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ORIGINAL RESEARCH

FAM105B Promotes Hepatocellular Carcinoma Progression and Metastasis by Activating the PI3K/AKT/MTOR Signaling Pathway and Inducing Epithelial-Mesenchymal Transition

Liu-Lin Yang[1,*], Xing Chen[2,*], Shao-Tong Tang[1], Kai-Ting Huang[1], Gui-Yan Ye[1], Ji-Long Wang[1]

[1]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China; [2]Department of Ultrasound, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Ji-Long Wang, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China, Email wangjilong-yucheng@163.com

Background: Recurrence and metastasis are major contributors to poor prognosis in hepatocellular carcinoma (HCC), yet the mechanisms remain unclear. FAM105B, a specific deubiquitinating enzyme, is critical in various biological processes, including cancer progression. However, its role in HCC is not well understood.

Methods: FAM105B expression in HCC patients was validated using public clinical datasets. Cox regression and Kaplan-Meier analyses assessed its association with clinicopathological features and prognosis. In vitro and in vivo experiments evaluated the effects of FAM105B on HCC cell proliferation and invasion. Its role in epithelial-mesenchymal transition (EMT) and the PI3K/AKT/MTOR pathway was analyzed via, Western blot, Reverse Transcription Quantitative Polymerase Chain Reaction (qRT-PCR), immunohistochemistry and immunofluorescence.

Results: FAM105B was significantly upregulated in HCC tissues and cell lines. High FAM105B expression correlated with aggressive features and poorer overall survival (OS) and disease-free survival (DFS). Functional studies revealed that FAM105B overexpression promoted, while knockdown inhibited, HCC cell proliferation and invasion. Mechanistically, FAM105B induced EMT and activated the PI3K/AKT/MTOR pathway.

Conclusion: FAM105B promotes HCC progression by inducing EMT and activating the PI3K/AKT/MTOR pathway, highlighting its potential as a therapeutic target and prognostic biomarker.

Keywords: FAM105B, hepatocellular carcinoma, proliferation, invasion, EMT, PI3K/AKT/MTOR

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer globally, with approximately 910,000 new cases annually. It ranks as the third leading cause of cancer-related mortality, accounting for around 830,000 deaths each year.[1] Over 80% of HCC cases are diagnosed at advanced stages, resulting in low surgical resection rates and a 5-year survival rate of less than 20%. These grim statistics highlight the urgent need for novel therapeutic strategies to improve prognosis and reduce the disease burden.[2,3] Invasion and metastasis are pivotal processes in HCC progression, contributing significantly to its high recurrence and mortality rates.[4–8] HCC frequently metastasizes to distant organs, such as the lungs and bones, via the bloodstream, drastically reducing patient survival.[9] These processes involve complex mechanisms, including epithelial-mesenchymal transition (EMT), extracellular matrix remodeling, and dysregulated signaling pathways, which compromise treatment efficacy and worsen prognosis.[10]

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Family with sequence similarity 105, member B (FAM105B), also known as Gumby or OTULIN (OUT domain-containing deubiquitinase with linear linkage specificity), is a protein-coding gene located on the human chromosome.[11,12] FAM105B plays crucial roles in angiogenesis, inflammation, and autoimmunity.[13] Studies have shown that mice lacking FAM105B or expressing catalytically inactive mutants die during the second trimester due to abnormal cell death and angiogenesis defects.[14,15] FAM105B also protects against hepatitis and HCC by inhibiting FADD- and RIPK1-mediated cell death.[16] Additionally, loss-of-function mutations in FAM105B are linked to OTULIN-associated autoinflammatory syndrome (ORAS), a systemic inflammatory condition.[17–19] Although the role of FAM105B in regulating tumor progression through the activation of the Wnt/β-catenin pathway has been well-established in breast cancer research,[20] its underlying mechanisms in HCC remain largely uncharacterized. To bridge this knowledge gap, we conducted a systematic bioinformatics analysis of the TCGA-LIHC dataset. Intriguingly, our analysis revealed a significant association between FAM105B expression and the PI3K/AKT/MTOR pathway. As a pivotal regulatory network governing cell proliferation and survival, dysregulation of this signaling axis has been implicated in the initiation and progression of HCC.[21] Based on these findings, this study aims to comprehensively investigate whether FAM105B contributes to the malignant progression of HCC by modulating the PI3K/AKT/MTOR signaling cascade.

This study aims to elucidate the role of FAM105B in HCC, focusing on its impact on tumor initiation, progression, invasion, and metastasis through the regulation of key signaling pathways and cellular processes. The findings are expected to provide a novel theoretical framework and identify potential targets for HCC diagnosis and treatment.

Materials and Methods

Bioinformatics Analysis

FAM105B expression across various cancers was analyzed using the TIMER2.0 database (http://timer.cistrome.org/). Expression levels in HCC tissues were assessed via TCGA, ICGC, and GEO databases. Kaplan–Meier survival curves were obtained from Kaplan–Meier Plotter (http://www.kmplot.com/) and UALCAN (http://ualcan.path.uab.edu). KEGG, GO, and HALLMARK pathway analyses were performed using R (https://bioconductor.org/biocLite.R), DAVID (https://david.ncifcrf.gov/), and GSEA (4.3.3).

Human Samples

A total of 95 HCC patients who underwent liver surgery at the First Affiliated Hospital of Guangxi Medical University between January 2015 and June 2016 were included in this study for prognostic analysis. Clinicopathological features are detailed in Supplementary Table 1. Patients were followed up every 1–2 months during the first two years and twice a year thereafter. Additionally, 24 paired fresh HCC samples and 10 portal vein tumor thrombus (PVTT) tissues were collected. The study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University (Approval NO: 2024-E181-01), and written informed consent was obtained from all participants.

Cell Culture and Transfection

The immortalized normal liver cell line L02 and HCC cell lines (PLC/PRF5, Hep3B, HepG2, SMMC7721, Huh7, and HCCLM3) were obtained from the Cell Culture Center, Chinese Academy of Medical Sciences. Luciferase transfection of HepG2 and Hep3B cells was conducted by Sciencelight Biology (China). All cell lines were cultured in dulbecco's modified eagle medium (DMEM, Servicebio, China) with 10% fetal bovine serum (FBS, Thermo, USA) at 37°C in a 5% carbon dioxide incubator. Lentiviruses for gene overexpression and knockdown were purchased from GeneChem, and stable clones were selected with 2μg/mL puromycin (Solarbio, China) for four weeks. Gene interference efficiency was verified via qRT-PCR and Western blot. In the functional experiments, HepG2 cells were divided into an overexpression control group (NC) and a FAM105B overexpression group (FAM105B), while Hep3B cells were divided into a knockdown control group (shCtl) and a FAM105B knockdown group (shFAM105B).

Western Blotting

Total protein was extracted with Radio immunoprecipitation assay lysis buffer (Beyotime Biotechnology, China). Protein lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane (Roche Life Sciences, Switzerland). After being blocked with 5% skim milk, the membranes were incubated with primary antibodies and then with the appropriate secondary antibodies. Protein signals were detected via enhanced chemiluminescence reagents (Thermo Scientific, USA) and quantified via densitometric analysis via ImageJ software (NIH, Bethesda, USA). The antibodies used are listed in Supplementary Table 3.

