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KIF3C inhibits the progression and proliferation of colorectal cancer

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Abstract

Background Evidence indicated that KIF3C, a member of the kinesin superfamily of motor proteins, exhibits significant upregulation across various cancer types. Consequently, its impact on cancer advancement, including cell proliferation, migration, and invasion, is evident. Nonetheless, the comprehension of KIF3C's expression and role in colorectal cancer (CRC) remains limited.

Methods Immunohistochemistry was used to evaluate the presence of KIF3C in CRC. The expression levels of KIF3C were assessed in CRC cells through western blot analysis (WB) and real-time polymerase chain reaction (RT-qPCR). KIF3C was knockdown and overexpressed using lentiviral vectors in the human CRC cell lines SW-480, HCT-116, and SW-620. In vitro experiments such as transwell assays, scratch wound healing, colony formation assays, counting cell CCK-8, and signaling pathway experiments were conducted to validate the KIF3C function in CRC cells.

Results We demonstrated that KIF3C is highly expressed in cells and tissues of CRC, and this expression is closely associated with tumor prognosis. It was shown that KIF3C knockdown significantly inhibited tumor cell proliferation and migration in CRC cells. Additionally, the KIF3C signaling pathway experiment in this study promoted the CRC progression by upregulating the PI3K/AKT, Bax, and Bcl-2 pathways.

Conclusions KIF3C knockdown promoted CRC proliferation, as it could be a potential therapeutic target for treating CRC.

Keywords KIF3C, Colorectal cancer, Cell cycle, Proliferation, Signaling pathway

Introduction

Colorectal cancer (CRC) is a prevalent malignancy, accounting for 6.1% of cancer incidences and 9.2% of cancer-related deaths [1, 2]. While surgery is the primary treatment for early-stage CRC, patients are often diagnosed at an advanced stage, sometimes with distant metastases already present. Despite the development of some antibodies and compounds for the treatment of colon cancer, surgical removal followed by chemotherapy and/or radiation therapy remains the standard of care after comprehensive treatment of the primary tumor [3]. The disseminated cells appear dormant and undetected, leaving the patients asymptomatic. Cancer stem cells have been attributed to being responsible for dormant cancer cells [3]. Chemotherapy and radiation therapy are believed to be ineffective against colon cancer stem-like cells (CSCs), which are a major cause of relapse. Identifying the detailed mechanisms that promote CSCs and considering targeted treatments are critical for improving patient outcomes [4].

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First identified in 1985, kinesin superfamily proteins (KIFs) are a conserved group of microtubule-dependent motor proteins involved in numerous cellular processes. These processes include the transport of intracellular organelles and macromolecules, maintenance of cell morphology, regulation of cytoskeletal dynamics, facilitation of cell migration, and control of cell division [5]. Three members of the KIF3 subfamily—KIF3A, KIF3B, and KIF3C—have been identified and characterized in mice [6]. The first member, KIF3A, acts as a microtubule-directed motor protein and plays a crucial role in the intracellular transport of the β-catenin–cadherin complex [7]. In prostate cancer, upregulation of KIF3A has been shown to enhance cell invasion and proliferation [8].

During cell mitosis, KIF3B, another member of the KIF3 subfamily, is involved in vesicle trafficking and membrane enlargement [9]. Due to its high expression in human hepatocellular carcinoma (HCC) tissues, downregulation of KIF3B may inhibit the proliferation of HCC [10]. Both KIF3C and KIF3B bind the KIF3A protein and share a similar amino acid sequence [6]. KIF3C is highly expressed in the nervous system and contributes to anterograde axonal transport in mouse neuronal cells [11]. According to a study by Gumy et al. [12] KIF3C KIF3C functions as an injury-specific kinesin, facilitating axon growth and regeneration by organizing and regulating the microtubule cytoskeleton within the growth cone. A study by Wang et al. reported that KIF3C exhibits elevated expression levels in breast cancer tissues. Furthermore, the study demonstrated that reducing KIF3C expression could mitigate tumor progression and metastasis in breast cancer by inhibiting TGF-β signaling [13]. However, the expression and potential impact of the KIF3C gene in colorectal cancer (CRC) remain unexplored. This study examines the expression of KIF3C in human colon cancer and investigates the functional consequences of therapeutically targeting KIF3C depletion on tumor growth, invasion, and metastasis in CRC.

Materials and methods

Clinical samples

From July 2015 to May 2017, 50 specimens of CRC and adjacent non-tumor tissues were procured from patients treated at Northern Jiangsu People's Hospital. Tumor-adjacent tissue was obtained from at least 5 cm away from the tumors. Tissues were immediately formalin-fixed and embedded in paraffin after isolation. No patient received radiotherapy or chemotherapy before surgery. At least two qualified pathologists adjudicated all tissues. American Joint Committee on Cancer TNM staging system (AJCC-8 TNM) was used to identify the stage of CRC. Tissue samples collected underwent pathological examination to confirm CRC diagnosis. Before participation, each patient provided informed consent, which was approved by the Ethics Committee of Northern Jiangsu People's Hospital. Furthermore, each participant provided informed consent for the collection of tissue (Table 1). The study follows the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline [14].

Immunohistochemical staining

Immunohistochemistry (IHC) was employed to assess protein expression in cancer specimens. Immunohistochemical staining was used to identify KIF3C expression. Tissue sections were dried at 60 ∘C for 2 h. Paraffin was dewaxed with xylene and samples were hydrated in a graded alcohol series and citrate buffer, then blocked with 3% hydrogen peroxide. The sections were left to incubate at 4 °C overnight with a rabbit monoclonal antibody targeting KIF3C (dilution 1:500; 14,333-1-AP; Proteintech, IL, USA). Subsequently, each section was washed three times with PBS, followed by a 30-min incubation with goat anti-rabbit IgG, then developed using 3,3'-diaminobenzidine. The ratings for staining intensity were as follows: 0 indicated no color, 1 indicated yellow, 2 indicated brown, and 3 indicated deep brown. The staining intensity score is as described: colorless (0), yellow (1), brown (2), dark brown (3). Scores were allocated based on the percentage of positive cells: 0 points for 0–5%, 1 point for 5–25%, 2 points for 25–50%, 3 points for 50–75%, and 4 points for > 75%. KIF3C expression levels were categorized as either low or high, determined by a total score of 5.

