Original Article

FAM60A promotes proliferation and invasion of colorectal cancer cells by regulating the Wnt/β-catenin signaling pathway

Zhikun Dong[1#], Shuwen Jin[1#], Kan Tang[1], Xiaomei Li[1], Yonglin Chen[2]

[1]The First Clinical Medical College of Lanzhou University, Lanzhou, China; [2]Department of Pathology, The First Hospital of Lanzhou University, Lanzhou, China

Contributions: (I) Conception and design: Z Dong, K Tang; (II) Administrative support: Y Chen; (III) Provision of study materials or patients: Z Dong, X Li; (IV) Collection and assembly of data: Z Dong; (V) Data analysis and interpretation: Z Dong, S Jin; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Yonglin Chen, MD. Department of Pathology, The First Hospital of Lanzhou University, 1 Donggang West Rd., Lanzhou 730000, China. Email: pathology88@163.com.

Background: Colorectal cancer (CRC) is one of the most detrimental tumors to human health. Although multimodal therapeutic approaches can improve patient survival rates, the prognosis for advanced-stage patients remains poor. It has been reported that family with sequence similarity 60, member A (FAM60A), a component of the SIN3 transcription regulator family member A (SIN3A)/histone deacetylase (HDAC) complex, plays a significant role in tumorigenesis. However, the precise function and mechanisms of action of FAM60A in CRC have not been fully elucidated. In this study, we aim to further delineate the role of FAM60A in CRC by assessing the protein expression levels of FAM60A and β-catenin in CRC tissues and to explore the potential mechanisms by which FAM60A may promote CRC cell proliferation and invasion through a suite of cellular assays.

Methods: Tumor tissues of 195 CRC patients and 65 adjacent non-neoplastic tissues were collected to construct tissue microarrays. The expression levels of FAM60A, c-Myc, cyclin D1, and β-catenin were detected using immunohistochemistry (IHC) staining, and the relationship between the results and the patients' clinicopathological characteristics and prognosis was analyzed. HCT116 and HT-29 cell lines with overexpression/knockdown of FAM60A were constructed. Western blot (WB) was used to detect the protein expression of FAM60A and β-catenin. Cell proliferation, apoptosis rate, cell cycle, and cell migration and invasion abilities were assessed using cell counting kit-8 (CCK-8) assay, flow cytometry, wound healing assay, and transwell assay, respectively.

Results: FAM60A demonstrated elevated expression in CRC tissues and was positively correlated with tumor infiltration depth, Ki67 proliferation index, and poor prognosis in patients. A positive correlation was observed between FAM60A and the expression of β-catenin, c-Myc, and cyclin D1, and patients with co-expression of FAM60A and β-catenin had a significantly higher rate of distant metastasis. The knockdown of FAM60A markedly reduced the proliferation, migration, and invasive capabilities of HCT116 cells, induced cell cycle arrest, and enhanced apoptosis, whereas its overexpression produced the converse effects. In HT-29 cells, FAM60A knockdown also reduced cell proliferation and impaired wound healing, with overexpression showing opposing outcomes. WB analysis revealed that modulation of FAM60A influenced β-catenin protein levels, suggesting a regulatory link between the two proteins.

Conclusions: FAM60A may be a key regulator factor that modulates proliferation and invasion in CRC cells via the Wnt/β-catenin signaling pathway. Elevated FAM60A expression is associated with an adverse prognosis in CRC, underscoring its potential as a prognostic biomarker.

Keywords: Colorectal cancer (CRC); family with sequence similarity 60, member A (FAM60A); Wnt/β-catenin signaling pathway; immunohistochemistry (IHC); clinicopathologic features

Submitted Sep 03, 2024. Accepted for publication Dec 17, 2024. Published online Feb 26, 2025.
doi: 10.21037/tcr-24-1608
View this article at: https://dx.doi.org/10.21037/tcr-24-1608

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy globally and the second leading cause of cancer-related mortality (1). A multimodal therapeutic approach, encompassing surgical resection, radiotherapy/chemotherapy, targeted therapy, and local ablation, has enhanced the survival rate in CRC patients. However, the prognosis for patients with advanced-stage CRC remains unfavorable due to high incidences of metastasis and recurrence (2,3). Consequently, there is an imperative need to dissect the molecular underpinnings of CRC development, pinpoint novel therapeutic targets, and uncover diagnostic biomarkers to augment patient prognoses.

Family with sequence similarity 60, member A (FAM60A) is an integral subunit of the SIN3 transcription regulator family member A (SIN3A)/histone deacetylase (HDAC) complex, a regulator of transcription (4), with significant implications in embryonic stem cell (ESC) function and tumorigenesis (5). It exerts pivotal effects on the regulation of ESC functions and promotes tumor development (6). FAM60A's influence over the cell cycle and its interactions with pluripotency pathways are areas ripe for further exploration, likely to yield novel perspectives on the underpinnings of cancer biology. Streubel *et al.* (5) reported that ESCs lacking FAM60A phenotype exhibited a decrease in SIN3A/HDAC complex levels, culminating in diminished cell proliferation, gap (G)1 phase prolongation, and lineage gene misregulation. FAM60A's regulation may be disrupted in various rapidly dividing cells, including tumor cells, potentially due to E2F transcription factor 1 (E2F1)-mediated transcriptional activation. Nevertheless, the actions of SIN3A/HDAC complex subunits are not uniformly oncogenic; for instance, breast cancer metastasis suppressor 1 (BRMS1), inhibitor of growth 1 (ING1), and retinoblastoma binding protein-1 (RBP1) are known to exert tumor-suppressive effects (7).

The Wnt/β-catenin signaling pathway plays an integral role in embryogenesis and adult homeostasis, with its dysregulation implicated in a spectrum of diseases, including cancer. Aberrant activation of this pathway is associated with growth-related diseases and cancers, particularly serving as a key driver in the initiation and progression of CRC (8). β-catenin serves as a central player within the Wnt/β-catenin signaling pathway. It participates not only in intercellular adhesion but also in Wnt-mediated cell signaling, regulating gene transcription. The levels of β-catenin activity can influence cell fate decisions, including cell proliferation, differentiation, and apoptosis. During embryonic development, the dynamic changes of β-catenin are crucial for the precise regulation of cell fate (9). Abnormal activation or stabilization of β-catenin is associated with a variety of diseases, particularly cancer. In many types of cancer, including CRC, liver cancer, and breast cancer, the stabilization and accumulation of β-catenin are closely related to the occurrence and development of tumors (10). c-Myc is a recognized downstream target gene of the Wnt/β-catenin signaling pathway. Upon Wnt signaling activation, β-catenin translocates to the cell nucleus and interacts with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) family transcription factors, subsequently activating the transcription of downstream genes, including c-Myc (8). c-Myc plays a pivotal role in cell proliferation, differentiation, and oncogenesis. It facilitates

Highlight box

Key findings

- Family with sequence similarity 60, member A (FAM60A) may modulate the proliferation and invasion of colorectal cancer (CRC) cells through the Wnt/β-catenin signaling pathway. Furthermore, elevated expression levels of FAM60A are correlated with an unfavorable prognosis in CRC, suggesting its potential as a prognostic biomarker for the disease.

What is known and what is new?

- FAM60A is not only crucial for maintaining the proliferation and self-renewal capabilities of embryonic stem cell, but also plays a key role in a variety of malignant tumors.
- Our study provides novel insights into the role of FAM60A in CRC and enhances our understanding of the molecular mechanisms involved in the development of CRC. Furthermore, FAM60A shows promise as a biomarker for predicting the prognosis of CRC patients.

What is the implication, and what should change now?

- FAM60A may serve as a potential therapeutic target for patients with CRC.

cell cycle progression, particularly during the transition from the G1 to synthesis (S) phase, thereby promoting cellular proliferation (11). Cyclin D1 is also a significant downstream target gene within the Wnt/β-catenin signaling pathway. Activation of β-catenin enhances the expression of cyclin D1. Cyclin D1 complexes with cyclin-dependent kinases 4 and 6 (CDK4/6), promoting the phosphorylation of the retinoblastoma (Rb) protein, which then leads to the release of E2F family transcription factors. This transition drives the cell cycle from the G1 phase into the S phase, facilitating DNA synthesis and cellular proliferation. Overexpression of cyclin D1 is correlated with the development of various tumors, including CRC (8). The precise function of FAM60A in CRC and its mechanisms of action are not well defined, with the Wnt/β-catenin signaling pathway being a key player in CRC growth and a potential target for FAM60A's regulatory effects (12,13). We hypothesize that FAM60A likely modulates the Wnt/β-catenin signaling pathway, thereby influencing CRC development and progression.

Our preliminary findings indicate that FAM60A is overexpressed in CRC and positively correlates with the Ki67 proliferation index, which suggests a positive association between FAM60A and CRC proliferation (14). This study is designed to further delineate the role of FAM60A in CRC by assessing the protein expression levels of FAM60A and β-catenin in CRC tissues, and to examine the correlation between these proteins. Furthermore, we aim to clarify the underlying mechanisms by which FAM60A may promote CRC cell proliferation and invasion using a suite of cellular assays. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1608/rc).

Methods

Materials

Tissue samples and clinical information

The study enrolled 195 patients diagnosed with CRC who underwent radical tumor resection at two tertiary medical centers: Gansu Provincial Cancer Hospital and the First Hospital of Lanzhou University, over a period spanning January 2013 to December 2021. Furthermore, 65 samples of normal adjacent tissue, at least 5 cm distant from the tumor margin, were also procured. Exclusion criteria included patients who had undergone chemotherapy or radiotherapy prior to surgery and those lost to follow-up. Comprehensive clinical and pathological data, along with follow-up notes, were compiled for each patient. Paraffin-embedded tissues from the aforementioned patients and controls were utilized to fabricate tissue microarrays for subsequent immunohistochemistry (IHC) analysis. The study was performed in line with the principles of the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The First Hospital of Lanzhou University, China (No. LDYYLL-2024-744). Informed consent to participate in this study was obtained from all individual participants. The requirement to obtain informed consent to the use of patient materials was waived by the same Ethics Committee.