Quantitative Real-Time PCR (qRT-PCR)

Total ribonucleic acid (RNA) was isolated using TRIzol® (Servicebio, China), complementary DNA (cDNA) was synthesized with a PrimeScript RT kit (Takara, Japan), and qRT-PCR was performed using SYBR Green PCR Master Mix (Takara, Japan), with GAPDH as the internal control. The prepared qRT-PCR reaction mixture was subjected to amplification using the Applied Biosystems 7500 Fast Real-Time PCR System. The thermal cycling conditions were as follows: initial denaturation at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 34 seconds. After completion of the reaction, data were analyzed based on the cycle threshold (CT) values. Data were analyzed using the $2^{-\Delta\Delta CT}$ method. Primer sequences are listed in Supplementary Table 5.

Immunohistochemistry (IHC)

Tissue samples were fixed in 4% tissue fixative solution (Servicebio, China) for 24 hours. Subsequently, the samples were dehydrated through a graded ethanol series, cleared in xylene, and infiltrated with molten paraffin at 60°C. After complete paraffin infiltration, the tissues were embedded in molds and allowed to solidify. The resulting paraffin blocks were then sectioned at a thickness of 4 μm for subsequent experimental analyses. The sections were deparaffinized and subjected to heat-induced epitope retrieval prior to immunohistochemical staining. After blocking endogenous peroxidase with 3% hydrogen peroxide (Solarbio, China) and nonspecific binding with 10% goat serum, sections were incubated overnight at 4°C with primary antibodies (Supplementary Table 3) and then with horseradish peroxidase conjugated secondary antibodies (Proteintech, China). 3,3′-Diaminobenzidine (DAB, Beyotime, China) was used for signal development, and hematoxylin for counterstaining. Immunohistochemical staining was scored based on intensity (0–3) and percentage of positive cells (0–4), with a final score ranging from 0 to 12. Scores of 0–4 were classified as low expression, and 5–12 as high expression.

Immunofluorescence

Cells were grown on sterile slides, fixed with 4% formaldehyde, permeabilized with 0.2% Triton X-100, blocked with 10% goat serum, and incubated overnight at 4°C with primary antibodies (Supplementary Table 3). After washing, cells were incubated with fluorescence-labeled secondary antibodies and 4′,6-diamidino-2-phenylindole (DAPI, Beyotime, China). For F-actin staining, rhodamine-conjugated phalloidin was used instead of secondary antibodies (Thermo, USA), with a 2-hour incubation. Images were captured using a Nikon 80i fluorescence microscope (Nikon, Japan).

MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) Assay

For the MTT assay, 5×10^3 cells were seeded into each well of a 96-well plates. 100μL fresh medium containing 0.5mg/mL MTT (Sigma, USA) was added to each well, and the mixture was incubated at 37°C for 4 hours. Then, the medium was replaced with 100μL of DMSO, and the mixture was incubated for 10 minutes at room temperature. The absorbance was measured at a wavelength of 490 nm via a Bio-Rad microplate reader (Thermo, USA).

Colony Formation Assays

For colony formation assays, 5×10^2 cells were seeded in 6-well plates, cultured in DMEM with 10% FBS for 14 days at 37°C, fixed with methanol, and stained with 0.1% crystal violet (Solarbio, China). Colonies were counted and compared. All experiments were performed in triplicate.

Wound Healing

A total of 5×10^5 cells were seeded into a 6-well plate. Once 90% confluence was reached, three parallel scratches were made using a sterile 10μL pipette tip, and wound closure was observed under a microscope at various time points.

Transwell Migration and Invasion

For migration assays, 5×10^4 cells in serum-free medium were seeded into the upper chamber with 8-μm pores. For invasion assays, 1×10^5 cells were added to Matrigel-coated (Sigma, USA) upper chambers. The lower chamber contained 500μL of medium with 10% FBS. After 12–48 h incubation, migrated/invaded cells were fixed with 4% paraformaldehyde, stained with 0.5% crystal violet (Solarbio, China), and counted in five random fields under a light microscope (magnificationx100, Japan).

Animal Experiments

Male BALB/c nude mice were maintained under specific pathogen free (SPF) conditions. For the orthotopic HCC model, HepG2 cells stably overexpressing FAM105B and their control cells, as well as Hep3B cells with stable knockdown of FAM105B and their control cells (2×10^6 cells in 20μL phosphate-buffered saline (PBS, Solarbio, China) with 25% Matrigel) were injected into the subcapsular region of the liver (n=6/group). Mice were divided into four groups: overexpression control (NC), FAM105B overexpression (FAM105B), knockdown control (shCtl), and FAM105B knockdown (shFAM105B). Tumor growth was monitored weekly using bioluminescence imaging. After 6 weeks, livers were harvested, imaged. HepG2 cells with stable overexpression of FAM105B and their control cells, as well as Hep3B cells with stable knockdown of FAM105B and their control cells (6×10^6 cells in 50μL PBS were injected via the tail vein, n=6/group). Similarly, mice were divided into four groups: NC, FAM105B, shCtl, and shFAM105B. After 4 weeks, lungs were collected, imaged, and paraffin-embedded. Tumor metastases were confirmed by H&E staining. All procedures were approved by the First Affiliated Hospital of Guangxi Medical University (Approval NO: 2024-E181-01).

Statistical Analysis

Statistical analyses were conducted using SPSS 22.0 (IBM Corporation, USA) and GraphPad Prism 6 (GraphPad Software Inc., USA). Data are expressed as mean ± standard deviation from at least three independent experiments. Continuous variables were analyzed by Student's *t*-test or one-way ANOVA, and categorical data by the chi-square test. Correlations were assessed using Spearman's rank correlation. Survival analysis was performed via the Kaplan–Meier method and Log rank test, while Cox regression identified independent prognostic factors with hazard ratios displayed in forest plots. $P<0.05$ was considered statistically significant.

Results

FAM105B Is Elevated in Human HCC Tissues and Cell Lines

To clarify the underlying role of FAM105B in HCC, our study first analyzed the distribution of expression levels in tumor and corresponding normal tissues in the TCGA pan-cancer cohort via the TIMER database and revealed that FAM105B was significantly upregulated in most cancers, including HCC (Figure 1A). Our study analyzed FAM105B expression using the GSE36376, GSE64041, and International Cancer Genome Consortium - Liver Cancer - RIKEN, Japan (ICGC-LIRI). The analysis consistently demonstrated that FAM105B expression levels were significantly higher in HCC tissues compared to adjacent nontumor liver tissues (Figure 1C). Our study subsequently examined the expression of FAM105B mRNA in 24 paired fresh HCC samples and 10 portal vein tumor thrombus (PVTT) tissues via qRT-PCR. The results revealed that FAM105B mRNA expression dramatically increased in HCC tissues compared with matched nontumor tissues, whereas PVTT tissues presented higher FAM105B mRNA levels than did HCC tissues (Figure 1B). Similar results were also observed via Western blot analyses (Figure 1D). In addition, IHC staining further confirmed the elevated expression of FAM105B in HCC tissues compared with nontumor tissues, with 64.2% HCC upregulated expression of FAM105B (Figure 1E and F). To explore the underlying mechanism of FAM105B in HCC progression, our study examined the expression of FAM105B in six human HCC cell lines (Huh7, Hep3B, PLC/PRF/5, HCCLM3,

