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Forkhead Box Protein J1 (FOXJ1) is Overexpressed in Colorectal Cancer and Promotes Nuclear Translocation of β-Catenin in SW620 Cells

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Background: FOXJ1, which is a forkhead transcription factor, has been previously studied mostly as a ciliary transcription factor. The role of FOXJ1 in cancer progression is still elusive and controversial. In the present study, the effect of FOXJ1 in progression of colorectal cancer (CRC) was investigated.

Material/Methods: The pattern of FOXJ1 expression was investigated using the method of immunohistochemistry (IHC) in a tissue microarray (TMA) incorporating 50 pairs of colon cancer specimens and adjacent normal tissue. In addition, the correlation of FOXJ1 expression with clinicopathological characteristics was evaluated in the other TMA containing 208 cases of colon cancer. Moreover, the influence of regulating FOXJ1 level on the proliferation, migration, and invasion ability of colorectal cancer (CRC) cells was evaluated.

Results: Increased expression of FOXJ1was significantly associated with clinical stage ($p<0.05$), metastasis of lymph node ($p<0.05$), and invasion depth ($p<0.001$) in colon cancer, suggesting FOXJ1 is a tumor promoter in CRC. Consistently, FOXJ1 overexpression significantly enhanced the proliferation, migration, and invasion of CRC cells, while silencing of FOXJ1 induced the opposite effect. Furthermore, up-regulation of FOXJ1 in SW620 cells markedly inhibited the level of truncated APC and the phosphorylation of β-catenin, while the level of cyclinD1 was decreased. In addition, overexpression of FOXJ1 significantly promoted nuclear translocation of β-catenin in SW620 cells.

Conclusions: These findings demonstrate that increased FOXJ1 contributes to the progression of CRC, which might be associated with the promotion effect of β-catenin nuclear translocation. FOXJ1 may be a novel therapeutic target in CRC.

MeSH Keywords: Colorectal Neoplasms • Forkhead Transcription Factors • Wnt Signaling Pathway

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Background

As the third most common cancer, colorectal cancer (CRC) is considered as one of the most common malignant causes of death in the world [1]. To develop more efficient treatment method and reduce the mortality rate, it is necessary to further explore the pathogenesis of CRC, especially the molecular mechanism underlying the tumor progression.

The forkhead box (Fox) gene family is a large and diverse group of transcription factors that share certain characteristics of a conserved DNA-binding motif known as the forkhead or winged helix domain; over 100 proteins with forkhead domains have been identified, comprising at least 17 subclasses to date [2]. FOX proteins are essential in normal biological processes [2], and are involved in carcinogenesis and cancer invasion [3,4]. The FOX family transcriptional factors play various roles in many malignancies, and the effect might be opposite even in the same tumor. For example, FOXC2, FOXM1, and FOXQ1 are all overexpressed in CRC [5–9]. Among them, FOXC2 promotes cell proliferation via down-regulating p27 and up-regulating cyclinD1 in CRC [5]; FOXM1 contributes to CRC progression through promoting the urokinase plasminogen activator receptor signaling pathway [6,7]; and FOXQ1 promotes the progression of CRC as a novel target of the Wnt pathway and is involved in several other crucial pathways, including transforming growth factor-β and vascular endothelial growth factor pathways [8,9]. In addition, the newly identified FOXK1 and FOXK2 are also increased in CRC and promote Wnt/β-catenin signaling [10]. In contrast, FOXO3 and FOXP1 are both decreased in CRC and the loss of expression correlates with poor prognosis [11,12].

FOXJ1 was identified about a decade ago. It is thought to be essential in multiple physiological processes, especially ciliogenesis, embryonic development, and spontaneous autoimmunity inhibition [13–16]. Recently, several studies have proposed that FOXJ1 also plays a role in malignancy [17–22]. FOXJ1 is reduced or hypermethylated in gastric cancer, ovarian cancer, breast cancer, aggressive ependymoma, and choroid plexus tumors, and its decreased expression is associated with poor prognosis [17–20], indicating its role as a novel tumor suppressor. However, in hepatocellular carcinoma and clear-cell renal cell carcinoma, FOXJ1 is overexpressed and the expression levels correlate with poor prognosis [21,22]. Overall, these findings indicate that FOXJ1 plays opposite roles in various tumor microenvironments; therefore, the effect of FOXJ1 in CRC needs further investigation.