Cell lines and reagents

In our investigation, antibodies targeting FAM60A (ab247130), β-catenin (ab227499), c-Myc (RMA-0803), cyclin D1 (RMA-0541), and β-actin (ab8226) were sourced from Abcam, Cambridge, UK. The human CRC cell lines HCT116 and HT-29 were sourced from the Cell Bank of the Chinese Academy of Sciences, Shanghai. McCoy's 5A medium was procured from Procell Life Science & Technology Co., Ltd., Wuhan, China. The RNAex Pro RNA extraction reagent, reverse transcription kit, and real-time quantitative polymerase chain reaction (RT-qPCR) kit were acquired from Accurate Biology, Hunan, China. Lentiviral particles for FAM60A overexpression and knockdown were obtained from GeneChem, Shanghai, China. The total protein extraction kit was procured from Beyotime Biotechnology, Shanghai, China. The bicinchoninic acid (BCA) protein assay kit was sourced from Ziker Biological Technology Co., Ltd., Shenzhen, China. The cell counting kit-8 (CCK-8) assay kit was acquired from Biosharp, Wuhan, China. The Annexin V-fluorescein isothiocyanate (V-FITC)/propidium iodide (PI) apoptosis detection kit was procured from Solarbio, Beijing, China. The cell cycle detection kit was sourced from Dojindo Laboratories (Shanghai, China).

Major instruments

In this study, the following major instruments were sourced from various manufacturers: a manual tissue microarray sampler from Beecher Instruments, USA; a CO_2 incubator from Amerex Instruments, USA; a gradient PCR machine from Bio-Rad, Hercules, CA, USA; the RT-qPCR instrument and 96-well microplate reader from Thermo Fisher Scientific, Waltham, MA, USA; the NanoDrop

spectrophotometer from ChenK Instrument, Shanghai, China; the gel imaging analysis system from Tianlong Technology, Shanghai, China; the flow cytometer, model NL-CLC 1L-3L, from Cytek Biosciences, Fremonnt, CA, USA; an optical microscope from Olympus Optical, Tokyo, Japan; and an inverted biological microscope from Sunny Optical Technology Co., Ltd., Ningbo, China.

Main methods

IHC staining and scoring

IHC staining was conducted using the streptavidin-peroxidase (SP) method. The FAM60A, β-catenin, c-Myc, and cyclin D1 antibodies were utilized as primary antibodies, employing the ethylenediaminetetraacetic acid (EDTA) antigen retrieval method. Scoring of FAM60A staining was based on the immunohistochemical scoring formula (15,16): immunoreactive score (IRS) = staining intensity × percentage of positive cells, with staining intensity and percentage scores detailed as previously described. An IRS of 4 or higher was indicative of positive expression, whereas an IRS below 4 indicated negative expression. Assessment of β-catenin staining adhered to the established criteria of Neumann *et al.* (17) and Buhmeida *et al.* (18), applying the IRS formula and using nuclear staining intensity and percentage of positive cells scores as outlined. An IRS of 2 or higher was indicative of positive expression, whereas an IRS below 2 indicated negative expression. The IHC-stained sections were independently assessed by three senior pathologists, with the average score being recorded.

RT-qPCR

Total cellular RNA was extracted using TRIzol reagent and subsequently reverse transcribed to complementary DNA (cDNA) according to the manufacturer's protocol. The primers for the *FAM60A* gene were as follows: upstream primer 5'-CTCCAGTTCTCGATTCACTGAC-3', downstream primer 5'-CGAGTCTCATGCAATCCAAAACA-3'. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene primers consisted of an upstream primer 5'-GCACCGTCAAGGCTGAGAAC-3' and a downstream primer 5'-TGGTGAAGACGCCAGTGGA-3'. qPCR was conducted using SYBR® Premix Ex Taq™ II following the manufacturer's instructions. Each reaction was conducted in triplicate to ensure reproducibility, and the experiment was conducted with a minimum of three replicates to ensure reliability of the results.

Cell transfection subsequent

RT-qPCR confirmed FAM60A messenger RNA (mRNA) expression levels subsequent to lentiviral transfection. The experimental groups comprised HCT116-OE-NC (HCT116 negative control cell line overexpressing FAM60A), HCT116-OE (HCT116 cell line overexpressing FAM60A), HCT116-sh-NC (HCT116 negative control cell line with FAM60A knockdown), HCT116-sh1 (HCT116 cell line with FAM60A knockdown), HT-29-OE-NC (HT-29 negative control cell line overexpressing FAM60A), HT-29-OE (HT-29 cell line overexpressing FAM60A), HT-29-sh-NC (HT-29 negative control cell line with FAM60A knockdown), and HT-29-sh1 (HT-29 cell line with FAM60A knockdown). The efficiency of transfection was assessed under a fluorescence microscope approximately 72 hours post-transfection.

Western blot (WB) for detection of FAM60A and β-catenin protein expression in CRC cells

Cells derived from multiple experimental groups of HCT116 and HT-29 were subjected to lysis using a protein lysis buffer. Protein concentrations were measured using the BCA assay. The protein samples were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were incubated in a blocking solution containing 5% non-fat milk at room temperature for 2 hours, followed by an overnight incubation at 4 ℃ with primary antibodies against FAM60A, β-catenin, and β-actin. After washing, membranes were incubated with secondary antibodies at room temperature for 2 hours. Protein expression was detected using a gel imaging system.

CCK-8 assay to measure CRC cell proliferation

Logarithmically growing CRC cells were plated in 96-well plates and cultured for 24, 48, and 72 hours. CCK-8 reagent was added to each well and incubated at 37 ℃ in a CO_2 incubator for 4 hours. The optical density (OD) at 450 nm was determined using a microplate reader to assess cell proliferation.

Scratch assay to evaluate CRC cell migration capability

Logarithmic phase HCT116 and HT-29 cells from each group were seeded in 6-well plates. Uniform scratches were created across the wells using a pipette tip after 24 hours of

culture. The progression of wound closure was monitored under an inverted microscope at 0, 24, and 48 hours, and the percentage of scratch closure was calculated.

Flow cytometry analysis of apoptosis in HCT116 cells

HCT116 cells from experimental groups were harvested and seeded in 6-well plates, then cultured at 37 ℃ in a CO_2 incubator. After 24 hours, cells were digested with trypsin, collected into single-cell suspensions, and washed with cold phosphate-buffered saline (PBS). After centrifugation, cells were resuspended in binding buffer and stained with Annexin V-FITC and PI at room temperature in the dark for 10 minutes. Flow cytometry was used to determine the rate of apoptosis.

Flow cytometry analysis of cell cycle in HCT116 cells

Logarithmically growing HCT116 cells were washed with PBS, digested with trypsin, and resuspended in medium containing serum to terminate digestion. After centrifugation, cells were fixed in 70% cold ethanol and stored at 4 ℃ for 12 hours. Following further centrifugation and washing with PBS, cells were resuspended in PBS containing 0.2 mg/mL ribonuclease A (RNase A) and stained with PI/polyoxyethylene octylphenyl ether (Triton X-100) staining solution at 37 ℃ for 15 minutes. Flow cytometry was used to analyze the cell cycle.

Transwell assay to evaluate migration and invasion of HCT116 cells

For the migration assay, logarithmic phase HCT116 cells were seeded at a density of 3×10^4 cells per well in the upper chambers of transwell inserts. The lower chamber of each insert was filled with 700 µL of complete culture medium. After 48 hours of incubation, cells that did not migrate were removed from the upper surface of the inserts. Migrated cells on the lower surface were fixed with 4% paraformaldehyde for 30 minutes and stained with 0.1% crystal violet for 20 minutes. For the invasion assay, the bottom surface of transwell inserts was coated with 100 µL of matrigel prior to the experiment, and the subsequent steps were similar to the migration assay to assess invasive capability.

Statistical analysis

The data were analyzed using statistical package for the social sciences (SPSS) 26.0 software. One-way analysis of variance (ANOVA) was applied to assess differences among multiple groups, and the *t*-test was used for pairwise comparisons. P value <0.05 was considered to indicate statistical significance. Kaplan-Meier method and log-rank test were employed for survival analysis, with a P value <0.05 indicating statistical significance.

Results

Expression of FAM60A in CRC tissues

IHC staining of FAM60A was primarily nuclear, appearing as brown granules. Among 195 cases of CRC tissues, 142 cases (72.8%) demonstrated positive expression of FAM60A (*Figure 1A,1B*), whereas 53 cases (27.2%) showed negative expression. In the 65 adjacent normal tissues, 12 cases (18.5%) exhibited positive FAM60A expression, and 53 cases (81.5%) showed negative expression (*Figure 1C*). This difference was statistically significant (P<0.001) (*Figure 1D*).

Relationship between FAM60A protein expression and clinicopathological features, and prognostic survival

We analyzed the clinical and pathological characteristics of 195 CRC cases. *Table 1* presents the association of FAM60A expression with clinicopathological features, where FAM60A expression significantly correlated with the depth of invasion (P=0.041) and was positively associated with the Ki67 proliferation index (P=0.008). No significant correlations were observed with age, sex, differentiation grade, tumor size, primary tumor site, lymph node metastasis, distant metastasis, or Dukes stage (all P>0.05) (*Figure 2A*).