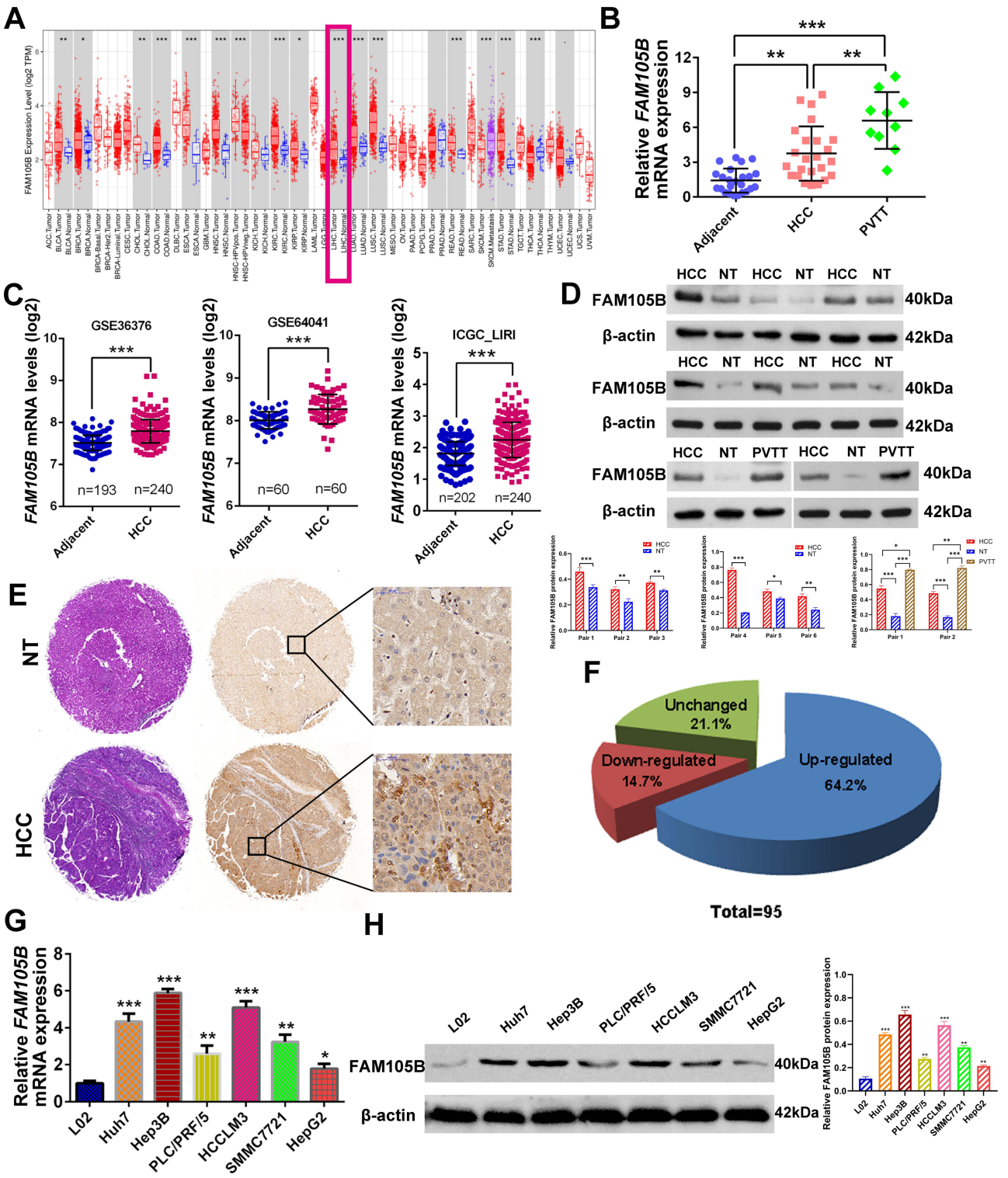

Figure 1 FAM105B is upregulated in HCC tissues and cell lines. (**A**) Differential expression analysis of FAM105B in various cancers in the TIMER database. (**B**) qRT-PCR was used to analyze FAM105B mRNA expression levels in PVTT (n=10) and HCC (n=24). (**C**) Relative mRNA expression of FAM105B in two GEO datasets (GSE36376 and GSE64041) and one ICGC dataset. (**D**) The protein expression of FAM105B in PVTT and HCC from HCC patients were investigated by Western blot. (**E**) Representative HE and IHC images of FAM105B expression in HCC and adjacent nontumor liver tissues. (**F**) Visualization of immunohistochemical data using a pie chart. (**G**) qRT-PCR was performed to analyze the transcriptional expression of FAM105B in HCC cell lines and L02. (**H**) Western blot was conducted to evaluate the protein expression of FAM105B in HCC cell lines and L02. (n=6/group, **P*<0.05, ***P*<0.01, ****P*<0.001).

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SMMC7721, and HepG2) and one immortalized normal liver cell line (L02) via qRT-PCR and Western blot. In line with the results from tissues, increased expression of FAM105B at the mRNA and protein levels was also detected in the aforementioned HCC cell lines compared with L02 cells (Figure 1G and H). Collectively, these results prove that FAM105B is highly expressed in HCC tissues and cell lines.

High FAM105B Expression Is Correlated with Aggressive Clinicopathological Characteristics and Unfavorable Prognosis in HCC Patients

To investigate the clinical significance of FAM105B upregulation in HCC, our study further analyzed the associations of FAM105B expression with the clinicopathological features and prognosis of HCC patients. High FAM105B expression was positively associated with multiple nodules, no encapsulation, microvascular invasion, and advanced TNM stage (Supplementary Table 1). HCC patients with high FAM105B expression presented a shorter OS and worse DFS than did those with low FAM105B expression (Figure 2A and B), which was further supported by the bioinformatics prognostic significance analysis from the online web portal UALCAN (Figure 2C) and Kaplan–Meier Plotter (Supplementary Figure 1A–D). Univariate and multivariate Cox proportional hazard regression analyses, which incorporated all significant variables identified by univariate analysis, further revealed that high FAM105B expression was an independent risk factor for both OS and DFS in HCC patients after liver resection, and the hazard ratios for OS and DFS in the high-FAM105B group were 2.763 and 2.510, respectively (Figure 2D, E and Supplementary Table 2). Analysis of the UALCAN database revealed that the transcriptional level of FAM105B is significantly correlated with tumor grade, stage, nodal metastasis and primary tumors. (Figure 2F–I). These findings collectively indicate that high FAM105B expression predicts poor prognosis in HCC patients and may contribute to HCC progression, which suggests that FAM105B may serve as a potential biomarker for the diagnosis and treatment of HCC.