Cell lines and culture

The normal colon cancer epithelial cell lines (NCM-460) and human colon cancer cell lines (HCT116, SW-480, and SW-620) were obtained from the Cell Bank of the Type Culture Collection at the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The different cell lines were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin and 100 μg/ml streptomycin (SV30010, Hyclone, Cytiva), and then incubated at 37 °C in a 5% CO2 environment.

Cell transfection

Small interfering RNA targeting KIF3C (si-KIF3C), control siRNA (si-Control), KIF3C overexpression construct (oe-KIF3C), and the empty control vector (Vector) were synthesized by Gene Chem (Shanghai, China). The sequences are as follows: si-Ctrl (forward:5′-UUCUCC

GAACGUGUCACGUTT-3′, reverse:5′ACGUGACAC GUUCGGAGAATT3′); siKIF3C(forward:5′GCAGCU CAUUCAGACCUAUTT-3′, reverse:5′AUAGGUCUG AAUGAGCUGCTT-3′). ApcDNA-KIF3C construct was cloned into the BamH1/EcoRI restriction digest site of the KIF3C.1 plasmid by Gene Pharma.

The primer sequence for KIF3C with the BamH 1/ EcoRI enzyme site is as follows: oe-KIF3 (forward:5′GCT TGGTACCGAGCTCGGATCCGCCACCATGCTGTG CGGCC-3′ reverse:5′TGCTGGATATCTGCAGAATTC TCAGGAGCGCTGGATGCGGATTTGGATGA-3′). CRC cells were plated in 6-well plates at a density of 1.5 million cells per well and cultured until reaching 75% confluence before transfection. According to the manufacturer's instructions, siRNA and pcDNA3.1-KIF3C plasmid were transfected using Lipofectamine® 2000 reagent (Thermo Fisher, Waltham, MA, USA). The efficiency of transfection was validated via western blotting and RT-qPCR after 48 h post-transfection.

Protein extraction and western blot

Proteins were extracted from normal CRC epithelial cells, CRC cells, and CRC tissues utilizing RIPA lysis buffer (catalog number R0010; Beijing Solarbio Science & Technology Co., Ltd.). The total protein content was quantified using the BCA kit. Subsequently, the proteins underwent separation on a 10% SDS-PAGE gel and were transferred onto a polyvinylidene difluoride (PVDF) membrane (Thermo Fisher Scientific, Waltham, MA, USA) through electro-transfer. After blocking with 5% skimmed milk for 1 h and 30 min at room temperature, the membranes were cut following each molecular size and were subsequently incubated overnight in a shaker at 4 °C with different primary antibodies. These included rabbit anti-KIF3C (1:3000; catalog no. ab108389) from Proteintech, PI3K, p-PI3K (1:1000), Akt, p-Akt (1:1000), Cyclin-D1 (1:1000), Bcl-2 (1:1000), Bax (1:1000), all obtained from Abmart, and P53 (1:1000) from Zenbio, as well as rabbit anti-GAPDH (1:5,000) and mouse anti-GAPDH (1:5,000, cat. no. AC002, both from ABclonal Biotech Co., Ltd.) were used as controls. After incubating with the HRP-conjugated secondary antibody for 2 h at room temperature, the membrane underwent three washes for 15 min each with TBST solution. Following that, the strips were enhanced using an ECL luminescent reagent (Applygen Technologies Inc., Beijing, China). The luminescent reagent consists of solution A and solution B mixed at a 1:1 ratio. The PVDF membrane protein strips were laid onto the development plate, evenly covered with the reagent mixture, and left for 1 min. Chemiluminescent bands were analyzed using image analysis. It's crucial to prevent exposure to light during this step. Band intensities on the western blot were quantified using Image J software. The experiments were conducted in triplicate.

Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

Colon cancer cell lines and normal cells obtained from colon cancer patients were lysed using TRIzol® reagent (Ambion; Thermo Fisher Scientific, Inc.), and total RNA extraction was carried out following the manufacturer's instructions. cDNA synthesis was performed using a kit with 1.0 μg of total RNA in a 20 μl reaction volume. Following that, qPCR was conducted utilizing the SYBR Green I kit (catalog no. rk21203; ABclonal Biotech Co., Ltd.) and the Step OnePlus Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's instructions (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences were as follows: KIF3C forward KIF3C-F: GAAGAGCAGCCGCAGGAAGAAG.

R: CGGTGGTTGTTGTTGTTGTCATCC. The outcomes of the real-time qPCR experiments were assessed using the $2-^{\Delta\Delta Ct}$ approach. Each experiment was independently conducted in triplicate.

Cell Counting Kit-8 (CCK-8)

$1\times10\hat{}3$ cells were seeded in a 96-well plate and cultured overnight at 37 °C. At 24, 48, 72, and 96 h. 10 μL of cell counting Kit-8 (CCK-8) reagent from Beyotime Institute of Biotechnology, Shanghai, China, was added to each well and incubated for 2 h. Following incubation, the absorbance at 450 nm was measured using a microplate reader.

Colony formation assay

Cells in the logarithmic growth phase were harvested, and 600 CRC cells were seeded into each well of six-well plates. The cells were cultured and incubated at 37 °C for two weeks in a 5% CO2 environment. After washing with PBS and fixation with 4% paraformaldehyde, the cells were stained using crystal violet. Cell counting was performed using ImageJ software.

Transwell assay

Transwell assays were conducted to evaluate cell migration and invasion. For the migration assay, a cell suspension was prepared using serum-free media. In the lower chamber of the transwell system (Costar, Cambridge, MA, USA), 500 mL of culture medium containing serum was added, while the upper compartment received

200 mL of cell suspension 5×10^4 cells. Subsequently, the cells were transferred to the incubator. Following a 24-h incubation period, the chambers were extracted, and the liquid in the upper chamber was drained. The cells in the upper chamber were meticulously eliminated using cotton balls. Following that, the cells beneath the membrane surface were stained with a crystal violet solution at room temperature for 15 min. After air-drying and PBS washing, further processing of the cells was conducted. After 24 h of incubation, the chambers were removed, and the liquid in the upper chamber was drained. Following that, the cells present in the upper chamber were carefully removed using cotton balls. The cells underneath the membrane's surface was then stained using a crystal violet solution for 15 min at room temperature. Subsequently, following air-drying and PBS washing, the cells were divided into 5 visual fields, with 200 cells counted and captured using a camera. In the cell invasion assay, the transwell chamber's membrane was pre-coated with matrigel, while the subsequent steps remained consistent with those of the migration assay.