Analysis of FAM60A protein expression in relation to overall survival (OS) revealed that patients with positive expression had a median survival time of 45 months, significantly shorter than the 71 months observed in patients with negative expression. The average survival time for patients with positive FAM60A expression was 60 months, notably shorter than the 72 months for patients with negative expression. The statistical analysis showed a significant difference (P=0.01), indicating that patients with positive FAM60A expression had a poorer prognosis and lower survival rates (*Figure 2B*). Similarly, the analysis of FAM60A protein expression in relation to progression-free survival (PFS) indicated a median survival time of 40 months for patients with positive expression, significantly shorter than the 60 months for those with negative expression. The average survival time for patients with

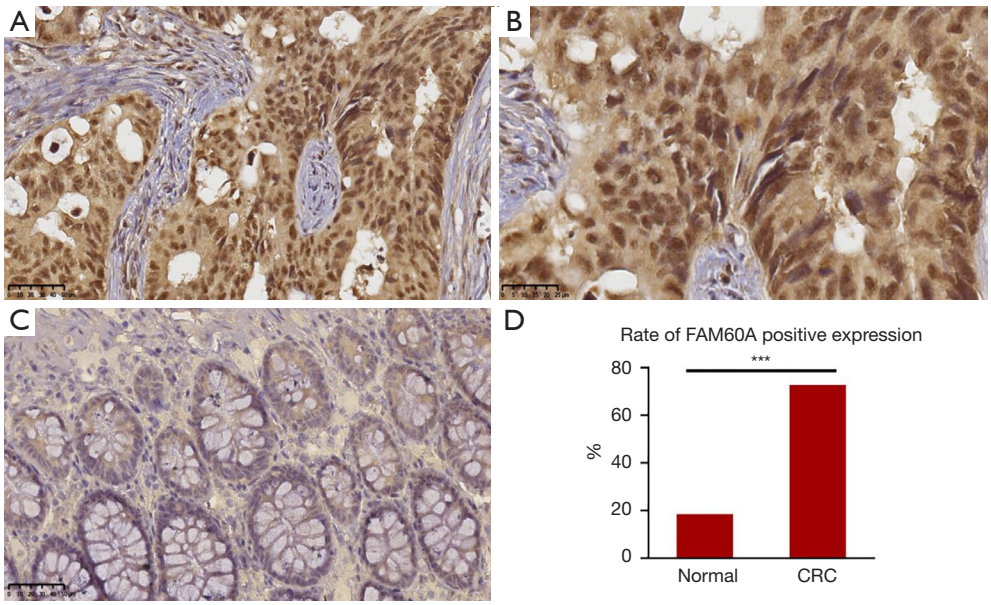

Figure 1 IHC staining analysis of FAM60A expression in CRC tissues. (A) Positive expression of FAM60A in CRC tissues. (B) Positive immunostaining for FAM60A was primarily observed in the cellular nucleus. (C) Negative expression of FAM60A in adjacent normal tissues. (D) Positive expression rates of FAM60A in each group. ***, P<0.001. FAM60A, family with sequence similarity 60, member A; CRC, colorectal cancer; IHC, immunohistochemistry.

positive FAM60A expression was 49 months, compared to 67 months for patients with negative expression. The statistical analysis showed a significant difference (P=0.01), indicating that positive FAM60A expression was associated with a higher risk of disease progression (*Figure 2C*).

Expression of β-catenin in different groups

Positive immunostaining for β-catenin was predominantly observed in the cellular cytoplasm or nucleus, with other locations being considered negative. In the analysis of 195 CRC cases, positive β-catenin expression was identified in 67 cases (34.4%) (*Figure 3A,3B*), while 128 cases (65.6%) demonstrated negative expression. In the control group of normal tissues, no positive β-catenin expression was detected (*Figure 3C*), indicating a stark contrast to the CRC cases and highlighting the statistical significance (P<0.001) (*Figure 3D*).

Correlation analysis of FAM60A and β-catenin expression, and their relationship with clinicopathological features

In CRC, the expression levels of both FAM60A and β-catenin were significantly increased. Among CRC cases positive for FAM60A, 39.4% (56/142) exhibited positive β-catenin expression, compared to 20.8% (11/53) in FAM60A-negative CRC. The statistical analysis demonstrated a significant positive correlation between FAM60A and β-catenin expression in CRC (P=0.02) (*Figure 4*). The co-expression of FAM60A and β-catenin was associated with elevated rates of distant metastasis (P=0.005), advanced stages (C and D) (P=0.02), and a high Ki67 proliferation index ≥50% (P=0.008), which were significantly different compared to non-co-expressors. However, the co-expression showed only a trend towards increased rates of lymph node metastasis and tumor invasion depth without reaching statistical significance (all P>0.05) (*Figure 5*).

Analysis of c-Myc and cyclin D1 expression in CRC tissues and correlation with FAM60A

Positive staining for c-Myc protein is primarily localized in the cell nucleus, with cytoplasm and other locations stained as negative. In a cohort of 195 CRC cases, 151 cases (77.4%) demonstrated positive c-Myc expression (*Figure 6A*), in contrast to 44 cases (22.6%) that were negative. Within a control group of 65 normal tissue samples, only 6 cases

Table 1 Relationship between FAM60A expression and clinicopathologic features

Clinicopathological features	FAM60A negative (n=53)	FAMN60A positive (n=142)	P value
Age (years)			0.60
<60	28	69	
≥60	25	73	
Gender			0.69
Male	33	84	
Female	20	58	
Maximum tumor diameter (cm)			0.13
<5	23	79	
≥5	30	63	
Infiltration depth			0.041*
T1 + T2	26	47	
T3 + T4	27	95	
Lymphatic transfer			0.20
No	36	82	
Yes	17	60	
Distant metastasis			0.18
No	46	111	
Yes	7	31	
Dukes instalment			0.15
A + B	33	72	
C + D	20	70	
Ki67			0.008**
<50%	13	14	
≥50%	40	128	
Degree of differentiation			0.61
High	10	19	
Middle	34	95	
Low	9	28	
Tumor location			0.28
Colon (right)	15	26	
Colon (left)	15	41	
Rectum	23	75	

Data are presented as number. *, P<0.05; **, P<0.01. FAM60A, family with sequence similarity 60, member A.

(9.2%) showed positive c-Myc staining, whereas 59 cases (90.8%) were negative (*Figure 6B*). The statistical analysis revealed a significant difference between the groups (P<0.001) (*Figure 6C*). Among adenocarcinomas that were FAM60A-positive, the rate of c-Myc positive expression was 88.0% (125/142), whereas in FAM60A-negative adenocarcinomas, the rate was 49.1% (26/53). Statistical analysis confirmed a significant positive correlation between FAM60A expression and the expression of c-Myc in CRC (P=0.01) (*Figure 6D*). Cyclin D1 positive staining is identified by the presence of brown granules within the nucleus, with other areas being negative. Of the 195 CRC cases, 163 (83.6%) exhibited positive cyclin D1 expression (*Figure 6E*), while 32 (16.4%) were negative. In the normal tissue group of 65, only 3 cases (4.6%) showed positive cyclin D1 expression, with 62 cases (95.4%) being negative (*Figure 6F*). The difference between the groups was found to be statistically significant (P<0.001) (*Figure 6G*). Similarly, the positive expression rate for cyclin D1 in FAM60A-positive CRC was 93.7% (133/142), compared to 56.6% (30/53) in FAM60A-negative adenocarcinomas. Statistical analysis confirmed a significant positive correlation between FAM60A expression and the expression of cyclin D1 in CRC (P=0.03) (*Figure 6H*).

Establishment of stable CRC cell lines with FAM60A knockdown and overexpression

To elucidate the relationship between FAM60A and β-catenin, and to determine if FAM60A regulates the biological behavior of CRC cells via the Wnt/β-catenin signaling pathway, lentiviral vectors for FAM60A overexpression were engineered and utilized to infect HCT116 and HT-29 cells, followed by puromycin selection to establish stable cell lines overexpressing FAM60A (HCT116-OE-NC, HCT116-OE, HT-29-OE-NC, and HT-29-OE). Validation using RT-qPCR demonstrated a significant upregulation of FAM60A mRNA levels in HCT116-OE and HT-29-OE cell lines compared to their respective controls (HCT116-OE-NC and HT-29-OE-NC) (all P<0.001) (*Figure 7A*,*7B*). Then, three distinct short hairpin RNA (shRNA) sequences were employed to generate shRNA constructs. RT-qPCR analysis confirmed efficient knockdown of FAM60A mRNA expression in HCT116 and HT-29 cell lines by all three shRNAs, with sh1 exhibiting the highest efficacy (P=0.002, P<0.001) (*Figure 7C*,*7D*). Immunofluorescence analysis confirmed the

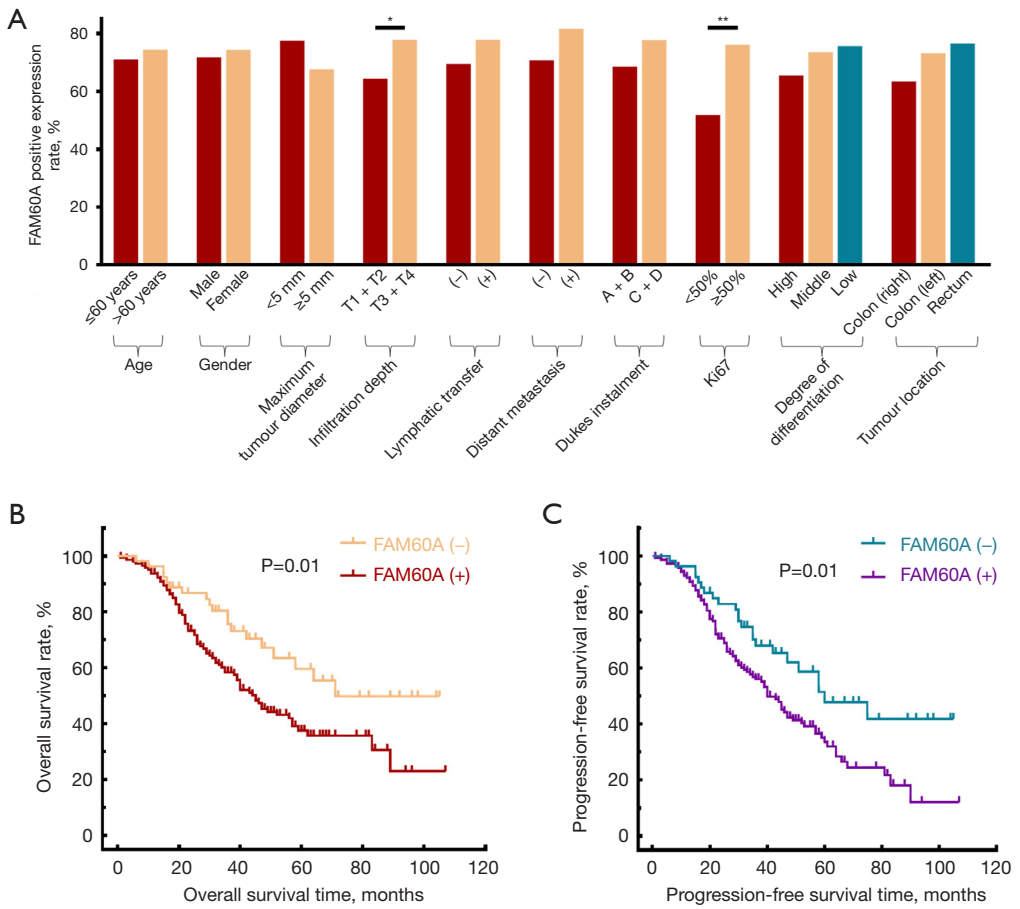

Figure 2 Relationship between FAM60A protein expression and clinicopathological features, and prognostic survival. (A) Relationship between FAM60A expression and clinicopathological features. (B) OS curve of patients with FAM60A protein expression. (C) PFS curve of patients with FAM60A protein expression. *, P<0.05; **, P<0.01. (–), negative; (+), positive. FAM60A, family with sequence similarity 60, member A; OS, overall survival; PFS, progression-free survival.

successful transfection of the cells (*Figure 7E*).