In the present study, FOXJ1 expression pattern and the clinicopathological correlation were investigated using the immunohistochemistry (IHC) method in 2 tissue microarrays (TMAs) of colon cancer, then FOXJ1 was overexpressed or silenced in CRC cells to explore its biological effect and the potential mechanisms. To the best of our knowledge, this is the first study to investigate the role of FOXJ1 in CRC.

Material and Methods

Tissue microarrays and ethics approval

Two commercial TMAs (BioMax Company, Rockville, MD, USA) containing 208 specimens of colon cancer and 50 pairs of colon cancer tissues and adjacent normal tissues, respectively, were purchased to investigate the expression pattern of FOXJ1 in colon cancer tissues. The clinicopathological data of patients and sample collection statement with informed consent was provided according to the manufacturer. Tumor node metastasis (TNM) stage was determined per the seventh edition of the TNM classification proposed by the American Joint Committee on Cancer. This study was approved by the Ethics Committee of Taizhou Hospital in Zhejiang Province, China.

Immunohistochemical staining and scoring

The staining protocol of IHC was performed using the two-step method described previously [23]. A rabbit polyclonal antibody of FOXJ1 [1: 100, Abcam, UK] was used as the primary antibody. The EnVision™+/HRP working solution was provided by DAKO (Agilent Technologies Inc., Glostrup, Denmark). Diaminobenzidine was added to visualize the reaction products and hematoxylin was used for counterstaining. The staining of FOXJ1 was independently scored by 2 pathology experts blinded to the clinical and pathological parameters according to the criteria previously described [23].

Preparation of cell lines

The cell lines of CRC (HCT116, SW480, and SW620) were all preserved in our laboratory and maintained at 37°C in a humidified atmosphere of 5% CO_2. Dulbecco's modified Eagle's medium/F12 (Neuronic Laboratories Corporation Limited, Beijing, China), Iscove's modified Dulbecco's (HyClone Laboratories Inc., Utah, USA), and Rosewell Park Memorial Institute (RPMI) 1640 (Gibco Life Technologies Inc., Grand Island, NY, USA) were supplemented with streptomycin (100 ng/mL) and penicillin (100 U/mL), as well as 10% fetal bovine serum (Gibco). Cells used for experiments were all in logarithmic growth phase.

Real-time polymerase chain reaction and Western blot analysis

TRIZOL reagent was used to extract total RNA from cells (Invitrogen Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary

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deoxyribonucleic acid (cDNA) was synthesized using the Prime Script Reverse Transcription Reagents kit (Takara Bio Inc., Shiga, Japan). Primer sequences of FOXJ1 and β-actin were as follows: FOXJ1 (forward 5'-AGTGGATCACGGACAACTTCTGCTA-3' and reverse 5'-TCTCCCGAGGCACTTTGATGAA-3'); β-actin (forward 5'-CGTGGACATCCGCAAAGA-3' and reverse 5'-CGTGGACATCCGCAAAGA-3'). Relative expression level (described as fold change) of target gene was evaluated using $2^{-\Delta\Delta CT}$ method. Mammalian protein extraction reagent (Pierce Biotechnology, Rockford, IL, USA) were used to extract proteins from total cell lysates. Subsequent Western blot (WB) analysis was performed as previously described [23]. For WB analysis of nuclear and cytoplasmicβ-catenin, nuclear and cytoplasmic proteins from cells were extracted using a nuclear-cytosol extraction kit according to procedures described by the manufacturer (Applygen Technologies Inc, Beijing, China). Primary antibodies were anti-FOXJ1 antibody (1: 500 dilution, mouse anti-human polyclonal antibody, #14-9965, eBioscience, San Diego, CA, USA), anti-phosphorylated-β-catenin antibody (1: 500, #9561, Cell Signaling Technology, Danvers, MA, USA), anti-APC antibody (1: 500, sc-53165), anti-β-catenin antibody (1: 500, sc-7963), anti-glyceraldehyde 3-phosphate dehydrogenase (1: 1000, sc-47724), and anti-cyclinD1 antibody (1: 500, sc-8396, all from Santa Cruz Biotechnology, CA, USA).