Wound healing assay

SW-480, SW-116, and SW-620 cells were seeded onto six-well plates and cultured in serum-free RPMI-1640 medium until they reached confluence as a monolayer. By the second day, the cells achieved 80–90% confluence as a monolayer. When reaching 80–90% confluence, a vertical wound was generated at the plate's base utilizing a 200-µl pipette tip. Following the scratching, the healed tissues were rinsed three times with PBS. Afterward, the medium containing 3% FBS was replaced to support the remaining cells. Following the scratch, images were taken at 0 and 24 h using a light microscope (magnification, x200). ImageJ software was used to evaluate wound closure.

Statistical analyses

Each set of data was replicated at least three times for all experiments. Statistical analysis was conducted using the T-test and GraphPad Prism 8 software (GraphPad 8.0.1 Software, La Jolla, CA, USA). The χ^2 test was employed to examine the association between the clinicopathological parameters of CRC, while Spearman's analysis was utilized for correlation analysis. Spearman statistical methods were applied to assess the correlation between gene expressions. Quantitative data were presented as means ± SD. A significance level of $P < 0.05$ denotes statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Results

KIF3C expression is increased in CRC cells lines and tissues

To explore the impact of KIF3C in CRC, we utilized immunohistochemistry (IHC) to evaluate KIF3C levels in 50 pairs of CRC and adjacent tissue samples. The results revealed significantly higher KIF3C expression in CRC tissues compared to adjacent tissues (Fig. 1A, B). Additionally, western blot and RT-qPCR analyses demonstrated a notable increase in KIF3C expression in CRC cell lines in comparison to normal colon epithelial cell line (NCM-460) and also western blot analysis of eight pairs of CRC tissue, which showed markedly elevated expression of CRC tissues compared to adjacent normal tissues (Fig. 1C, E, D). Furthermore, we investigated the correlation between KIF3C inhibition and clinicopathological characteristics. The study demonstrated that KIF3C inhibition was correlated with parameters such as tumor size, T stage, degree of differentiation, Lauren type, nerve invasion, pathological stage, and vascular invasion ($p < 0.05$; Table 1).

Moreover, we conducted univariate and multivariate analyses to identify potential risk factors associated with CRC. The univariate regression analysis identified factors influencing overall survival (OS), including KIF3C expression, pathological stage, T stage, tumor size, vascular invasion, and nerve invasion. Subsequent multivariate analysis revealed that KIF3C expression, tumor size, T stage, pathological stage, and nerve invasion independently served as risk factors for CRC (Table 2).

Furthermore, we investigated the relationship between KIF3C expression and CRC prognosis by examining the overall survival (OS) in a cohort of 50 CRC patients. The Kaplan–Meier curve analysis indicated a relationship between heightened KIF3C protein expression and diminished survival outcomes in CRC patients (Fig. 1F).

Knockdown of KIF3C inhibits the proliferation and invasion of CRC cells

We selected SW-480 and HCT-116 cell lines to investigate the consequences of reduced KIF3C expression, while the SW-620 cell line was employed to examine the effects of KIF3C overexpression. The si-KIF3C was utilized to decrease KIF3C levels in SW-480 and HCT-116 cells and augment oe-KIF3C expression in SW-620 cells. The effectiveness of KIF3C knockdown was validated through RT-qPCR analyses and western blot (Fig. 2A, B, C, D). Furthermore, to assess whether KIF3C affects migration on the CRC cells, colony formation assays and CCK8 assays were conducted to measure the proliferation of SW-480 and HCT-116 cells. The findings