Regulation of Wnt/β-catenin signaling pathway expression by FAM60A in CRC cell lines HCT116 and HT-29

WB analysis was performed to evaluate the effects of FAM60A overexpression and knockdown on β-catenin expression levels in HCT116 and HT-29 CRC cell lines. As shown in *Figure 8*, overexpression of FAM60A led to a significant increase in β-catenin expression in HCT116 cells (P=001), while knockdown resulted in a decrease (P=0.002) (*Figure 8A-8C*). Consistent with the findings in HCT116 cells, HT-29 cells also demonstrated similar β-catenin expression patterns (OE, P<0.001; sh1, P=0.004) (*Figure 8D-8F*), suggesting upstream regulation of β-catenin by FAM60A, which in turn modulates the Wnt/β-catenin signaling pathway expression in CRC cells.

Promotion of proliferation capacity of CRC cell lines HCT116 and HT-29 by FAM60A

The proliferation capacity of HCT116 and HT-29 CRC cell lines with FAM60A overexpression (OE-NC and OE) and knockdown (sh-NC, sh1) was assessed using the CCK-8 assay at 0, 12, 24, 48, and 72 hours (*Figure 9A,9B*). The sh1 group exhibited a significant reduction in average OD values at 72 hours (all P<0.001) in both HCT116 and HT-29 cell lines, indicating reduced proliferation capacity. In contrast, the OE group showed a significant increase in average OD values at 72 hours (all P<0.001) in both cell lines, suggesting enhanced proliferation capacity due to FAM60A overexpression.

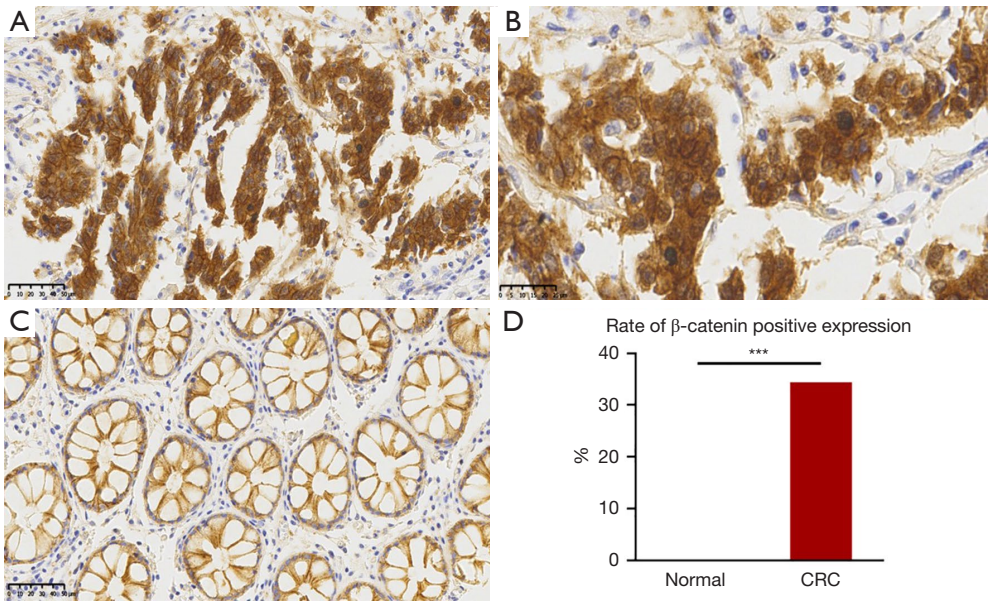

Figure 3 IHC staining analysis of β-catenin expression in CRC tissues. (A) Positive expression of β-catenin in CRC tissues. (B) Positive immunostaining for β-catenin was predominantly observed in the cellular cytoplasm or nucleus. (C) Negative expression of β-catenin in adjacent normal tissues. (D) Positive expression rates of β-catenin in each group. ***, P<0.001. CRC, colorectal cancer; IHC, immunohistochemistry.

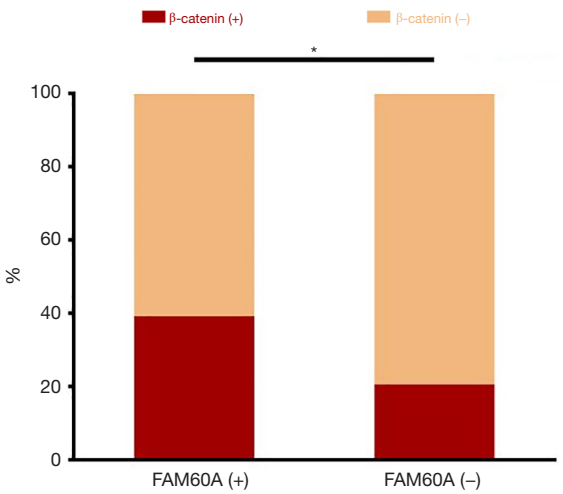

Figure 4 Correlation analysis of FAM60A and β-catenin expression. *, P<0.05. (–), negative; (+), positive. FAM60A, family with sequence similarity 60, member A.

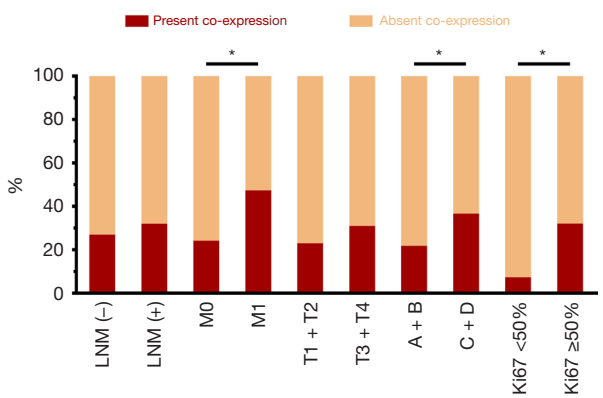

Figure 5 Relationship between co-expression of FAM60A and β-catenin and clinicopathological features. *, P<0.05. (–), negative; (+), positive. LNM, lymph node metastasis; FAM60A, family with sequence similarity 60, member A.

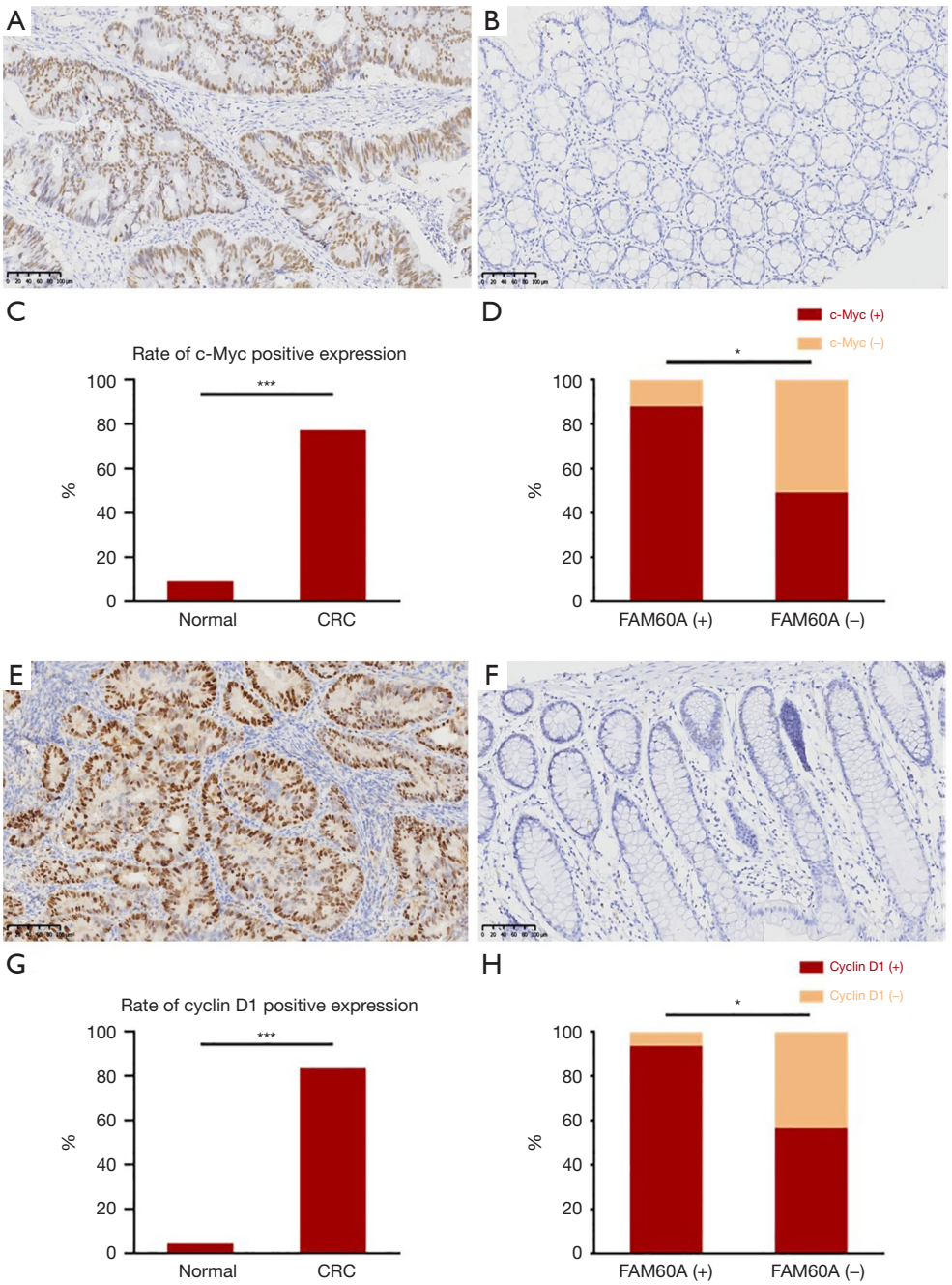

Figure 6 IHC analysis of c-Myc and cyclin D1 expression in CRC tissues and their correlation with FAM60A. (A) Positive expression of c-Myc in CRC tissues. (B) Negative expression of c-Myc in adjacent normal tissues. (C) Positive expression rates of c-Myc in each group. (D) Correlation analysis of FAM60A and c-Myc expression. (E) Positive expression of cyclin D1 in CRC tissues. (F) Negative expression of cyclin D1 in adjacent normal tissues. (G) Positive expression rates of cyclin D1 in each group; (H) Correlation analysis of FAM60A and cyclin D1 expression. *, P<0.05; ***, P<0.001. (–), negative; (+), positive. CRC, colorectal cancer; IHC, immunohistochemistry; FAM60A, family with sequence similarity 60, member A.