FAM105B Enhances HCC Cell Proliferation, Migration and Invasion Capacities in vitro

To further elucidate the functions of FAM105B in HCC progression, our study established stable overexpression cell lines via lentiviral infection (Supplementary Table 4) in HepG2 cells, which presented relatively low endogenous FAM105B levels. Additionally, our study knocked down FAM105B in Hep3B cells, which presented relatively high endogenous FAM105B levels. qRT–PCR and Western blot assays were used to confirm the interference efficiency (Figure 3A and B). Among the three shRNAs, shRNA2, which produced the greatest reduction in endogenous FAM105B expression, was chosen for further study. MTT and colony formation assays revealed that, compared with the control, the overexpression of FAM105B in HepG2 cells resulted in greater tumorigenic ability by increasing the cell proliferation rate and frequency of colony formation (Figure 3C and D). Conversely, silencing FAM105B in Hep3B cells significantly inhibited cell proliferation and colony formation (Figure 3C and D). Moreover, wound healing, transwell migration and invasion assays revealed a marked increase in the motility of HCC cells after overexpression of FAM105B. Consistent with these findings, knockdown of FAM105B suppressed cell migration and invasion (Figure 3E and F). Taken together, these data indicate that FAM105B enhances HCC cell proliferation, migration and invasion capacities in vitro.

FAM105B Promotes HCC Growth and Metastasis in vivo

To investigate the role of FAM105B in tumor progression in vivo, orthotopic HCC and lung metastasis models were established using luciferase-labeled HepG2-NC, HepG2-FAM105B, Hep3B-shCtl, and Hep3B-shFAM105B cells. Bioluminescence imaging showed significantly enhanced fluorescence intensities in the FAM105B-overexpressing group and reduced intensities in the Hep3B-shFAM105B group compared to controls (Figure 4A). Gross examination revealed that the livers from the HepG2-FAM105B and Hep3B-shCtl groups exhibited more prominent metastatic nodules on the surface, whereas the Hep3B-shFAM105B group showed almost no visible nodules (Figure 4B). These findings are consistent with the results of the orthotopic tumor intervention., demonstrating that FAM105B promotes HCC growth and metastasis, highlighting its oncogenic potential. HE staining revealed increased size and number of intrahepatic metastatic foci in the HepG2-FAM105B and Hep3B-shCtl groups, indicating that FAM105B promotes intrahepatic metastasis (Figure 4C and D).

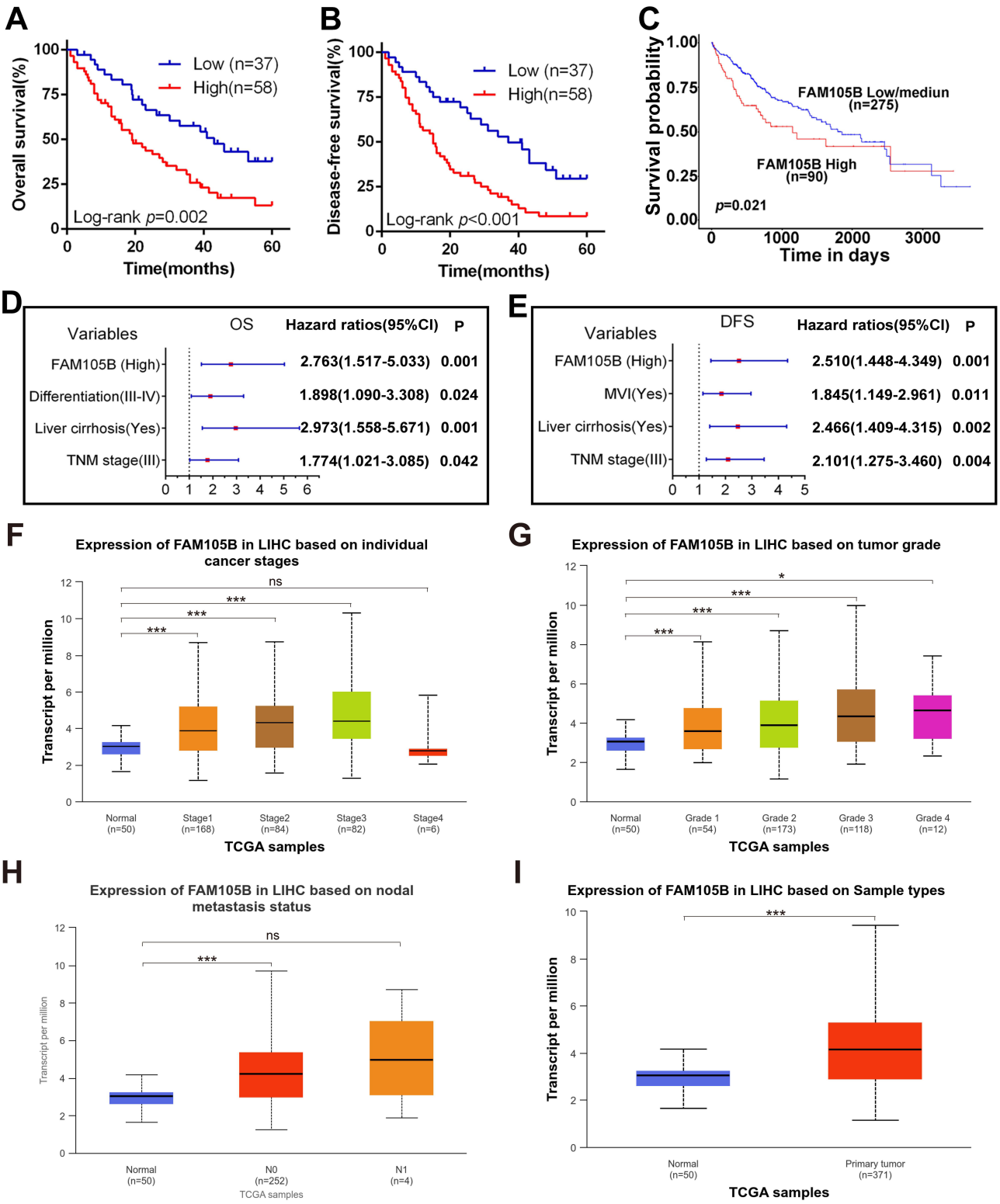

Figure 2 High FAM105B expression in HCC tissues predicts a poor prognosis in HCC patients. (**A** and **B**) Kaplan–Meier curves for OS and DFS based on the results of the IHC analysis of FAM105B expression in 95 HCC patients. (**C**) The prognostic significance of FAM105B was analyzed via UALCAN. (**D** and **E**) Forest plot based on the results of multivariate analysis of the factors associated with the OS and DFS of HCC patients. (**F–I**) UALCAN analysis of FAM105B expression in HCC. (*P<0.05, ***P<0.001, ns: not significant (P>0.05)).