Construction and transfection of FOXJ1 overexpressing vector

The cDNA of FOXJ1 in pCMV-SPORT6 vector (purchased from YRbio Company, Changsha, Hunan, China) was amplified and then cloned into vector of pcDNA 3.1(+), as previously described [23]. Then, transfection of pcDNA 3.1-FOXJ1 plasmid into SW480 and SW620 cells was completed using Lipofectamine 2000 transfection reagent (Invitrogen Life Technologies Corporation, Carlsbad, CA, USA). FOXJ1 mRNA and protein were verified using reverse transcription-polymerase chain reaction (RT-PCR) and WB analysis.

Preparation and transfection of small interfering RNAs

Chemically constructed small interfering RNAs (siRNAs) against FOXJ1 and control siRNAs were purchased from GenePharma Corporation Limited (Shanghai, China). Four siRNA sequences (siRNA1, 2, 3, and 4) targeting FOXJ1 were designed to select the most efficient sequence. Sequences of 2 siRNAs (siRNA1 and siRNA2) used for further transfection and negative control siRNA were as follows: siRNA1 (sense 5'-CCACCUGGCAGAAUUCAAUTT-3' and antisense 5'-AUUGAAUUCUGCCAGGUGGTT-3'); siRNA2 (5'-UCUCCAUUCUCAACGCCAATT-3' and antisense 5'-UUGGCGUUGAGAAUGGAGATT-3'); control siRNA (sense 5'-UUCUCCGAACGUGUCACGUTT-3' and antisense 5'-ACGUG ACACGUUCGGAGAATT-3'). For silencing FOXJ1 *in vitro*, siRNA1, siRNA2 and control were introduced into HCT116 using transfection reagents of Lipofectamine 2000.

Cell proliferation, Transwell migration, and invasion assays

In proliferation assays, cells (5×10^3/well) were incubated using the Cell Counting Kit (CCK)-8 (Dojindo Laboratories, Kumamoto, Japan). Four hours later, cell viability was evaluated according to the absorbance at 450 nm at designated times. In Transwell migration and invasion assays, 5×10^3 cells incubated in RPMI 1640 medium were seeded into the top insert chambers of Transwell devices with non-coated or matrigel-coated membrane (Corning, NY, USA). After 24 h, tumor cells on top chambers of Transwell devices were removed, while tumor cells remaining on the underside were fixed and then stained using 0.1% crystal violet. Every insert was counted using a microscope (Olympus Corporation, Tokyo, Japan) on 3 random fields to calculate the number of cells.

Immunofluorescence

SW620 cells grown on coverslips were fixed with 4% paraformaldehyde for 30 min, then treated with 1% Triton X-100 for 5 min, and incubated with 5% bovine serum albumin for 1 h. Then, the cells were incubated overnight at 4°C with anti-β-catenin primary antibody (1: 500 dilution, sc-7963, Santa Cruz Biotechnology, CA, USA). After being rinsed with PBS, the cells were incubated with goat-anti-rabbit-596nm (1: 400) and goat-anti-mouse-488nm (1: 400) fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (Jackson,711-585-152). Cell nuclei were stained with DAPI (4', 6-diamidino-2-phenylindole). The stained cells were then detected using a Zeiss Confocal Laser Scanning Microscope.

Statistical analysis

SPSS 17.0 (Chicago, IL, USA) software was used in statistical analysis. All data are reported as mean ± standard deviation. Intergroup comparison was performed with Pearson's chi-square test or Fisher's exact test (as applicable). For multiple comparisons, one-way ANOVA was used. $p<0.05$ was considered as statistically significant.