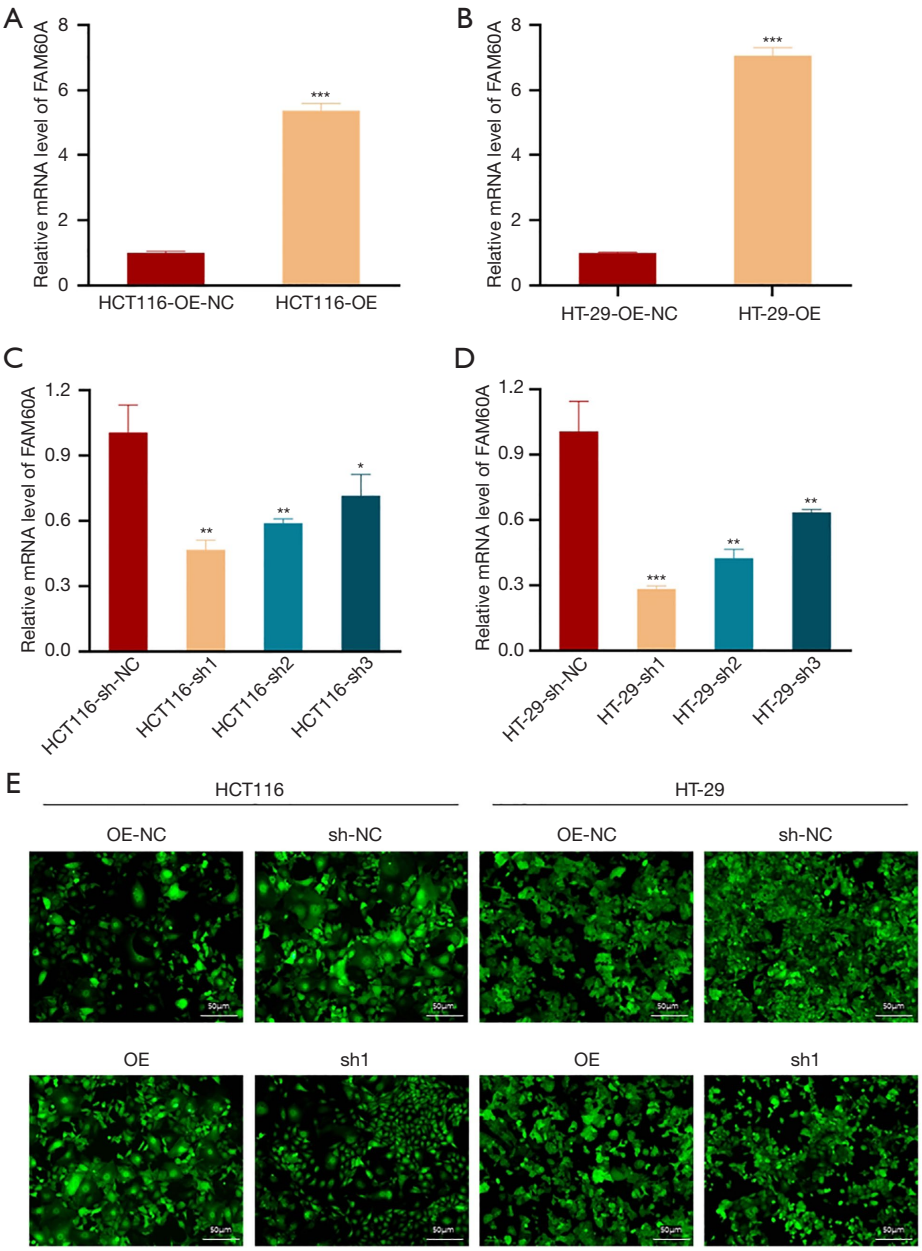

Figure 7 Construction and validation of stable overexpression and knockdown of FAM60A lentivirus in CRC cell lines. (A) RT-qPCR detection of FAM60A mRNA levels in overexpression CRC cell lines HCT116. (B) RT-qPCR detection of FAM60A mRNA levels in overexpression CRC cell lines HT-29. (C) RT-qPCR detection of FAM60A mRNA levels in knockdown CRC cell lines HCT116. (D) RT-qPCR detection of FAM60A mRNA levels in knockdown CRC cell lines HT-29. (E) Immunofluorescence staining to observe transfection efficiency in different groups. *, P<0.05; **, P<0.01; ***, P<0.001. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown; HT-29-OE-NC, HT-29 negative control cell line overexpressing FAM60A; HT-29-OE, HT-29 cell line overexpressing FAM60A; HT-29-sh-NC, HT-29 negative control cell line with FAM60A knockdown; HT-29-sh1, HT-29 cell line with FAM60A knockdown. mRNA, messenger RNA; FAM60A, family with sequence similarity 60, member A; CRC, colorectal cancer; RT-qPCR, real-time quantitative polymerase chain reaction.

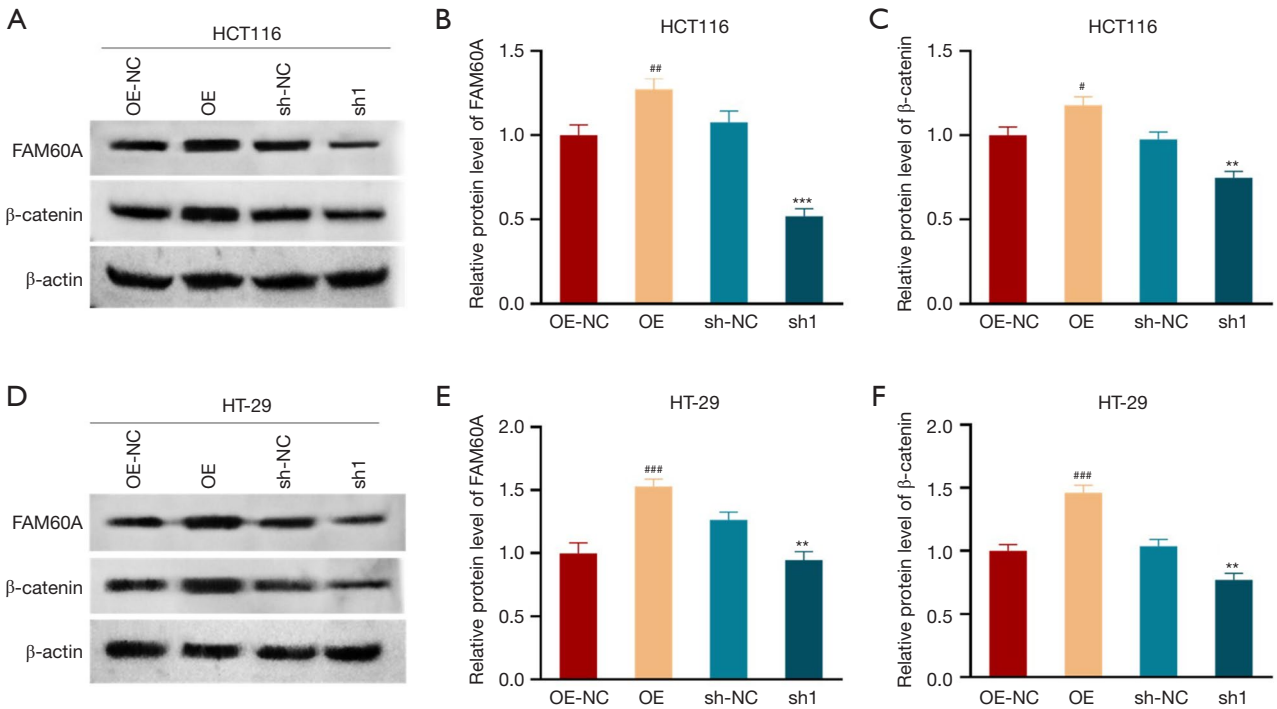

Figure 8 Regulation of Wnt/β-catenin signaling pathway expression by FAM60A in CRC cell lines HCT116 and HT-29. (A-C) WB analysis of FAM60A and β-catenin protein expression levels in CRC cell lines HCT116. (D-F) WB analysis of FAM60A and β-catenin protein expression levels in CRC cell lines HT-29. #, P<0.05 *vs.* OE-NC; ##, P<0.01 *vs.* OE-NC; ###, P<0.001 *vs.* OE-NC; **, P<0.01 *vs.* sh-NC; ***, P<0.001 *vs.* sh-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown; HT-29-OE-NC, HT-29 negative control cell line overexpressing FAM60A; HT-29-OE, HT-29 cell line overexpressing FAM60A; HT-29-sh-NC, HT-29 negative control cell line with FAM60A knockdown; HT-29-sh1, HT-29 cell line with FAM60A knockdown. FAM60A, family with sequence similarity 60, member A; CRC, colorectal cancer; WB, western blot.

Promotion of scratch healing capacity of CRC cell lines HCT116 and HT-29 by FAM60A

The scratch healing assay was conducted to determine the influence of FAM60A expression levels on the migration capacity of CRC cell lines HCT116 (*Figure 10A*,*10B*) and HT-29 (*Figure 10C*,*10D*). FAM60A overexpression significantly enhanced scratch healing at 24 and 48 hours (all P<0.05). In contrast, knockdown of FAM60A significantly impaired scratch healing at 24 and 48 hours (all P<0.001) in both cell lines.

Inhibition of apoptosis in CRC cell line HCT116 by FAM60A

To elucidate the effects of FAM60A expression on apoptosis in HCT116 cells, flow cytometry was utilized. The results indicated that the sh1 group displayed a higher rate of apoptosis compared to the sh-NC group (P<0.001). In contrast, the OE group exhibited a significantly lower rate of apoptosis compared to the OE-NC group (P=0.009) (*Figure 11A*,*11B*).