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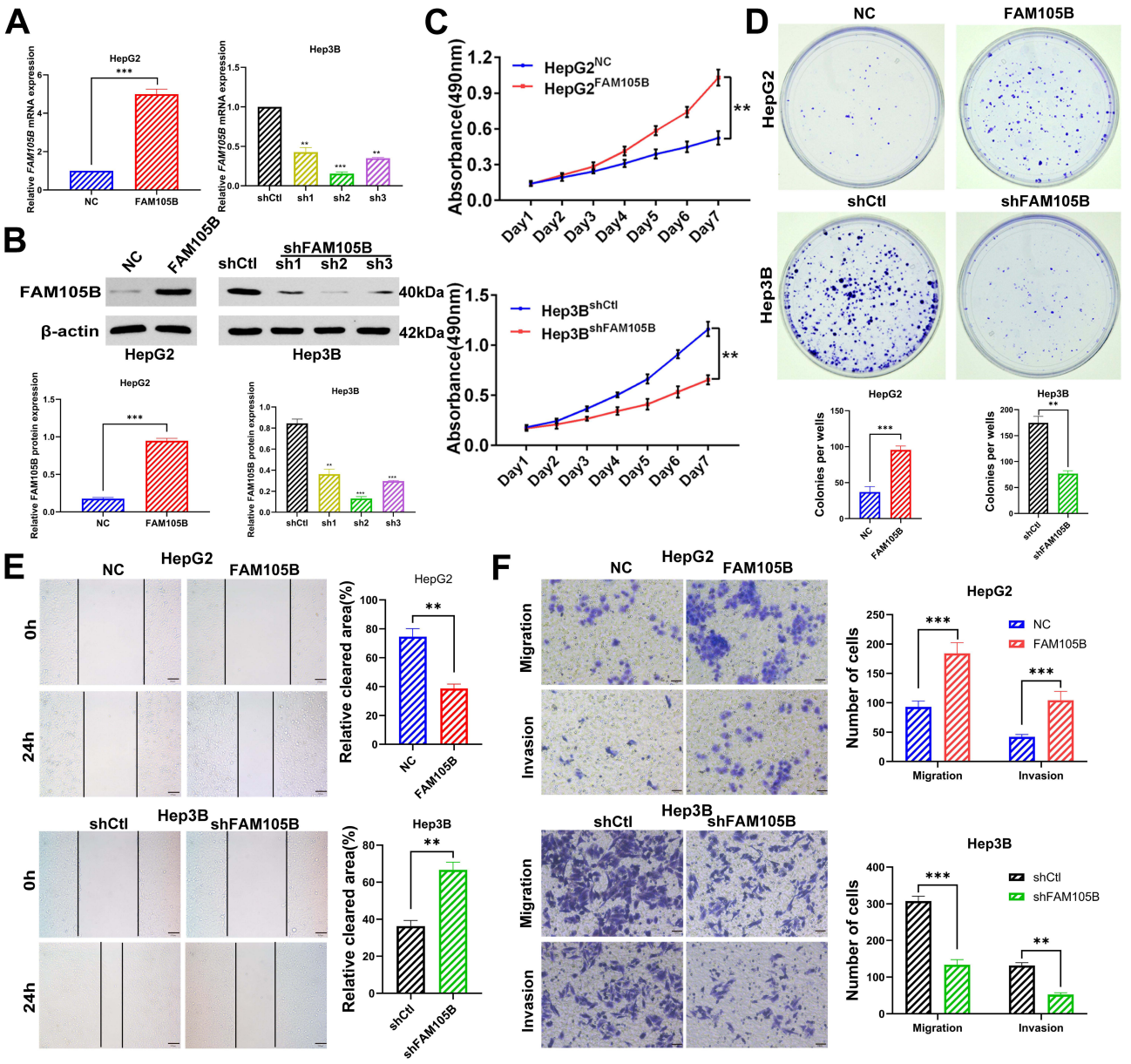

Figure 3 FAM105B promotes the proliferation and invasion of HCC cells in vitro. (**A**) qRT-PCR was conducted to evaluate the mRNA expression levels. (**B**) Western blot was performed to assess the protein expression levels. (**C**) The MTT assay was conducted to evaluate the effect of FAM105B on the proliferation. (**D**) Colony formation assay was performed to assess the impact of FAM105B on the clonogenic ability. (**E**) Wound healing assay was conducted to determine the migration ability, scale bar=200μm. (**F**) Transwell assay was performed to evaluate the migration and invasion abilities, scale bar=100μm. (n=6/group, ***P*<0.01, ****P*<0.001).

FAM105B Induces EMT in HCC Cells

EMT is crucial for tumor invasion and metastasis, characterized by the loss of cell polarity, acquisition of spindle morphology, and enhanced motility and invasiveness.[20–23] In our study, HepG2-NC cells exhibited a cobblestone shape with disorganized F-actin, while HepG2-FAM105B cells displayed spindle morphology and elongated F-actin. Silencing FAM105B reversed Hep3B cells' morphology from spindle to cobblestone (Figure 5A), indicating a potential role of FAM105B in EMT. QRT-PCR revealed that FAM105B overexpression reduced the epithelial marker E-cadherin and increased mesenchymal markers Vimentin and N-cadherin. Conversely, FAM105B knockdown led to elevated E-cadherin and decreased Vimentin and N-cadherin expression (Figure 5B). Western blot and IF analyses confirmed these findings at the protein level (Figure 5C and D). Furthermore, IHC staining of xenograft tumors showed that FAM105B promoted Vimentin expression while suppressing E-cadherin expression (Figure 5E). In addition, we