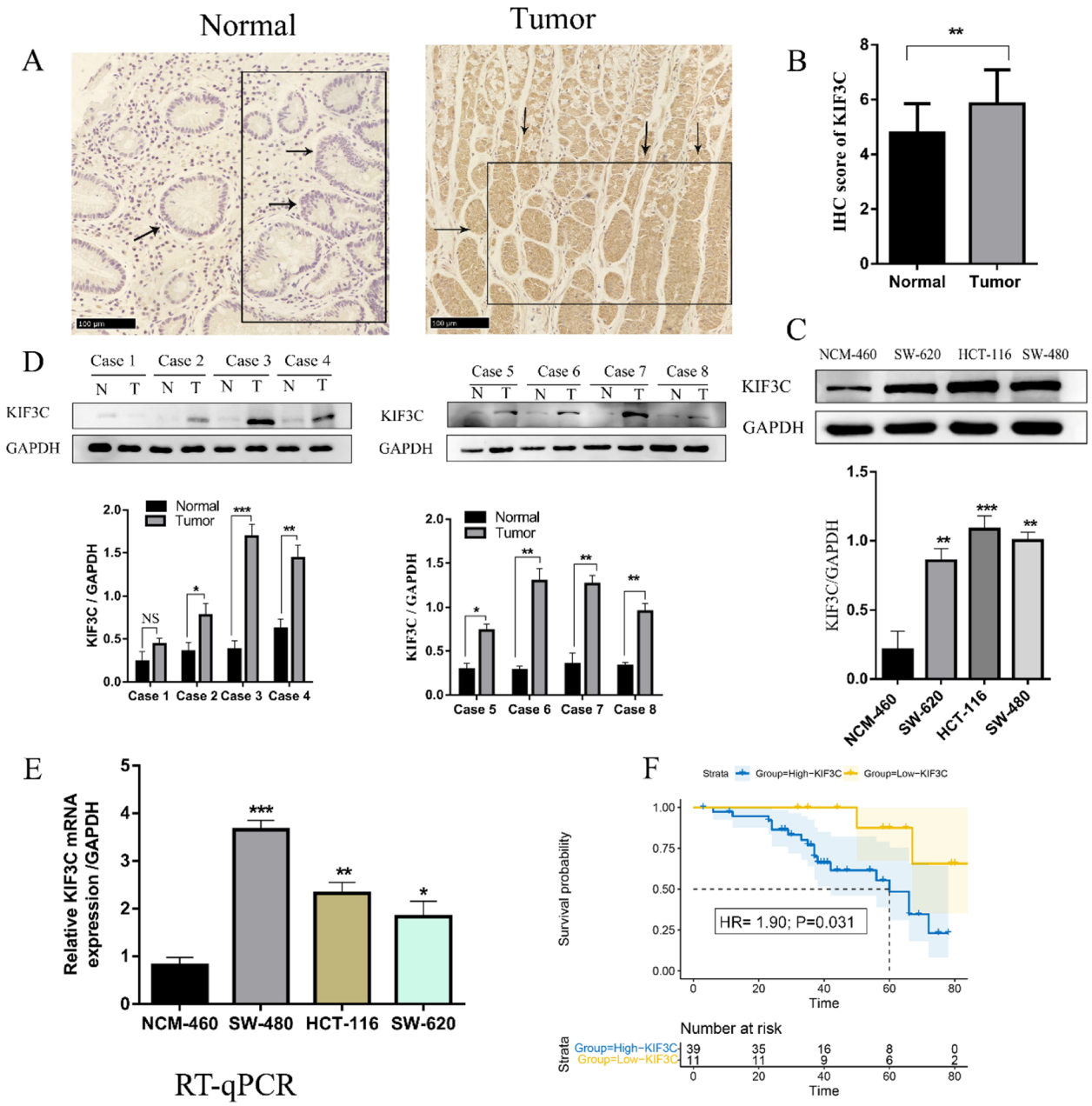

Fig. 1 KIF3C was increased in colorectal cancer tissues and colorectal cancer cell lines and has a poor prognosis. IHC expression in tissue arrays of 50 CRC patients (**A**). (N: normal colon tissue; T: tumor; scale bar = 100 μM); IHC scores for 50 pairs of normal and CRC samples (**B**). Western blot and RT-qPCR revealed KIF3C expression in normal CRC cell line (NCM-460) and cancer cell lines (SW-480, SW-620, and HCT-116) and western blot analysis of KIF3C protein in 8 paired CRC tissues in comparison to the adjacent normal tissues. **C**, **E**, **D** Survival analysis was conducted on CRC patients with varying expression levels of KIF3C (**F**)

demonstrated a significant inhibition in the proliferation rate of SW-480 and HCT-116 cells treated with si-KIF3C compared to the si-Ctrl group (Fig. 2E, F) and (Fig. 3A, B). Moreover, wound-healing assays and transwell assays were performed to investigate the KIF3C knockdown in SW-480 and HCT-116 cells. The findings from wound-healing assays and transwell assays indicated a notable decrease in ability in the si-KIF3C group, which was different from the si-Ctrl group in SW-480 and HCT-116 cell lines (Fig. 3C, D, E, and F).

Table 1 The correlation of KIF3C expression and clinical-pathological characteristics of CRC

Parameter	Total Number	KIF3C Expression		χ^2	*P*-value
	(*n* = 50)	Low(11)	High(39)		
Age (years)					
≤65	22	5	17	< 0.001	> 0.999
>65	28	6	22		
Gender					
Female	22	4	18	0.055	0.815
Male	28	7	21		
Tumor size (cm)					
<5	17	5	12	< 0.001	> 0.999
≥5	33	6	27		
Histological type Differentiated					
Low	24	7	16	0.973	0.324
High	27	4	23		
TNM stage					
I/II	20	9	11	8.163	0.004
III/IV	30	2	28		
Lymph node metastasis					
Negative	24	6	18	0.242	0.623
Positive	26	5	21		
Depth of invasion					
T1/T2	21	9	12	7.203	0.007
T3/T4	29	2	27		
Venous invasion					
Negative	24	8	16	3.455	0.063
Positive	26	3	23		

KIF3C overexpression accelerated migratory and invasive ability of CRC cell lines

We examined whether KIF3C overexpression enhances CRC cell proliferation and invasion by performing RT-qPCR analyses and a western blot of KIF3C overexpression in SW-620 cells (Fig. 4A, B). Moreover, CCK8 and colony formation assay experiments established that overexpression elevated the proliferation of SW-620 cells (Fig. 4C, D). Additionally, scratch wound healing and transwell assay were employed to assess the migratory and invasive abilities of SW-620 cells that overexpressed KIF3C (Fig. 4E, F). The results indicated that the oe-KIF3C group significantly increased migration and invasion ability compared to the vector group.

KIF3C promoted CRC cell proliferation via the PI3K/AKT/cyclin-D1signaling pathway

A western blot investigated the regulatory relationship between KIF3C and the PI3K/Akt pathway, with Bax and Bcl-2proteins associated with apoptosis. The KIF3C knockdown results revealed that in CRC SW-480 cells, si-KIF3C downregulation displayed diminished levels of p-PI3K/PI3K and phosphorylated p-AKT/AKT, cyclin D-1, and Bcl-2, and elevated the levels of Bax and p53 proteins (Fig. 5A). Consequently, KIF3C overexpression upregulated protein p-AKT/AKT, cyclin D-1, and Bcl-2, while the protein levels of Bax and p53 were reduced in CRC SW-620 cells. Our results indicated the possible engagement of KIF3C in the PI3K/AKT pathway, influencing CRC cell function and progression (Fig. 5B).

Discussion

Currently, tumor metastasis and invasion are the leading cause of decreased tumor therapeutic effectiveness and eventual patient death in CRC patients. Hence, additional, comprehensive molecular investigations are necessary to elucidate the mechanisms underlying CRC metastasis, facilitating the advancement of early intervention strategies for high-risk metastatic cases [15]. Several investigations have shown that KIF3C is notably abundant in neural tissues such as the retina, spinal cord, and brain, where it regulates axon regeneration at growth cones after neuronal injury. Additionally, it performs a vital function in regulating various biological processes, encompassing cell morphology, intracellular transport of large molecules, cytoskeleton dynamics, cell division, and migration [12].