Promotion of cell cycle progression in CRC cell line HCT116 by FAM60A

Flow cytometric analysis was conducted to assess the effects of FAM60A modulation on the cell cycle progression in the HCT116 cell line. The findings revealed that FAM60A knockdown significantly arrested cells in the G0/G1 phase and reduced the S phase cell population (all P<0.001), ultimately affecting the proliferation capacity. In contrast, FAM60A overexpression decreased the proportion of cells in the G0/G1 phase (P<0.001) and increased the S and

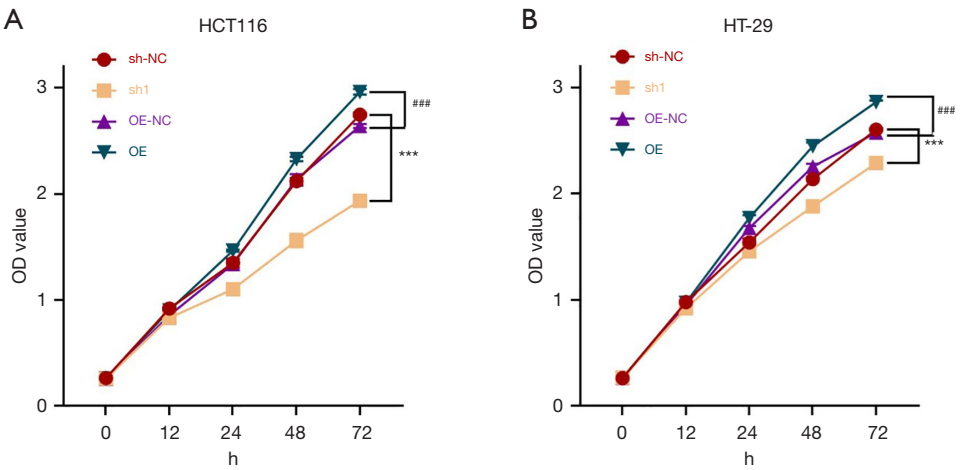

Figure 9 Promotion of proliferation of CRC cell lines HCT116 and HT-29 by FAM60A. (A) CCK-8 assay was used to evaluate the effect of FAM60A overexpression and knockdown on proliferation capacity of CRC cell lines HCT116. (B) CCK-8 assay was used to evaluate the effect of FAM60A overexpression and knockdown on proliferation capacity of CRC cell lines HT-29. ***, P<0.001 *vs.* sh-NC; ###, P<0.001 *vs.* OE-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown; HT-29-OE-NC, HT-29 negative control cell line overexpressing FAM60A; HT-29-OE, HT-29 cell line overexpressing FAM60A; HT-29-sh-NC, HT-29 negative control cell line with FAM60A knockdown; HT-29-sh1, HT-29 cell line with FAM60A knockdown. OD, optical density; CRC, colorectal cancer; FAM60A, family with sequence similarity 60, member A; CCK-8, cell counting kit-8.

G2/mitosis (M) phase populations in HCT116 cells (all P<0.001) (*Figure 12A,12B*).

Promotion of invasion and migration of CRC cell line HCT116 by FAM60A

Prior research has established a positive correlation between FAM60A expression levels and tumor invasion depth in CRC patients. To explore the potential of FAM60A to enhance the invasive and migratory capabilities of HCT116 cells, transwell assays were performed (*Figure 13A*). The results demonstrated that FAM60A knockdown inhibited the migration (P=0.005) and invasion (P=0.004) capabilities of HCT116 cells compared to the sh-NC group, while overexpression promoted these capabilities (all P<0.01) (*Figure 13B,13C*).

Discussion

The recognition of FAM60A's significant role in regulating stem cell function and its implications in cancer has grown among researchers in recent years. FAM60A is not only integral to maintaining the proliferation and self-renewal capacity of ESC, but also plays critical roles in a range of malignancies, including esophageal (19), gastric (20), lung (21), liver (22), gliomas (23), and pancreatic (24) cancer, where its effects are intricately linked to cell cycle regulation and signaling pathways. FAM60A demonstrates a biphasic role within tumors, capable of both fostering and suppressing carcinogenesis, contingent upon the cellular microenvironment.

Our investigation revealed significantly elevated expression levels of FAM60A in CRC tissues relative to adjacent non-neoplastic tissues. Subsequent analyses demonstrated a significant association between FAM60A expression levels and clinical pathological features in CRC patients, particularly in relation to tumor invasion depth and the Ki67 proliferation index. PFS and OS indicated that patients with FAM60A-positive protein expression exhibited an increased risk of disease progression and diminished survival rates. Collectively, these observations implicate a potential oncogenic role for FAM60A in the development and progression of CRC. Considering the essential role of the Wnt/β-catenin signaling pathway in the growth of

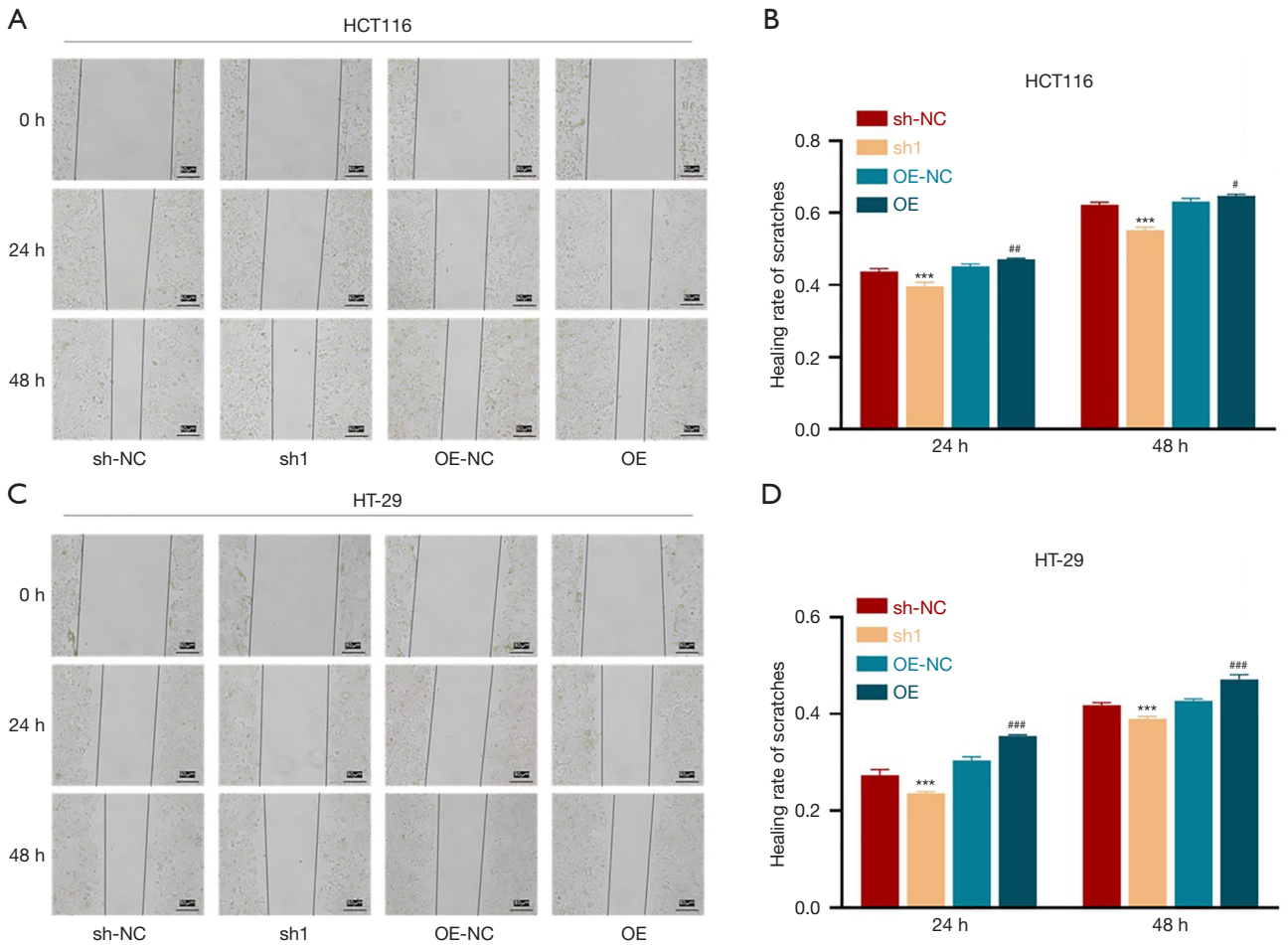

Figure 10 Promotion of scratch healing capacity of CRC cell lines HCT116 and HT-29 by FAM60A. (A) Scratch healing assay was conducted to evaluate migration capacity of HCT116 cell lines following overexpression and knockdown of FAM60A. (B) Statistical results of the scratch healing assay in the HCT116 cell line. (C) Scratch healing assay was conducted to evaluate migration capacity of HT-29 cell lines following overexpression and knockdown of FAM60A. (D) Statistical results of the scratch healing assay in the HT-29 cell line. ***, P<0.001 *vs.* sh-NC; #, P<0.05 *vs.* OE-NC; ##, P<0.01 *vs.* OE-NC; ###, P<0.0001 *vs.* OE-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown; HT-29-OE-NC, HT-29 negative control cell line overexpressing FAM60A; HT-29-OE, HT-29 cell line overexpressing FAM60A; HT-29-sh-NC, HT-29 negative control cell line with FAM60A knockdown; HT-29-sh1, HT-29 cell line with FAM60A knockdown. CRC, colorectal cancer; FAM60A, family with sequence similarity 60, member A.