Results

The overexpression of FOXJ1 in colon cancer was associated with TNM stage

In the TMA, constituting of 50 cases of colon carcinoma and paired normal tissues (TMA1), the expression of FOXJ1 was categorized as strong in 41(83.7%) of 49 cases (1 tissue spot was

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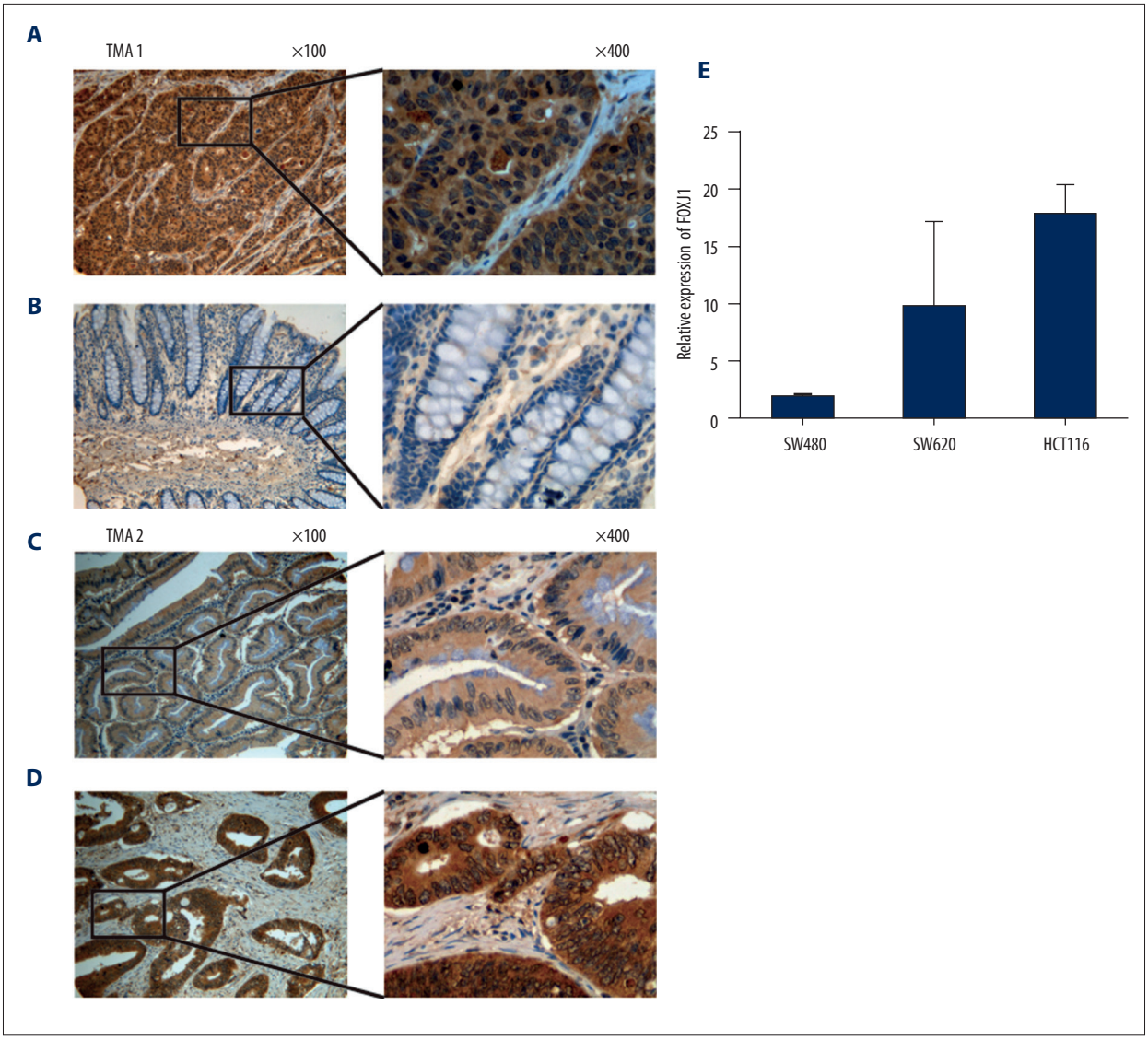

Figure 1. The expression of FOXJ1 in tissue microaarrays (TMAs) of colon cancer and colorectal cancer (CRC) cells. (**A**) The strong staining of FOXJ1 in cytoplasm of tumor cells of colon cancer and (**B**) the negative staining of FOXJ3 in normal colon tissue in TMA1 containing 50 pairs of colon cancer and adjacent normal tissue; (**C**) the weak staining of FOXJ1 in colon cancer and (**D**) the strong staining FOXJ1 in colon cancer in TMA2 containing 206 cases of colon cancer. (**E**) The relative expression levels of FOXJ1 mRNA in CRC cells.

missed in the staining procedure) according to the hierarchical staining score, and was strong in only 29 (58%) of 50 peritumor normal tissues, suggesting that the expression of FOXJ1 is remarkably upregulated in colon cancer ($p<0.001$) (Figure 1).