Nevertheless, the majority of research relied on accumulating evidence that substantiates the regulatory role of KIF3C in cancer biology. Some researchers have established a link between different KIFs and various human malignancies, suggesting that aberrant expression and activity of KIFs might be crucial for the proliferation or invasion of cancer cells [16]. Elevated expression of KIF2A or KIF2C has been linked to tumor progression and an unfavorable prognosis in patients with oral carcinoma, gliomas, gastric cancer, colorectal cancer, and breast cancer [16]. Liu et al. [8] explored the relationship between the expression of KIF3A or KIF3B and malignant tumors. Subsequently, due to its similarity and association with KIF3A, we were intrigued to explore whether KIF3C exhibits distinct roles in the initiation and advancement of human breast cancer [6].

In this research, immunohistochemistry staining assays conducted on 50 paired colon carcinoma tissues and adjacent normal tissues revealed a significant correlation between elevated levels of KIF3C and the advanced clinicopathological stage of CRC. Moreover, we observed elevated heightened KIF3C mRNA and protein in CRC cell lines and eight patient tissues, with a notable high expression. Additionally, KIF3C overexpression significantly enhanced CRC cells' proliferation, invasion, and migration. Our study revealed consistent overexpression of KIF3C in CRC cell lines and tissues. This heightened

Table 2 Prognostic factors for the overall survival of 50 colorectal cancer patients were analyzed by univariate and multivariate Cox proportional hazards models

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age				
< = 65	Reference			
> 65	1.267 (0.942—1.791)	0.118		
Gender				
Female	Reference		Reference	
Male	1.462 (0.995—2.334)	0.053	1.663(0.717—3.551)	0.701
Lauren type				
Intestinal type	Reference			
Diffuse type	0.912 (0.716—1.348)	0.701		
T stage				
T1&T2	Reference		Reference	
T3&T4	1.723 (1.110—2.515)	0.009	2.394 (1.051—5.515)	0.009
N stage				
N0&N1	Reference		Reference	
N2&N3	2.567 (1.077—5.763)	0.037	1.507 (0.713—3.221)	0.258
M stage				
M0	Reference		Reference	
M1	5.357 (1.747—18.255)	0.001	5.513 (2.179—16.012)	< 0.001
TNM stage				
Stage I&Stage II	Reference		Reference	
Stage III&Stage IV	2.216 (1.217—4.218)	0.003	4.339(1.813—8.129)	< 0.001
Histological grade				
G1&G2	Reference			
G3	1.357 (0.811—2.274)	0.215		
Venous invasion				
No	Reference			
Yes	1.712 (0.611—4.173)	0.526		
Nerve invasion				
No	Reference			
Yes	1.142(0.667—2.312)	0.612		
KIF3C				
Low	Reference		Reference	
High	1.986 (1.215—3.519)	0.007	2.235 (1.189- 4.336)	< 0.001

expression exhibited a significant correlation with tumor recurrence and lymph node metastasis, highlighting a pronounced link between KIF3C expression and the advancement of CRC, particularly in tumor growth and metastasis. Furthermore, depletion of KIF3C inhibited these malignant characteristics of CRC cells. Moreover, the degree of KIF3C expression was associated with numerous adverse prognostic factors, including tumor size, pathological stage, T stage, Lauren type, degree of differentiation, vascular invasion, and nerve invasion. Our multivariate analysis found that KIF3C inhibition, T stage, tumor size, pathological stage, and nerve invasion were independent risk factors for CRC progression. A survival analysis was performed to assess the predictive significance of KIF3C. Kaplan–Meier analysis revealed that CRC patients exhibiting increased KIF3C expression experienced a reduced overall survival time. The si-KIF3C was separately transfected into SW-480 and HCT-116 CRC cell lines to investigate the effects of reduced KIF3C expression, while the oe-KIF3C overexpression was also transfected to investigate the elevated effects in SW-620 cell line. Consequently, the qRT-PCR and WB analyses were performed, and the results showed that KIF3C expression decreased after si-KIF3C

