CROSSREF
25. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8:793-800.
PUBMED | CROSSREF
26. Wang Y, Wahafu A, Wu W, Xiang J, Huo L, Ma X, et al. FABP5 enhances malignancies of lower-grade gliomas via canonical activation of NF-κB signaling. J Cell Mol Med 2021;25:4487-4500.
PUBMED | CROSSREF

27. Li B, Reynolds JM, Stout RD, Bernlohr DA, Suttles J. Regulation of Th17 differentiation by epidermal fatty acid-binding protein. J Immunol 2009;182:7625-7633.
PUBMED | CROSSREF

28. Lee J, Kim B, Chu H, Zhang K, Kim H, Kim JH, et al. FABP5 as a possible biomarker in atopic march: FABP5-induced Th17 polarization, both in mouse model and human samples. EBioMedicine 2020;58:102879.
PUBMED | CROSSREF

29. Senga S, Kobayashi N, Kawaguchi K, Ando A, Fujii H. Fatty acid-binding protein 5 (FABP5) promotes lipolysis of lipid droplets, de novo fatty acid (FA) synthesis and activation of nuclear factor-kappa B (NF-κB) signaling in cancer cells. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863:1057-1067.
PUBMED | CROSSREF