CRC, aberrant activation of β-catenin can result in the dysregulation of cell proliferation and adenoma formation, thereby promoting carcinogenesis. Our analysis also included the expression levels of β-catenin in CRC tissues, which were found to be significantly elevated compared to adjacent non-neoplastic tissues. Additional analyses demonstrated a significant positive correlation between the expression of FAM60A and β-catenin. Patients with CRC exhibiting co-expression of FAM60A and β-catenin showed significantly higher rates of distant metastasis and advanced tumor stages (C and D), as well as an elevated Ki67 proliferation index compared to those not co-expressing these proteins. Similarly, downstream factors c-Myc and cyclin D1 in the Wnt/β-catenin signaling pathway also

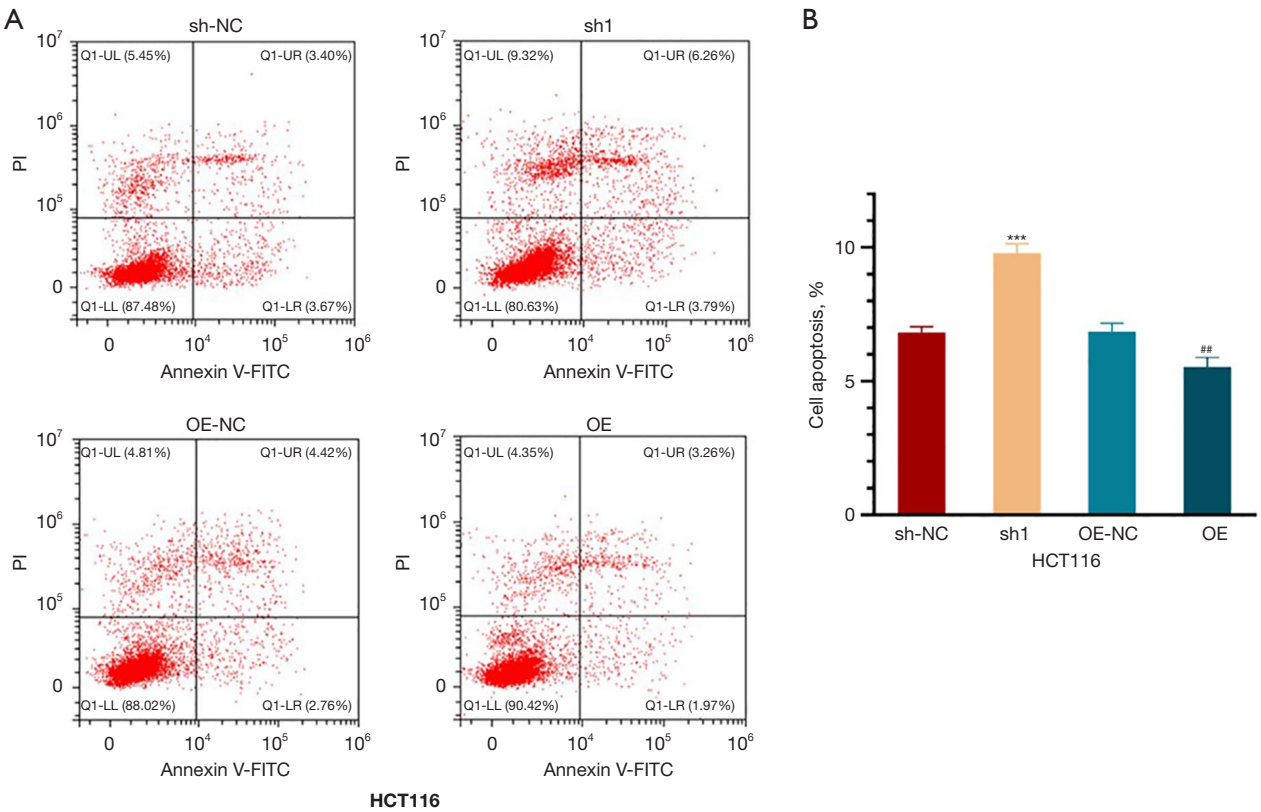

Figure 11 Inhibition of apoptosis in CRC cell line HCT116 by FAM60A. (A) Flow cytometry analysis of the impact of FAM60A overexpression and knockdown on apoptosis levels in CRC cell line HCT116. (B) Presentation of flow cytometry analysis results. ***, P<0.001 *vs.* sh-NC; ##, P<0.01 *vs.* OE-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown. PI, propidium iodide; FITC, fluorescein isothiocyanate; UL, upper left; UR, upper right; LL, lower left; LR, lower right; CRC, colorectal cancer; FAM60A, family with sequence similarity 60, member A.

show a significant positive correlation with FAM60A.These findings suggest a significant interaction between FAM60A and the Wnt/β-catenin signaling pathway, which may modulate CRC development by affecting cell proliferation, invasion, and the potential for distant metastasis. To substantiate the link between FAM60A and β-catenin, we developed stable CRC cell lines featuring FAM60A knockdown and overexpression. WB analysis indicated that changes in FAM60A expression levels were accompanied by corresponding changes in β-catenin protein levels, suggesting FAM60A acts upstream to regulate β-catenin in CRC. This implicates FAM60A as a potential therapeutic target, potentially interacting with β-catenin to induce the overexpression of downstream effectors such as c-Myc and cyclin D1, thus facilitating CRC development.

Following this, we conducted a battery of *in vitro* experiments to ascertain the influence of FAM60A on the biological behavior of CRC cells via modulation of the Wnt/β-catenin signaling pathway. The CCK-8 assay and scratch wound healing assay demonstrated that overexpression of FAM60A enhanced cell viability and proliferation in the HCT116 and HT-29 cell lines, while knockdown produced the converse effect. Additional flow cytometry analysis revealed that FAM60A knockdown induced cell cycle arrest at the G0/G1 phase and promoted apoptosis, thereby inhibiting cell growth. In contrast, FAM60A overexpression decreased the proportion of cells in the G0/G1 phase and increased the population in the S and G2/M phases, thus inhibiting apoptosis and promoting cell growth. This aligns with findings from prior research

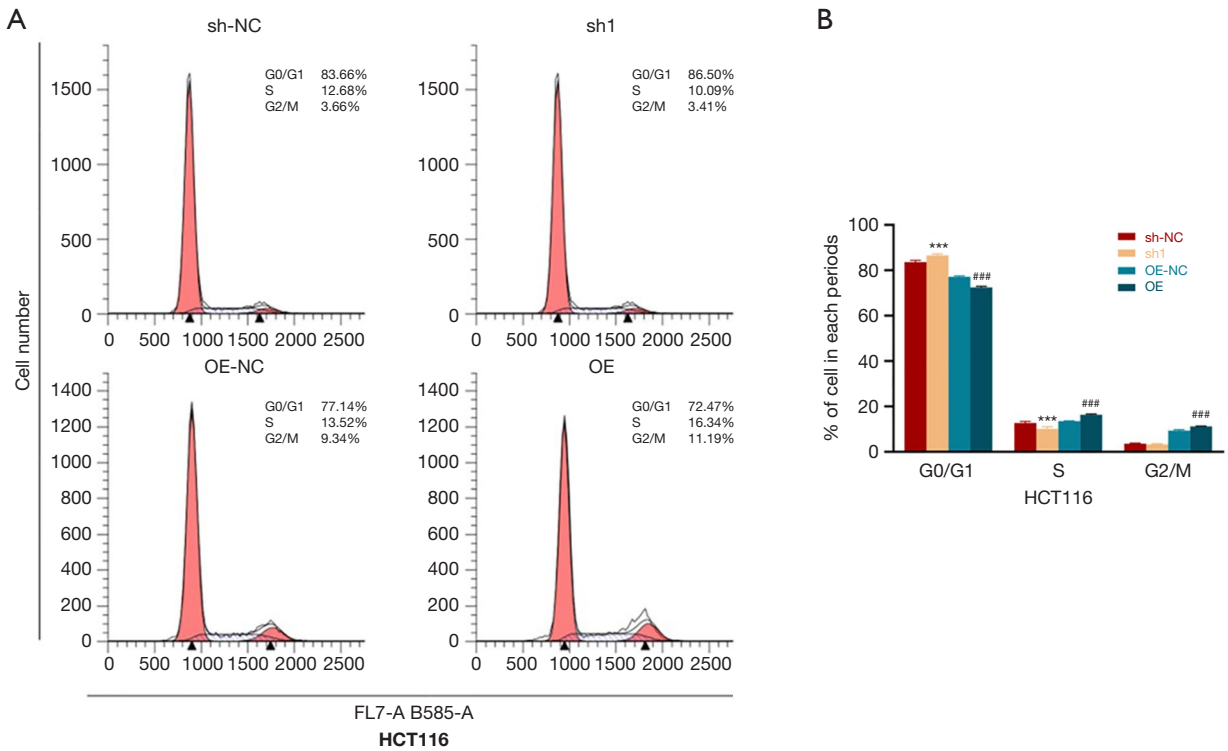

Figure 12 Promotion of cell cycle progression in CRC cell line HCT116 by FAM60A. (A) Flow cytometry analysis of the impact of FAM60A overexpression and knockdown on cell cycle progression in CRC cell line HCT116. (B) Presentation of flow cytometry analysis results. ***, P<0.001 *vs.* sh-NC; ###, P<0.001 *vs.* OE-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown. G, gap; S, synthesis; M, mitosis; CRC, colorectal cancer; FAM60A, family with sequence similarity 60, member A.

in esophageal (25) and gastric (20) cancers, which suggest that FAM60A knockdown can prevent tumor cells from entering the G2/M phase, thereby inhibiting proliferation and promoting apoptosis. Furthermore, functional assays evaluating CRC cell invasion and migration using transwell revealed that overexpression of FAM60A promoted these capabilities, whereas FAM60A knockdown exerted an opposing effect. Prior research has documented substantial anti-neoplastic effects of FAM60A knockdown in esophageal (25), gastric (20), gliomas (23), and pancreatic (24) cancers, corroborating our results. According to IHC and WB data indicating FAM60A's upstream regulatory role on β-catenin, combined with experimental evidence of FAM60A promoting malignant biological behavior in CRC cells, our findings provide partial validation for the role of FAM60A in promoting CRC proliferation and invasion through modulation of the Wnt/β-catenin signaling pathway.

It is noteworthy that the role of FAM60A may vary across different tumors due to variations in intracellular environments, suggesting potential tumor-specific biological functions. Earlier investigations have proposed a tumor-suppressive role for FAM60A in certain cancers (7), contrasting with our study, which identified a distinct oncogenic role for FAM60A in CRC. This discrepancy could be due to tumor-specific signaling networks and microenvironments, highlighting the need for further research to elucidate these mechanisms.

Conclusions

To conclude, our research offers novel perspectives on the role of FAM60A in CRC, enhancing our comprehension of the molecular mechanisms involved in CRC development.