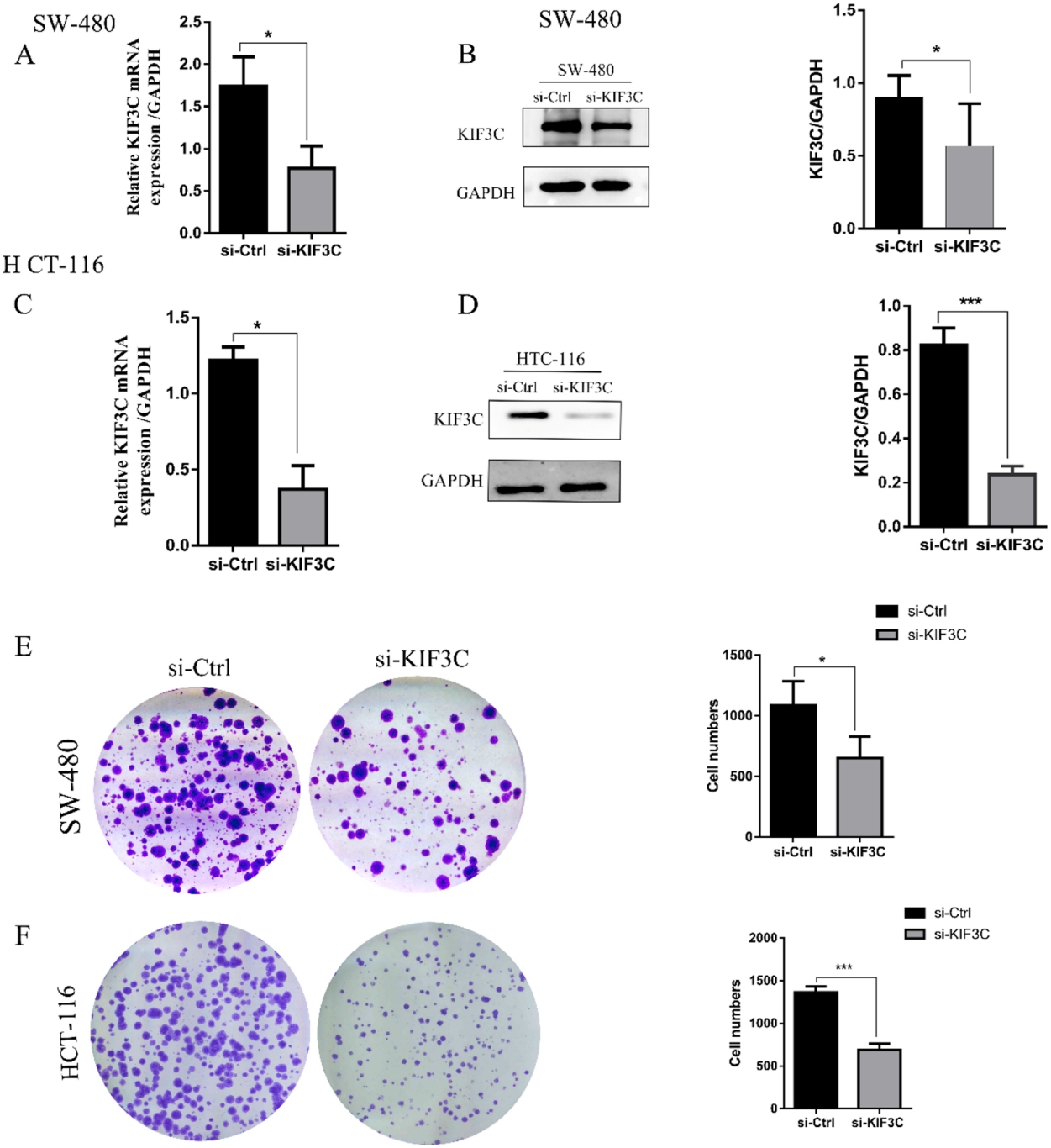

Fig. 2 KIF3C knockdown inhibits the proliferation and invasion of CRC cells. The expression of KIF3C was significantly diminished in SW-480 and HCT-116 cells when KIF3C was knockdown with si-KIF3C compared to the si-Ctrl group through RT-qPCR and western blot experiments (**A**, **B** and **C**, **D**). Representative images from the colony formation assay in SW-480 and HCT-116 cells following KIF3C knockdown (**E**, **F**)

transfection and KIF3C overexpression increased after treatment with oe-KIF3C, indicating that the KIF3C knockdown cell model was successfully established. Son et al. [17] highlighted that mutations could potentially deactivate the functions of KIF3C, a phenomenon that could play a critical role in cancer development. Additionally, research has indicated that the KIF3C peptide effectively stimulates glioma-reactive cytotoxic T lymphocytes (CTLs) in human glioma, suggesting that peptide-based immunotherapy could be promising for

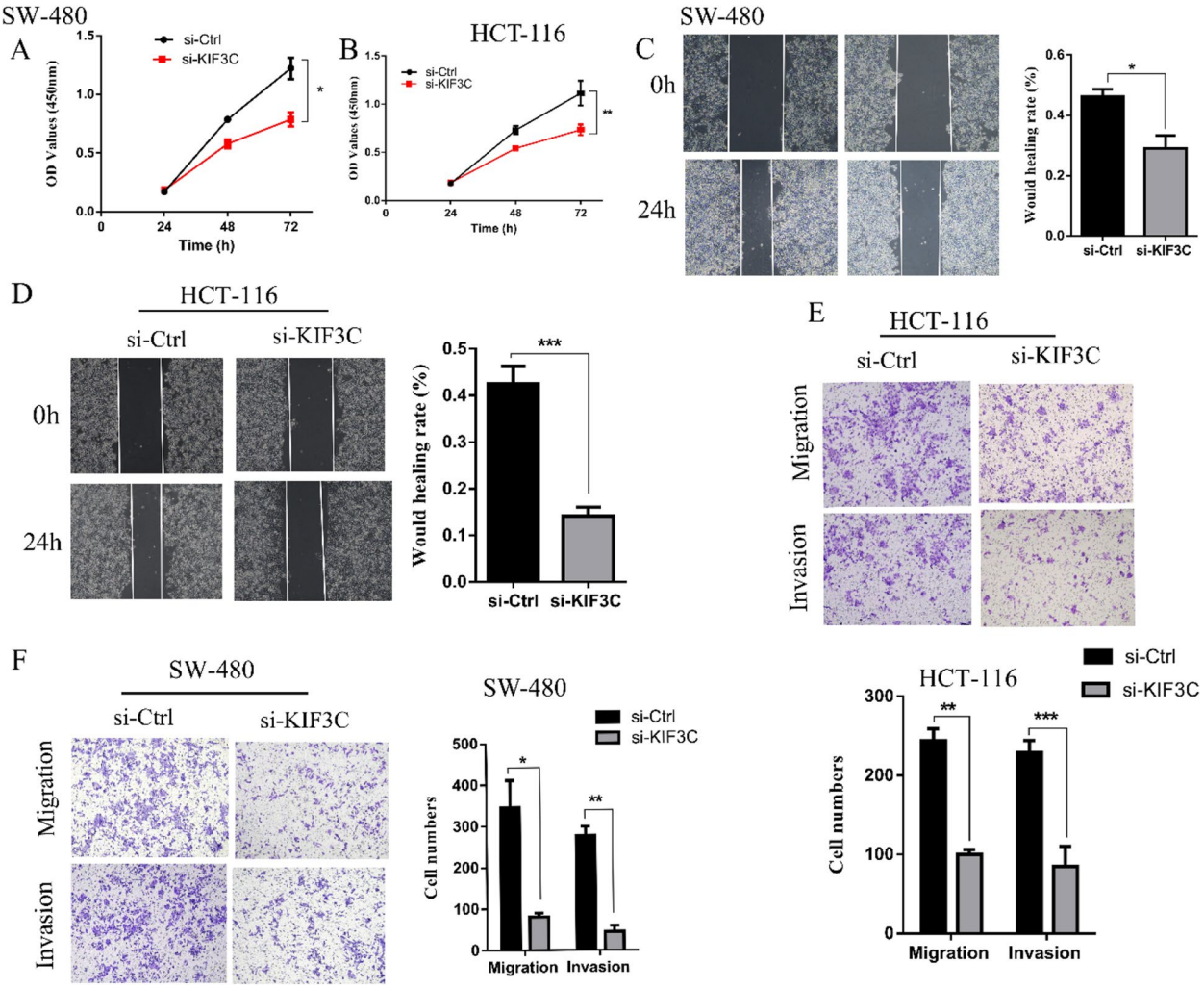

Fig. 3 KIF3C knockdown upregulation effectively inhibited CRC cell proliferation, migration, and invasion. The proliferation of SW-480 and HCT-116 cells with KIF3C knockdown were evaluated using the cell counting Kit-8 (**A**, **B**). Wound healing assays and transwells were utilized to evaluate how KIF3C knockdown influenced the proliferation of SW-480 and HCT-116 cells (**C**, **D**, **E**, **F**)