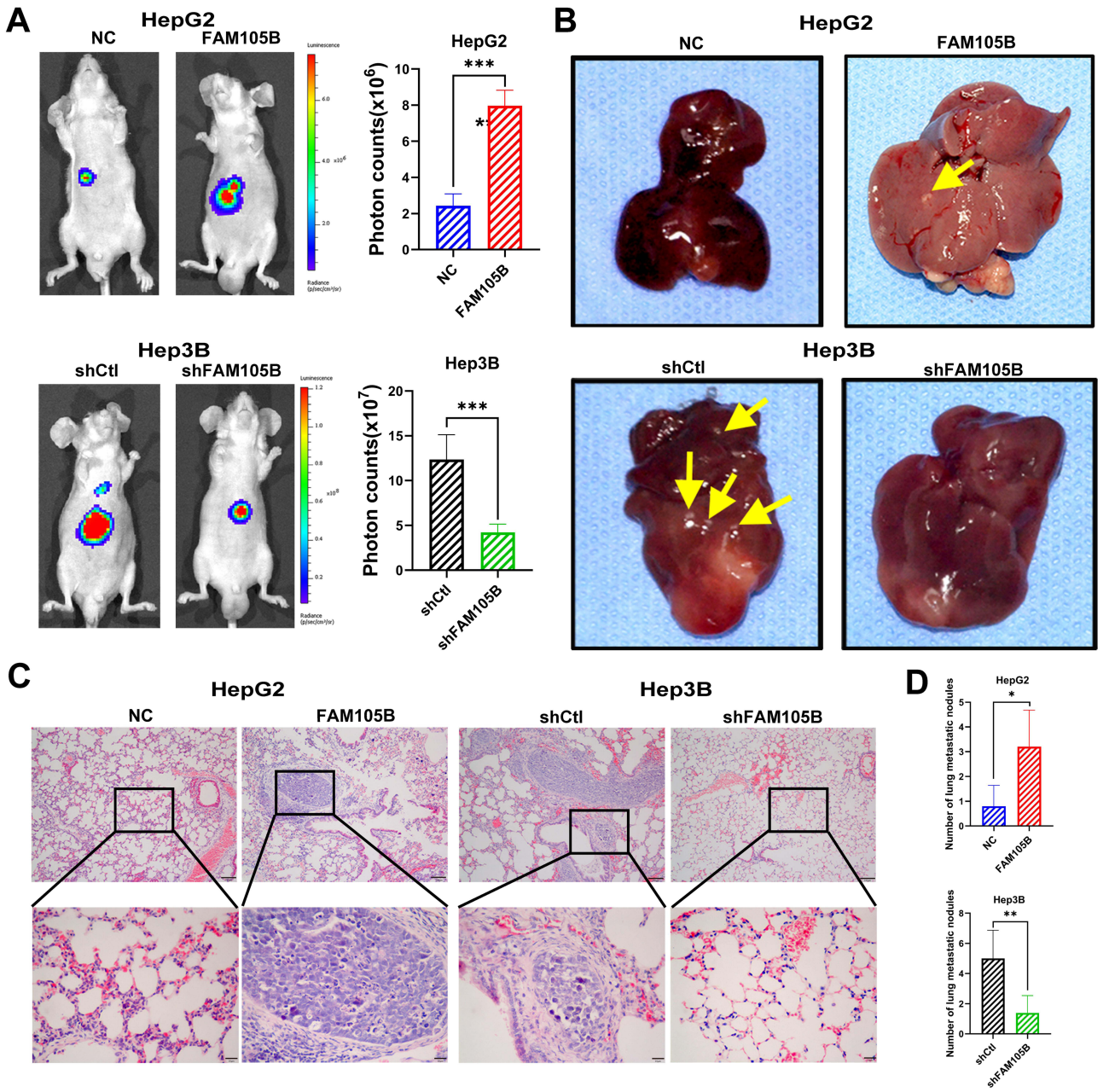

Figure 4 FAM105B promotes HCC growth and metastasis in mice. (**A**) Representative images of the murine liver orthotopic-implanted HCC models with the indicated HCC cells were captured with the In Vivo Fx Pro Imaging System. The signal intensity of the luciferase activity in tumors was quantified as the mean density of photon flux (p/sec/mm2). (**B**) Representative images of intrahepatic metastases, the yellow arrow indicates representative metastatic nodules on the liver surface. (**C**) Representative images of HE staining of pulmonary metastatic nodules in different groups of mice (scale bar=100μm). (**D**) Lung metastasis nodules were counted and plotted. (n=6/group, **P*<0.05, ***P*<0.01, ****P*<0.001).

conducted a correlation analysis and found that FAM105B expression in the nude mouse intrahepatic metastasis model was significantly positively correlated with the EMT pathway (Figure 5F). These results establish FAM105B as an inducer of EMT in HCC cells, driving changes in cell morphology, motility, and cytoskeletal organization.

FAM105B Regulates HCC Progression and Metastasis via the PI3K/AKT/MTOR Signaling Pathway

To elucidate the mechanism of FAM105B in HCC, GSEA analysis of the TCGA-LIHC dataset revealed that FAM105B is positively correlated with multiple tumor-associated pathways, particularly the PI3K/AKT/MTOR pathway (Figure 6A).

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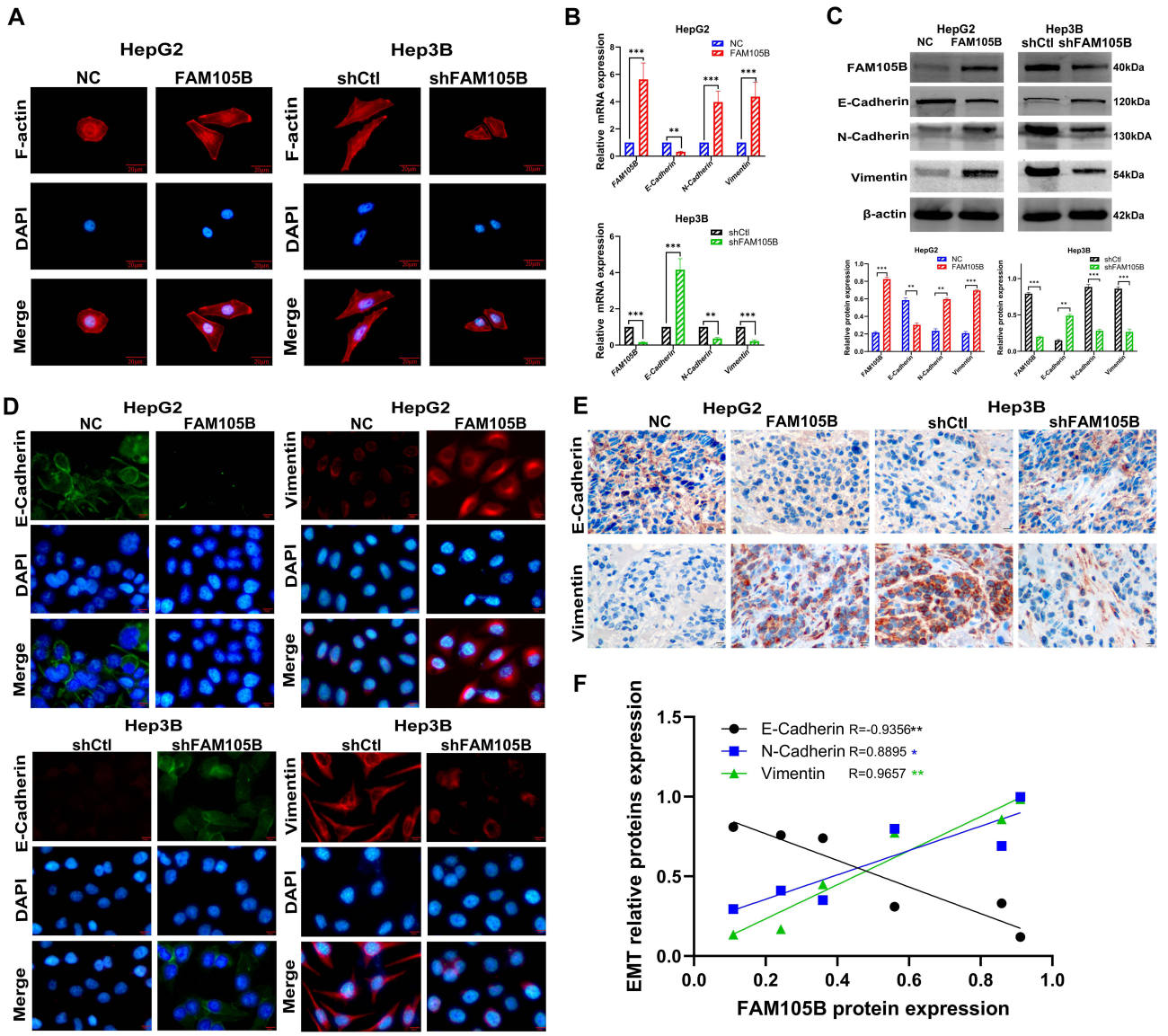

Figure 5 FAM105B promotes EMT in HCC. (**A**) F-actin was visualized using rhodamine-phalloidin staining (red), while nuclei were counterstained with DAPI (blue), scale bar=20μm. (**B**) qRT-PCR was performed to validate the mRNA expression levels of key proteins involved in EMT. (**C**) Protein expression levels of EMT marker detected by Western blot. (**D**) IF colocalization analysis was performed to visualize the intracellular localization of FAM105B and EMT marker proteins, scale bar=20μm. (**E**) The protein expression of E-cadherin and Vimentin in tumor xenografts from the two different groups was determined by IHC analysis, scale bar=20μm. (**F**) A correlation analysis was performed between FAM105B expression and the expression levels of EMT-related markers in intrahepatic metastatic lesions of nude mice. (n=6/group, **P*<0.05, ***P*<0.01, ****P*<0.001).