In the other commercial TMA, including 208 cases of colon cancer (TMA2), the clinicopathological significance of FOXJ1 expression was evaluated, showing that the overexpression of FOXJ1 was significantly associated with lymphatic metastasis ($p<0.05$), infiltration depth ($p<0.001$), and TNM staging ($p<0.05$), but we found that FOXJ1 expression has no correlation with sex, age, tumor histology type, or distant metastatic lesions (Table 1). The levels of FOXJ1 mRNA were also evaluated in SW480, SW620, and HCT116 cells, showing that FOXJ1 mRNA was highest in HCT116 cells (Figure 1).

Verification of FOXJ1 overexpression plasmid and FOXJ1 small interfering RNA in colorectal cancer cells

After transfection of FOXJ1-pcDNA3.1 plasmid into SW480 and SW620 cells, proteins of FOXJ1 were significantly increased. After transfection of 4 designed siRNAs, siRNA 1 and siRNA 2 showed more significant silencing effect; therefore, we selected these 2 siRNAs for subsequent experiments (Figure 2).

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Table 1. Correlation between the expression level of FOXJ1 and the clinicopathological indexes in TMA2.

Cases	n	FOXJ1 expression		χ^2	*P* value
		Weak	Strong		
Gender					
Male	118	56	62	1.523	0.217
Female	90	35	55		
Age (years)				2.058	0.151
≤60	121	58	63		
>60	87	33	54		
Histological type				1.82	0.177
Adenocarcinoma	177	74	103		
Mucinous adenocarcinoma	31	17	14		
Infiltration depth				12.14	0.000*
T1+T2	23	18	5		
T3+T4	185	74	111		
Lymphatic metastasis				5.01	0.025*
Absence	153	74	79		
Presence	55	17	38		
Distant metastasis				0.257	0.612
Absence	197	87	110		
Presence	11	4	7		
TNM stage				3.048	0.047*
I+II	150	72	78		
III+IV	58	19	39		

* *P*<0.05; TMA – tissue microarray; TNM – tumor node metastasis; T – tumor.

FOXJ1 promoted the proliferation of colorectal cancer cells

In CCK-8 assays, overexpression of FOXJ1 significantly increased the proliferation of SW620 cells, although proliferation of SW480 cells was not influenced. Accordingly, down-regulation of FOXJ1 using siRNA1 and siRNA2 in HCT116 cells remarkably inhibited proliferation of HCT116. These results indicate that up-regulation of FOXJ1 may enhance the proliferative ability of CRC cells (Figure 3).

FOXJ1 promoted migration and invasion ability of colorectal cancer cells

In Transwell migration and invasion assays, overexpression of FOXJ1 using FOXJ1-pcDNA3.1 plasmid in SW480 and SW620 cells significantly promoted the migration of SW480 and SW620 cells through Transwell chambers that were either coated or not coated with matrigel. Consistently, siRNA-1 and siRNA-2 against FOXJ1 both remarkably inhibited the migration of HCT116 cells through Transwell chambers that were either coated or not coated with matrigel. These findings demonstrate that the overexpression of FOXJ1 may contribute to the malignant progression of CRC by promoting the migration and invasion ability of tumor cells (Figure 4).