glioma patients [18]. Another investigation conducted by Cabibbo et al. [19] revealed that anti-proliferative treatments resulted in the upregulation of KIF3C transcripts in various tumor cell lines. This observation hints at a potential functional link between KIF3C and the arrest of tumor cell growth. These findings suggest varying roles for KIF3C across different types of cancers. Conversely, we noted a notable reduction in KIF3C expression in cells transfected with KIF3C knockdown (SW-480 and HCT-116), while it was different in those transfected with si-Ctrl. Compared to cells transfected with the empty vector, oe-KIF3C significantly increased in SW-620 cells after KIF3C overexpression. Overall, these results suggest a significant involvement of KIF3C in advancing and growing CRC.

Moreover, we identified an association between the cell cycle signaling pathway and KIF3C. However, Gao et al. [20] documented a relation between KIF3C and the PI3K/AKT pathway, though without supporting experimental confirmation. Similarly, it was revealed that KIF proteins influence the behavior of various types of human tumor cells via diverse pathways [20]. The advancement of CRC could have correlated with the activation of the PI3K/AKT pathway, while colon carcinogenesis may be connected with the overexpression of AKT. Moreover, it has been observed that reducing KIF3C expression in breast cancer tumors inhibits metastasis and tumor growth by suppressing TGF-signaling [13]. Therefore, from the present study, we indicated that the PI3K/AKT pathway controls tumor growth and metastasis and might serve as a

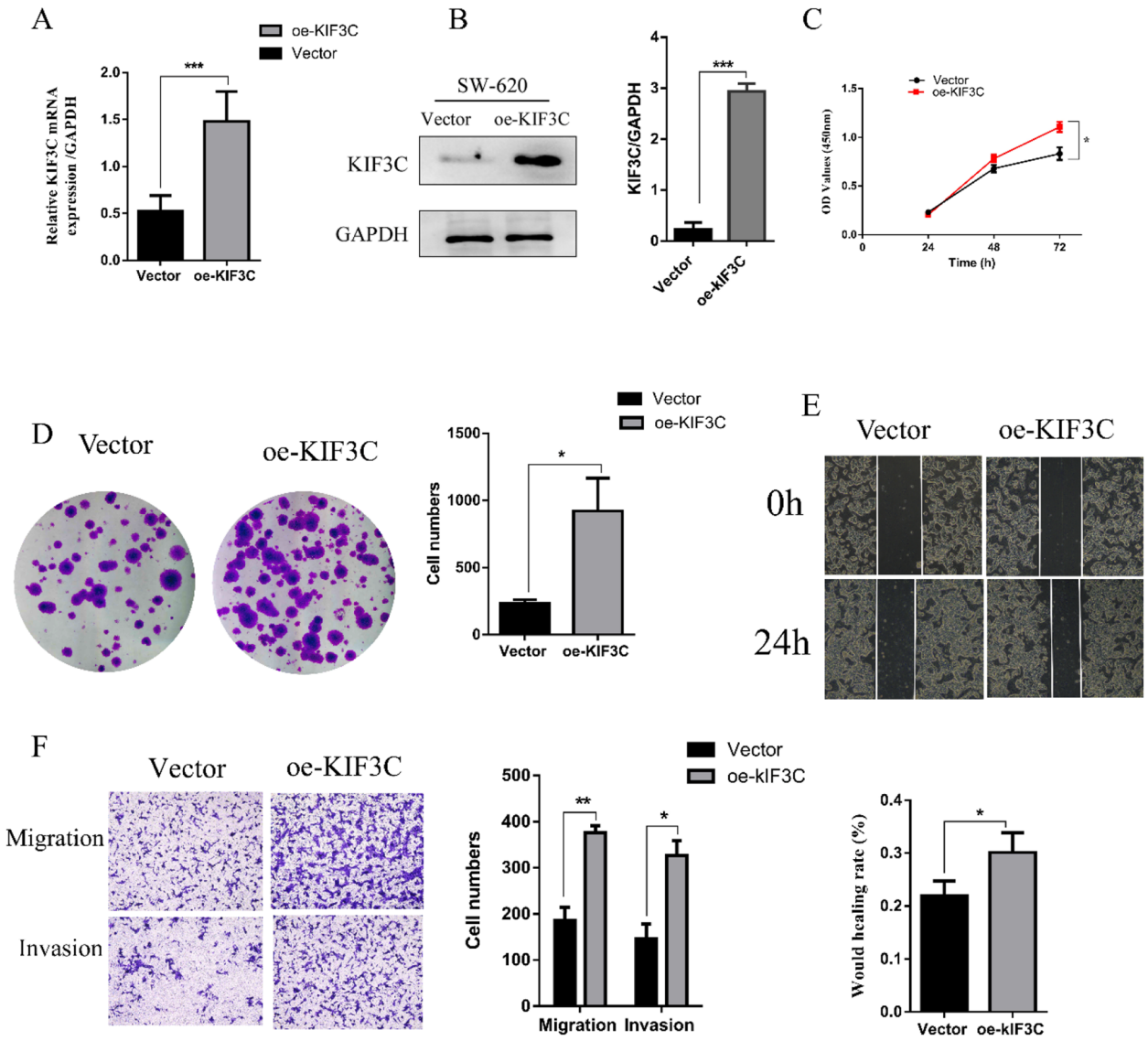

Fig. 4 The overexpression of KIF3C promoted the proliferation of CRC cells. Reverse transcription-quantitative PCR (qRT-PCR) and western blot analysis were employed to assess the efficacy of KIF3C overexpression in SW-620 cells transfected with oe-KIF3C overexpression (**A**, **B**). Cell counting Kit-8 and colony formation assays were analyzed in SW-620 cells transfected with oe-KIF3C compared to the transfected vector (**C**, **D**). The wound healing assay and transwell assays were performed to determine the proliferation of SW-620 cells after oe-KIF3C overexpression (**E**, **F**)

prospective tumor promoter and prognostic indicator in CRC. Nonetheless, cancer cells displayed enhance proliferation and resistance to apoptosis.