KEGG analysis further confirmed this association (Figure 6B). TIMER2.0 identified positive correlations between FAM105B and PIK3CA, AKT1, and MTOR in LIHC (Figure 6C), suggesting that FAM105B may drive HCC progression via the PI3K/AKT/MTOR pathway. Western blot analysis validated this hypothesis by showing increased phosphorylation levels of PI3K (p-PI3K), AKT (p-AKT), and MTOR (p-MTOR) in HepG2 cells overexpressing FAM105B, while knockdown of FAM105B in Hep3B cells reduced these phosphorylation levels. Notably, total PI3K, AKT, and MTOR protein levels remained unchanged (Figure 6D). We performed a correlation analysis between FAM105B expression and the phosphorylation levels of the PI3K/AKT/MTOR pathway in two human HCC cell lines and found that FAM105B expression was significantly positively correlated with PI3K/AKT/MTOR phosphorylation (Figure 6E). These findings highlight FAM105B as a regulator of HCC progression and metastasis through activation of the PI3K/AKT/MTOR pathway.

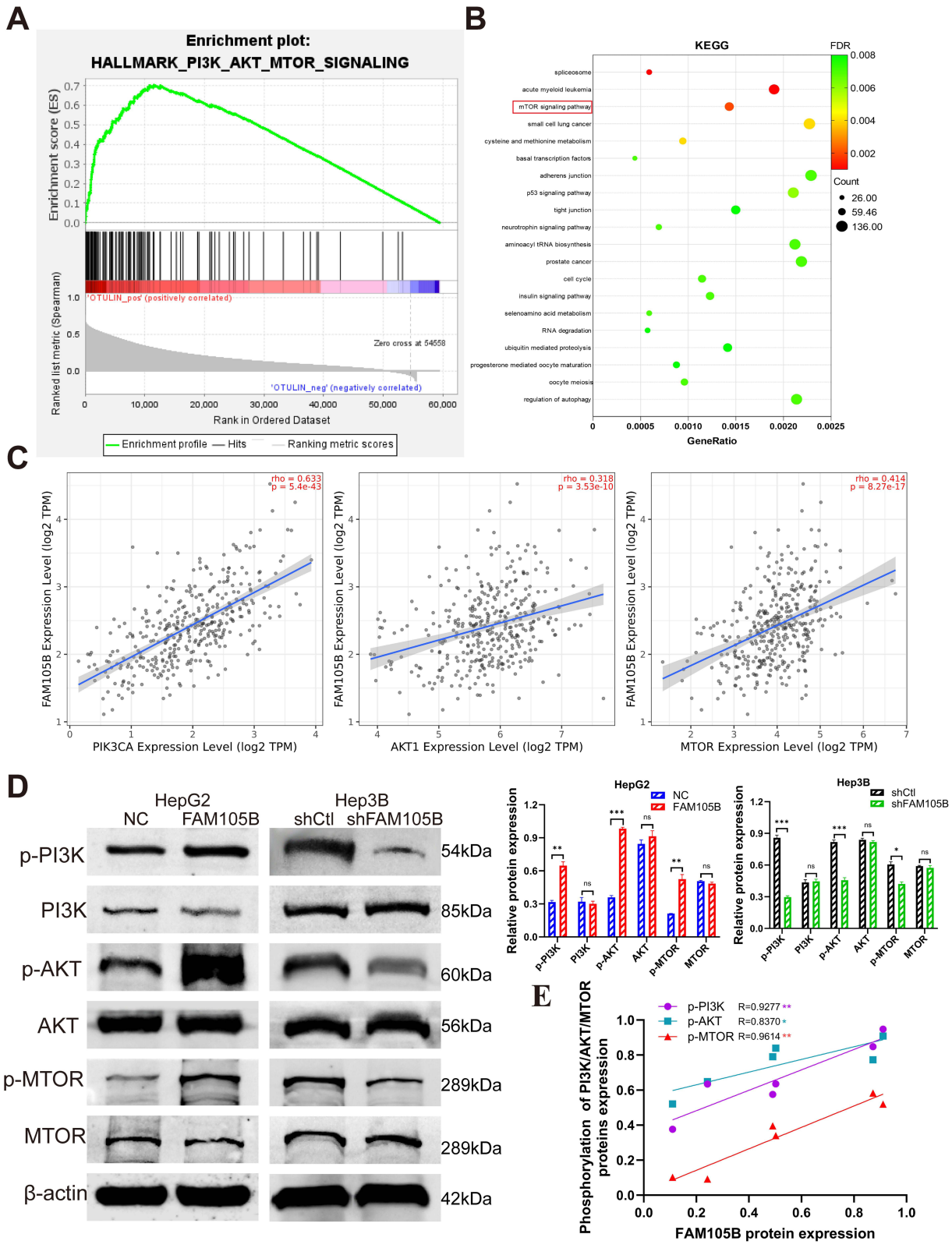

Figure 6 FAM105B activates the PI3K/AKT/MTOR signaling pathway in HCC cells. (**A**) HALLMARK analysis of FAM105B expression in the TCGA-LIHC database via GSEA 4.3.3 software. (**B**) KEGG analyses of FAM105B expression in the TCGA-LIHC database. (**C**) Correlation analysis of PIK3CA, AKT1 and MTOR with FAM105B in LIHC via TIMER2.0 database. (**D**) The protein levels of PI3K, AKT, MTOR, p-PI3K, p-AKT and p-MTOR in cells were detected via Western blot. (**E**) A correlation analysis between FAM105B expression and PI3K/AKT/MTOR phosphorylation in HCC cell lines revealed statistically significant positive correlations. (n=6/group, **P*<0.05, ***P*<0.01, ****P*<0.001, ns: not significant (*P*>0.05)).