FOXJ1 promoted nuclear translocation of β-catenin by inhibiting truncated adenomatous polyposis coli protein and β-catenin phosphorylation

To further clarify the mechanism of FOXJ1 as a tumor promoter in CRC, Agilent human gene microarrays were used to explore gene alterations after silencing FOXJ1 in HCT116 cells. Microarray analysis and PCR verification revealed that the adenomatous polyposis coli (APC) gene was most significantly inhibited by the overexpression of FOXJ1 in CRC cells (data not shown). It is well known that wild-type APC encodes a

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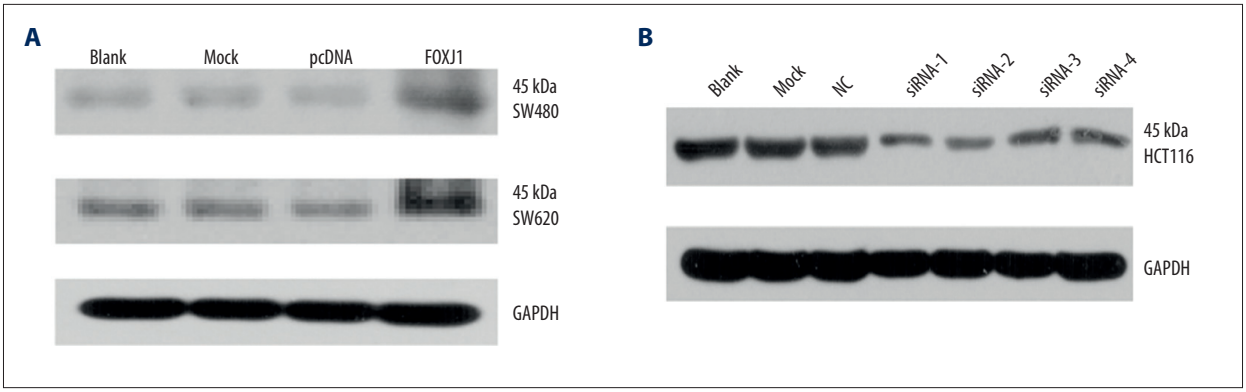

Figure 2. Validation of trasfection using FOXJ1-pcDNA3.1 overexpression vector and small interfering RNA s(siRNA) against FOXJ1. (**A**) FOXJ1 protein was increased in total protein lysates of SW480 and SW620 cells after transfection of FOXJ1-pcDNA3.1 pasamid for 48 h. (**B**) FOXJ1 protein was decreased in total protein lysates of HCT116 cells after transfection of siRNAs against FOXJ1 for 24 h, in which siRNA-1 and siRNA-2 exhibited the most protein interfering effect. FOXJ1 – transfection of FOXJ1-pcDNA3.1 plasmid; pcDNA – transfection of pcDNA3.1 empty plasmid; NC – transfection of siRNA as negative control; Mock – mock transfection with lipofectamine 2000; Blank – no treatment to cells.