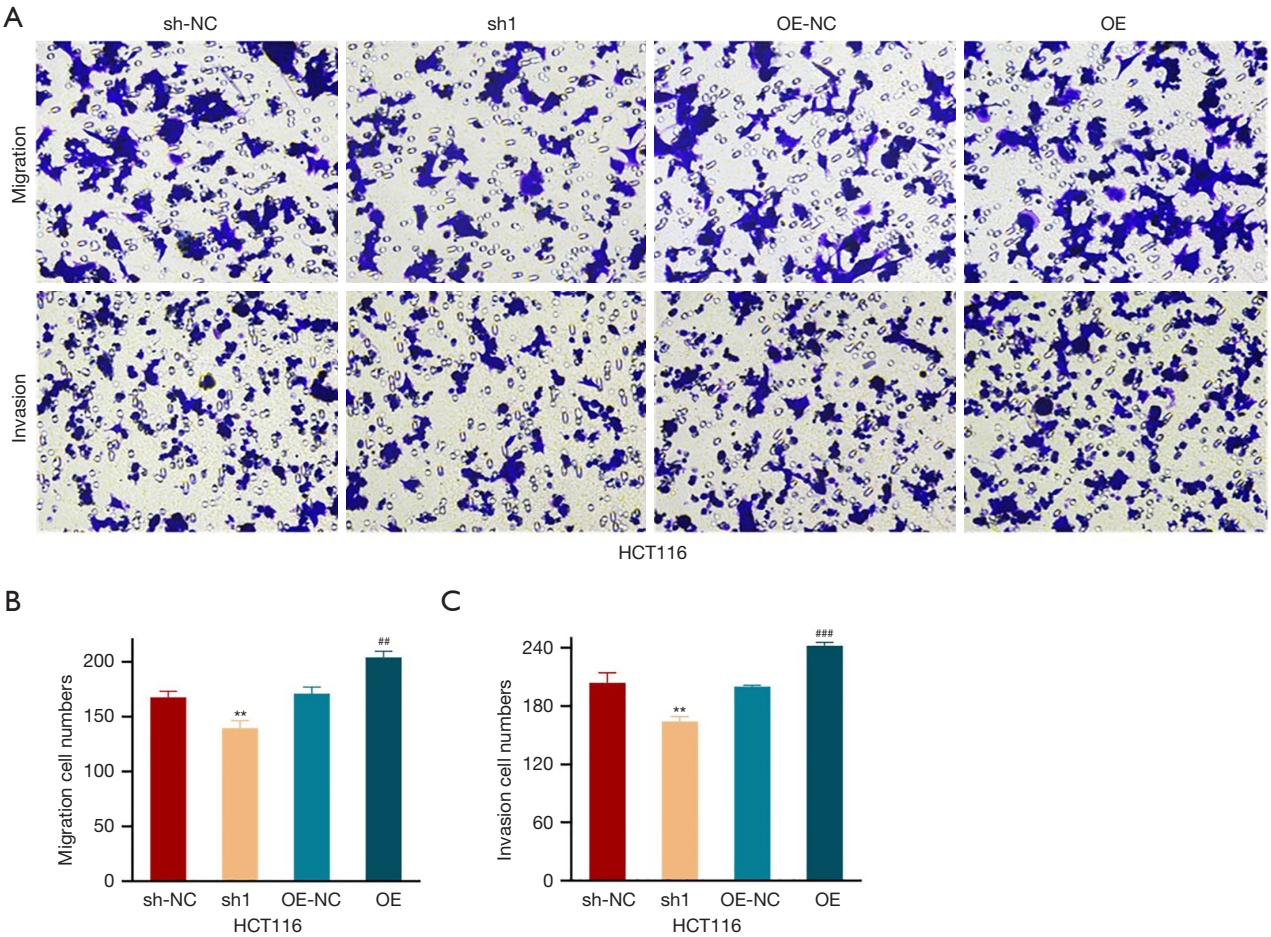

Figure 13 Promotion of invasion and migration of CRC cell line HCT116 by FAM60A. (A) Transwell assay evaluating the impact of FAM60A overexpression and knockdown on migration and invasion of CRC cell line HCT116 (magnification, ×40). (B) Presentation of migration assay results. (C) Presentation of invasion assay results. Staining method for transwell assays: crystal violet staining. **, P<0.01 *vs.* sh-NC; ##, P<0.01 *vs.* OE-NC; ###, P<0.001 *vs.* OE-NC. HCT116-OE-NC, HCT116 negative control cell line overexpressing FAM60A; HCT116-OE, HCT116 cell line overexpressing FAM60A; HCT116-sh-NC, HCT116 negative control cell line with FAM60A knockdown; HCT116-sh1, HCT116 cell line with FAM60A knockdown. CRC, colorectal cancer; FAM60A, family with sequence similarity 60, member A.

Furthermore, these findings hold promise for identifying specific molecular biomarkers with practical value for predicting prognosis in CRC patients.

Acknowledgments

We are grateful for the convenience provided by The First Hospital of Lanzhou University for collecting tissue specimen.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1608/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1608/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1608/prf

Funding: This work was supported by the Health Industry Scientific Research Project of Gansu Province (No. GSWSKY2023-02), the Science and Technology Project of Chengguan District Lanzhou City (No. 2022JSCX0031),

and the First Hospital of Lanzhou University Fund Projects (No. ldyyyn2022-2).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1608/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was performed in line with the principles of the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The First Hospital of Lanzhou University, China (No. LDYYLL-2024-744). Informed consent to participate in this study was obtained from all individual participants. The requirement to obtain informed consent to the use of patient materials was waived by the same Ethics Committee.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
3. Wang DS, Yang H, Liu XY, et al. Dynamic monitoring of circulating tumor DNA to predict prognosis and efficacy of adjuvant chemotherapy after resection of colorectal liver metastases. Theranostics 2021;11:7018-28.
4. Muñoz IM, MacArtney T, Sanchez-Pulido L, et al. Family with sequence similarity 60A (FAM60A) protein is a cell cycle-fluctuating regulator of the SIN3-HDAC1 histone deacetylase complex. J Biol Chem 2012;287:32346-53.
5. Streubel G, Fitzpatrick DJ, Oliviero G, et al. Fam60a defines a variant Sin3a-Hdac complex in embryonic stem cells required for self-renewal. EMBO J 2017;36:2216-32.
6. Kareta MS, Sage J, Wernig M. Crosstalk between stem cell and cell cycle machineries. Curr Opin Cell Biol 2015;37:68-74.
7. Sun YH, Sun XW, Yang L, et al. Research Progress of FAM60A in the Regulation of Cellular Function. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2021;43:469-74.
8. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther 2022;7:3.
9. Gu JW, Niu GM, Ke CW. Research progress on the mechanism regulating the cellular sub-localization of β-catenin and potential associated therapeutic targets in canonical Wnt signaling pathway. Fudan University Journal of Medical Sciences 2022;49:300-8.
10. Liu YJ, Wu JZ, Yu Y, et al. Phosphorylation of β-Catenin and its biological function. Available online: https://www.paper.edu.cn/releasepaper/content/201311-110
11. A Najafi SMA. The Canonical Wnt Signaling (Wnt/β-Catenin Pathway): A Potential Target for Cancer Prevention and Therapy. Iran Biomed J 2020;24:269-80.
12. Matly A, Quinn JA, McMillan DC, et al. The relationship between β-catenin and patient survival in colorectal cancer systematic review and meta-analysis. Crit Rev Oncol Hematol 2021;163:103337.
13. Dow LE, O'Rourke KP, Simon J, et al. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. Cell 2015;161:1539-52.
14. Yue X, Xi X, Cheng B, et al. SIN3A/HDAC complex subunit FAM60A is associated with the proliferation in colorectal cancer. Asian J Surg 2023;46:2794-5.
15. Liu Z, Sun DX, Teng XY, et al. Expression of stromal cell-derived factor 1 and CXCR7 in papillary thyroid carcinoma. Endocr Pathol 2012;23:247-53.
16. Friedrichs K, Gluba S, Eidtmann H, et al. Overexpression of p53 and prognosis in breast cancer. Cancer 1993;72:3641-7.
17. Neumann J, Horst D, Kriegl L, et al. A simple immunohistochemical algorithm predicts the risk of distant metastases in right-sided colon cancer. Histopathology 2012;60:416-26.
18. Buhmeida A, Elzagheid A, Algars A, et al. Expression of the cell-cell adhesion molecule beta-catenin in colorectal carcinomas and their metastases. APMIS 2008;116:1-9.
19. Dong G, Mao Q, Yu D, et al. Integrative analysis of copy number and transcriptional expression profiles in esophageal cancer to identify a novel driver gene for

therapy. Sci Rep 2017;7:42060.
20. Yao X, Liu D, Zhou L, et al. FAM60A, increased by Helicobacter pylori, promotes proliferation and suppresses apoptosis of gastric cancer cells by targeting the PI3K/AKT pathway. Biochem Biophys Res Commun 2020;521:1003-9.
21. Hou Q, Jiang Z, Li Y, et al. FAM60A promotes cisplatin resistance in lung cancer cells by activating SKP2 expression. Anticancer Drugs 2020;31:776-84.
22. Smith KT, Sardiu ME, Martin-Brown SA, et al. Human family with sequence similarity 60 member A (FAM60A) protein: a new subunit of the Sin3 deacetylase complex. Mol Cell Proteomics 2012;11:1815-28.
23. Liu X, Zhang M, Zhu X, et al. Loss of FAM60A attenuates cell proliferation in glioma via suppression of PI3K/Akt/mTOR signaling pathways. Transl Oncol 2021;14:101196.
24. Zhang Y, Li JH, Yuan QG, et al. Restraint of FAM60A has a cancer-inhibiting role in pancreatic carcinoma via the effects on the Akt/GSK-3β/β-catenin signaling pathway. Environ Toxicol 2022;37:1432-44.
25. Yang Y, Liu S, Xie C, et al. Trafficking protein TMED3 promotes esophageal squamous cell carcinoma. Biomed J 2023;46:100528.

Cite this article as: Dong Z, Jin S, Tang K, Li X, Chen Y. FAM60A promotes proliferation and invasion of colorectal cancer cells by regulating the Wnt/β-catenin signaling pathway. Transl Cancer Res 2025;14(2):1171-1189. doi: 10.21037/tcr-24-1608