Apoptosis is essential for both cancer progression and its treatment [21]. We also showed that KIF3C initiates the activation of Bax and BCL-2 signaling pathways, which play an essential role in the pathogenesis and progression of CRC. Nevertheless, the antiapoptotic, Bcl-2 and cell cycle regulator, Cyclin D1 are two important proteins involved in cell regulation, but they have distinct roles in the processes of cell survival and proliferation [22]. Furthermore, KIF3C knockdown elevated p53 levels by interfering with the interaction between p53 and Bax protein expression, consequently diminishing CRC proliferation. These findings underscore the pro-oncogenic function of KIF3C in CRC. P53 gene restrains tumor growth through its proapoptotic effects [23, 24]. Typically, the activation of genes from the p53 and Bcl-2 families is involved in controlling this type of cell death [21]. Bcl-2 protein family, which includes both anti-apoptotic such as Bcl-2 and pro-apoptotic like Bax members, is acknowledged as critical

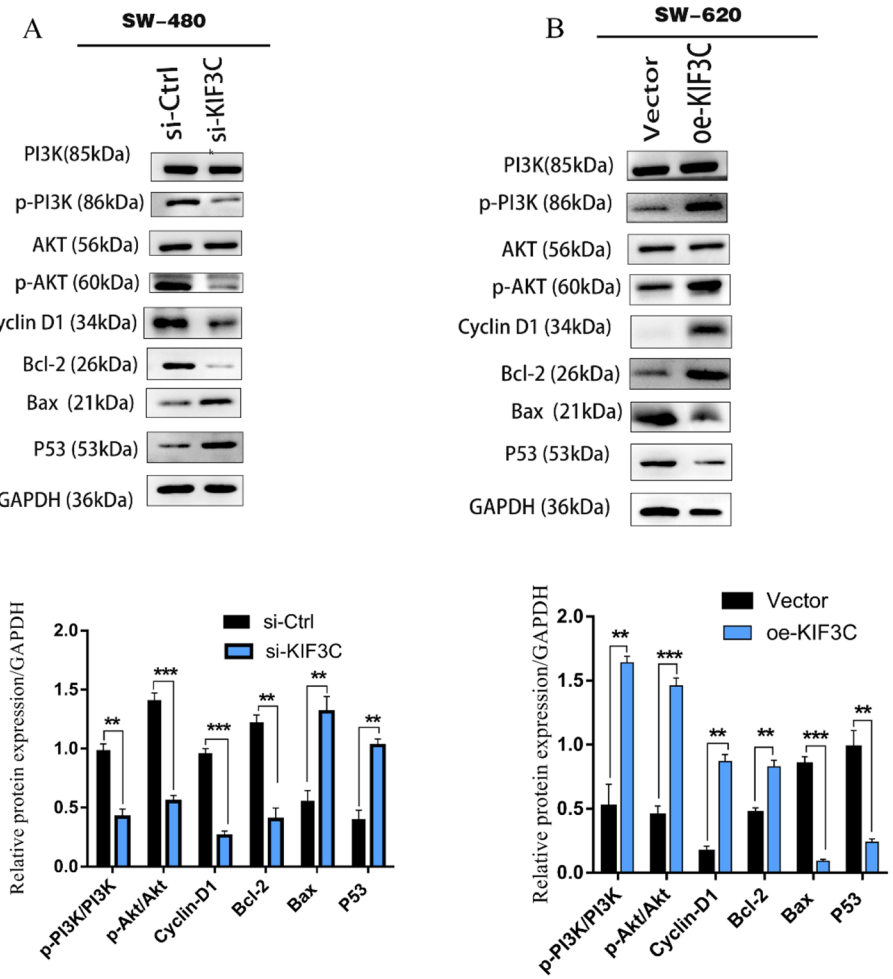

Fig. 5 Effect of KIF3C overexpression on proteins related to the PI3K/AKT/ Bax/Bcl-2 signaling pathway CRC cells (SW-480 and SW-620). Protein levels of p-PI3K, PI3K, p-Akt, Akt, Bax in SW-480 and SW-620 cells were determined through western blotting after treatment with si-Ctrl and si-KIF3C, as well as oe-KIF3C and vector (**A**, **B**)

regulators of apoptosis [25]. Studies indicate that p53 promoted the expression of Bax while suppressing the transcription of Bcl-2 [26]. The inhibition or upregulation of KIF3C could have promoted the growth and progression of CRC by activating the PI3K/AKT pathway. These effects exhibit a positive correlation with cyclin D1 and Bcl-2 expression and a negative correlation with p53 and Bax expression. Li et al. [27] demonstrated that the PI3K/AKT pathway promotes cancer development and inhibits the activity of p53 and its proapoptotic functions. Our western blot analysis revealed that KIF3 expression influences CRC progression by inhibiting the PI3K/AKT signaling pathway.

Our study has several limitations due to unavoidable factors. First, it tracked only patients' overall survival (OS) without monitoring disease-free survival. Second, it did not investigate the impact of KIF3C on the cell cycle. Finally, additional animal experiments are required to corroborate the current findings and provide new insights into the role of KIF3C in colorectal cancer. This represents an important area for future research.

Conclusion

KIF3C expression showed a significant increase in CRC. KIF3C knockdown inhibited migration and invasion in CRC, while overexpression stimulated migration, invasion, and proliferations. Additionally, KIF3C could be a promising therapeutic target for inhibiting tumor progression.

Supplementary Information

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Supplementary Material 1.

Authors' contributions

Maladho Tanta Diallo: Conceptualization, Methodology, Data curation, Validation, Writing – original draft, Bangquan Chen: Visualization, Investigation, Data curation, Qing Yao: Data curation, Investigation, Zhang Yan: Investigation, Format analysis, Qiannan Sun: Data curation ,investigation, Daorong Wang: Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

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Data availability

Data will be made available on request.

Declarations

Ethics approval and consent to participate

The studies involving human patients provided informed consent and were approved (approval no. 2019KY-022) by the Ethics Committee of Northern Jiangsu People's Hospital, the Clinical Medical School, and the and the Yangzhou University Ethics Committee (Yangzhou, China). Written, informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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