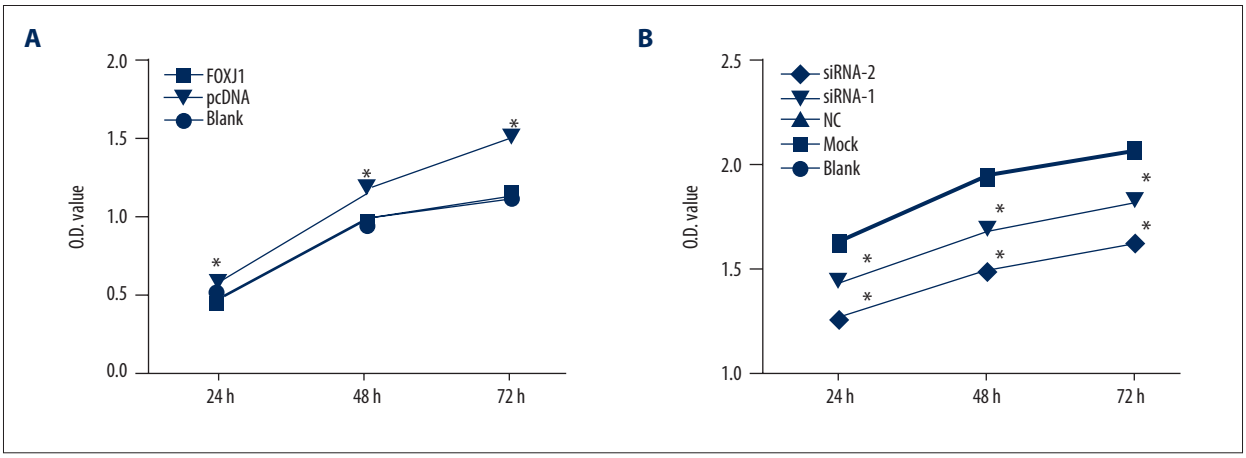

Figure 3. The effect of FOXJ1 on proliferation of colorectal cancer cells. (**A**) The proliferation of SW620 cells were markedly increased after transfection of FOXJ1-pcDNA3.1 overexpression vector on 24 h, 48 h, and 72 h. (**B**) The proliferation of HCT 116 cells were markedly inhibited after transfection of siRNA-1 and siRNA-2 against FOXJ1 on 24 h, 48 h, and 72 h. * $P<0.001$ compared with pcDNA, NC, nock and blank group. FOXJ1 – transfection of FOXJ1-pcDNA3.1 plasmid; pcDNA – transfection of pcDNA3.1 empty plasmid; NC – transfection of siRNA as negative control; Mock – mock transfection with lipofectamine 2000; Blank – no treatment to cells.

full-length protein with a molecular weight of about 310 kDa, while mutated APC encodes truncated proteins with molecular weight between 80–180 kDa in CRC cells [24]. In SW620 cells, truncated APC of approximately 110–130 kDa is expressed, and up- or downregulation of FOXJ1 significantly decreased or increased the expression of truncated APC. Considering the crucial role of β-catenin phosphorylation in the APC/β-catenin pathway, the phosphorylation level of β-catenin was evaluated in SW620 cells after up-regulation or downregulation of FOXJ1. We found that FOXJ1 overexpression markedly inhibited the phosphorylation level of β-catenin at sites of Ser33/37/Thr41, and increased the level of intranuclear β-catenin, although total β-catenin level was only slightly affected. Accordingly, silencing of FOXJ1 using previously identified siRNA-2 promoted the phosphorylation level of β-catenin and decreased the level of intranuclear β-catenin. In addition, cyclin D1, a downstream target gene of Wnt/β-catenin pathway was significantly upregulated after overexpression of FOXJ1. Furthermore, the promotion effect of FOXJ1 on β-catenin intranuclear translocation was confirmed in immunofluorescence assays. All these data suggest that FOXJ1 may be involved in the activation of Wnt/β-catenin signaling in CRC by inhibiting the expression of truncated APC, consequently decreasing phosphorylation of β-catenin and promoting the nuclear translocation of β-catenin (Figure 5).