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Discussion

HCC development is a complex, multistage process involving interactions between various genes and pathways. Dysregulated cellular signaling is central to cancer initiation, progression, and metastasis, with ubiquitination playing a pivotal regulatory role in many signaling pathways.[24] Ubiquitination ensures protein stability and coordinates cellular responses to stimuli and stress, making it a potential therapeutic target in HCC. FAM105B, a member of the OTU deubiquitinating enzyme family, specifically cleaves linear ubiquitin chain assembly complex (LUBAC), restricting its function in an enzyme activity-dependent manner.[11,12] LUBAC-mediated NF-κB signaling is critical in various cancers, including diffuse large B-cell lymphoma (ABC-DLBCL), where its upregulation promotes tumor progression by enhancing NF-κB activity.[25–27] In vivo studies have shown that inhibiting LUBAC activity can suppress tumor growth in xenograft models.[26] Additionally, LUBAC, regulated by β-catenin, drives NF-κB activation via NF-κB essential modulator (NEMO) linear ubiquitination, contributing to estrogen receptor-negative breast cancer progression.[28] FAM105B acts as a negative regulator of NF-κB signaling by limiting LUBAC activity.[29] However, its elevated expression is associated with aggressive subtypes and poor survival in breast cancer, where it promotes drug resistance and metastasis through Wnt/β-catenin signaling.[20] In gastric cancer, FAM105B overexpression correlates with poor prognosis and enhances proliferation, migration, and invasion.[30] Conversely, reduced FAM105B expression protects against hepatocyte death, inflammation, and metabolic dysregulation in mice and humans, functioning as a tumor suppressor in liver diseases.[31] Despite evidence implicating FAM105B in tumor progression, its role in tumorigenesis, particularly in HCC, remains underexplored.

This study investigates the role of FAM105B in HCC progression, focusing on its impact on tumor proliferation, migration, and invasion. Bioinformatics analysis using TIMER2.0 revealed significant upregulation of FAM105B in most cancers, including HCC. Further validation through GEO datasets (GSE36376, GSE64041) and ICGC data confirmed elevated FAM105B expression in HCC tissues compared to adjacent normal tissues. Western blot analysis of clinical samples demonstrated higher FAM105B expression in HCC tissues, with further elevation in PVTT tissues. Similarly, FAM105B expression was significantly higher in six HCC cell lines than in the immortalized normal liver cell line L02. Clinical pathology and survival data revealed that high FAM105B expression correlates with poor clinical features, including increased nodule count, absence of tumor capsule, microvascular invasion, and advanced TNM stage, but not with sex, age, HBsAg status, cirrhosis, AFP levels, or tumor size. Kaplan-Meier survival analysis showed that both OS and DFS were significantly lower in the high-FAM105B expression group. Cox regression analysis identified FAM105B as an independent prognostic risk factor for OS and DFS. Functional assays demonstrated that FAM105B overexpression enhanced proliferation, migration, and invasion of HepG2 cells, whereas its knockdown reduced these capabilities in Hep3B cells. MTT and colony formation assays confirmed that FAM105B promotes HCC cell proliferation, while wound healing and Transwell assays revealed its role in enhancing migration and invasion. Collectively, these findings suggest that FAM105B plays a critical role in the malignant progression of HCC by promoting proliferation, migration, and invasion. However, the use of a single HCC cell line to verify the knockdown effect limits the generalizability of these results. Future studies should include multiple HCC cell lines to confirm the robustness and reproducibility of FAM105B's function.

FAM105B has been shown to regulate tumor progression through multiple mechanisms. In triple-negative breast cancer, FAM105B inhibits linear ubiquitination of the Wnt receptor complex downstream of β-catenin, stabilizing β-catenin after DNA damage and activating the Wnt/β-catenin pathway, which promotes EMT and tumor migration.[10,20,32–34] Similarly, in gastric cancer, FAM105B enhances tumor proliferation and adhesion by inhibiting RACK1 ubiquitination and activating FAK signaling.[35,36] EMT, a hallmark of tumor invasion and metastasis, is characterized by downregulation of epithelial markers (E-cadherin) and upregulation of mesenchymal markers (Vimentin, N-cadherin), weakening cell adhesion and increasing motility.[37] In HCC, overexpression of FAM105B induced cytoskeletal changes and transformed HepG2 cells from an epithelial to a mesenchymal phenotype, while FAM105B knockdown reversed this effect. EMT-related marker changes, including reduced E-cadherin and increased Vimentin and N-cadherin expression, were validated by qRT-PCR, Western blot, and immunofluorescence. These findings suggest that FAM105B promotes HCC invasion and metastasis by driving EMT, consistent with studies in other cancers, such as breast cancer, where silencing FAM105B suppressed tumor growth and lung metastasis in mouse models.[20] To explore its underlying mechanisms, GSEA of the TCGA-LIHC dataset identified the PI3K/AKT/MTOR pathway as significantly associated with FAM105B expression. This pathway is critical in regulating cell cycle, proliferation, apoptosis, and metastasis and is frequently dysregulated in cancers, including HCC.[21,38–41] Activation of the

PI3K/AKT/MTOR pathway promotes EMT in colorectal cancer and metastasis in gastric cancer.[42,43] Western blot analysis confirmed that FAM105B overexpression activates the PI3K/AKT/MTOR pathway, while FAM105B knockdown suppresses it, supporting the hypothesis that FAM105B drives HCC invasion and metastasis through this pathway. These findings highlight the potential of FAM105B as a therapeutic target in cancer treatment.

However, the precise mechanism by which FAM105B activates the PI3K/AKT/MTOR pathway remains unclear. It is still undetermined whether FAM105B directly interacts with key components of the pathway or activates it indirectly through intermediary molecules. To elucidate its functional mechanism, future studies should employ immunoprecipitation combined with mass spectrometry to identify FAM105B-interacting proteins. This could be complemented by CRISPR-Cas9-mediated gene editing and site-directed mutagenesis to define the essential structural domains and key amino acid residues required for its activity. In addition, integrative multi-omics analyses—such as transcriptomics, proteomics, and metabolomics—may help to comprehensively map its regulatory network and downstream biological effects. However, this study did not utilize pharmacological inhibitors or genetic interventions to block the PI3K/AKT/MTOR pathway, thus lacking direct evidence to establish a causal relationship. Therefore, future research should incorporate the use of specific inhibitors to further confirm whether FAM105B exerts its tumor-promoting effects through this signaling axis. Moreover, in vivo validation, particularly the assessment of PI3K/AKT/MTOR pathway activity in mouse lung metastases, is necessary to enhance the credibility and translational relevance of these findings.

Conclusion

In summary, this study identifies FAM105B as a novel oncogene in HCC. It is significantly upregulated in tumor tissues and cell lines, with high expression associated with aggressive clinicopathological features and poorer OS and DFS. Functional assays confirm that FAM105B promotes proliferation, migration, and invasion of HCC cells by inducing EMT, both in vitro and in vivo. Mechanistically, FAM105B activates the PI3K/AKT/MTOR pathway, contributing to tumor progression, although the precise regulatory mechanism remains to be clarified. Collectively, these findings suggest that FAM105B may serve as a potential prognostic biomarker and therapeutic target in HCC.

Ethics Approval and Informed Consent

All human and animal experiments involved in this study were approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Approval No.: 2024-E181-01). All participants voluntarily provided written informed consent prior to enrollment. The human study was conducted in accordance with the Declaration of Helsinki. All animal experiments were performed in compliance with the GB/T 43051-2023 "General Requirements for Biosafety in Animal Experiments of Laboratory Animals in China", and all efforts were made to minimize animal suffering and ensure animal welfare.

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Disclosure

The authors report no conflicts of interest in this work.

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