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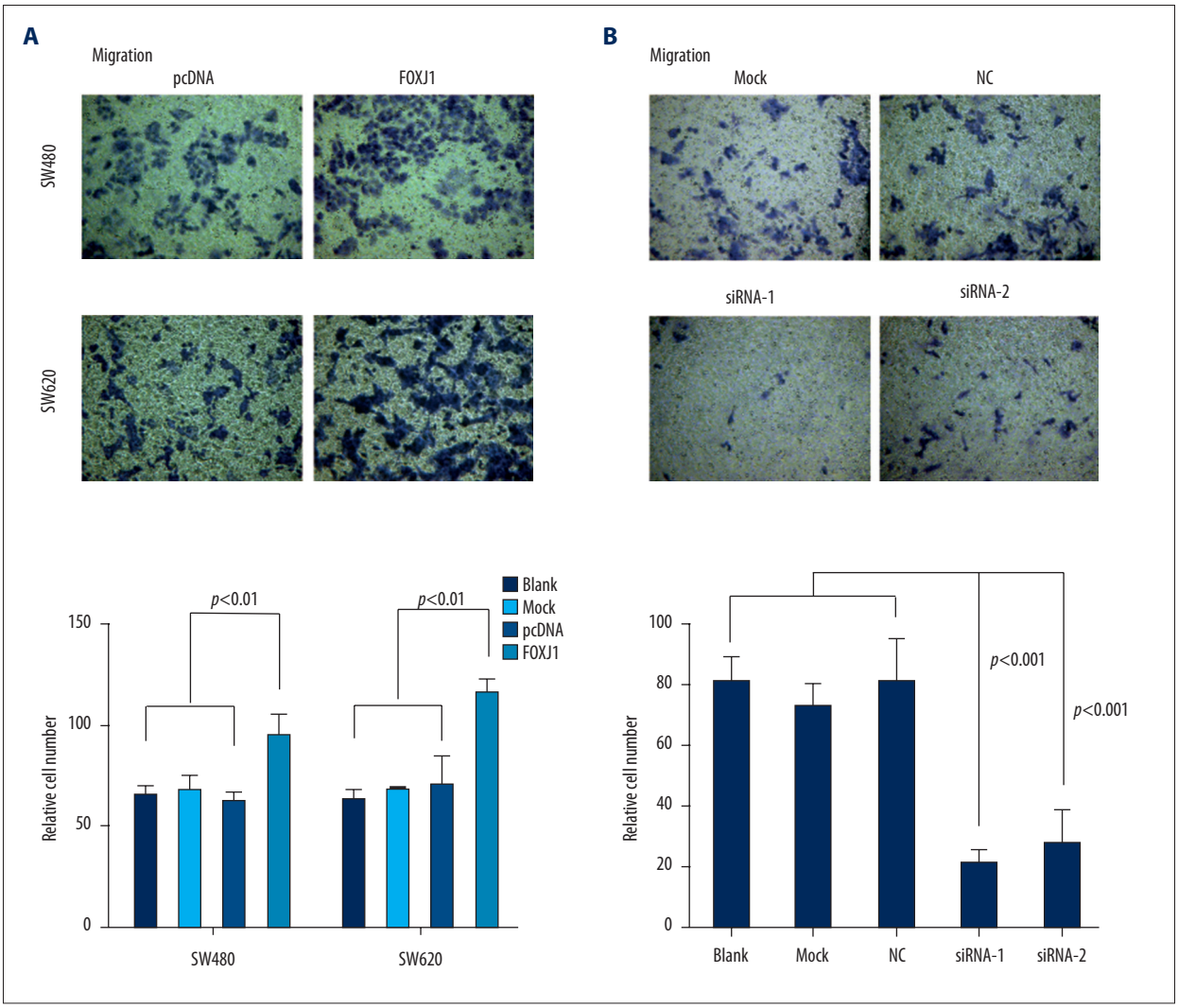
Discussion

The FOX transcriptors family has important functions in several crucial processes of cancer development, including the Wnt pathway [10,25], epithelial- to-mesenchymal transition [4,26,27], and angiogenesis [9]. According to previous reports, FOX transcriptors exert differential effects in various tumors, possibly due to different microenvironments. Similarly, it was suggested that FOXJ1 exerts the opposite effects in various carcinomas [28]. In stomach cancer, it was suggested that decreased FOXJ1 was associated with poor prognosis and metastasis of tumors [16]. In addition, up-regulation of FOXJ1 in stomach cancer cells inhibited the proliferation, migration, and invasion of tumor cells [16]. In contrast, downregulation of FOXJ1 enhanced the proliferation, migration, and invasion of tumor cells [16]. In ovarian cancer, FOXJ1 functioned as a mediator in downstream of NANOG, a stem cell transcription factor, and it was found that Nanog-mediated cell migration and invasion are involved its downregulation of E-cadherin and FOXJ1 [16]. In contrast, it was reported that FOXJ1 is increased in hepatocellular cancer and renal carcinoma [20,21]. In hepatocellular carcinoma, overexpression of FOXJ1 was associated with histological grade and poor prognosis, and overexpression of FOXJ1 in hepatocellular cancer cells can promote tumor cell proliferation and cell-cycle transition, whereas FOXJ1 siRNA exhibited the opposite effects [20]. In clear-cell renal cell carcinoma, FOXJ1 expression was also associated with tumor stage, histologic grade, and size. The overexpression or silencing FOXJ1 in renal cancer cells significantly enhanced or inhibited the proliferation of cancer cells, respectively, and these *in vitro* results were supported by *in vivo* investigations in BALB/c nude mice [21].

The expression and function of FOXJ1 in CRC still remains largely unknown. Hence, in the present study, the expression pattern of FOXJ1 in CRC and its clinical significance was first evaluated using 2 TMAs. In TMA1 with colon cancer and paired adjacent noncancerous specimens, the overexpression rate of



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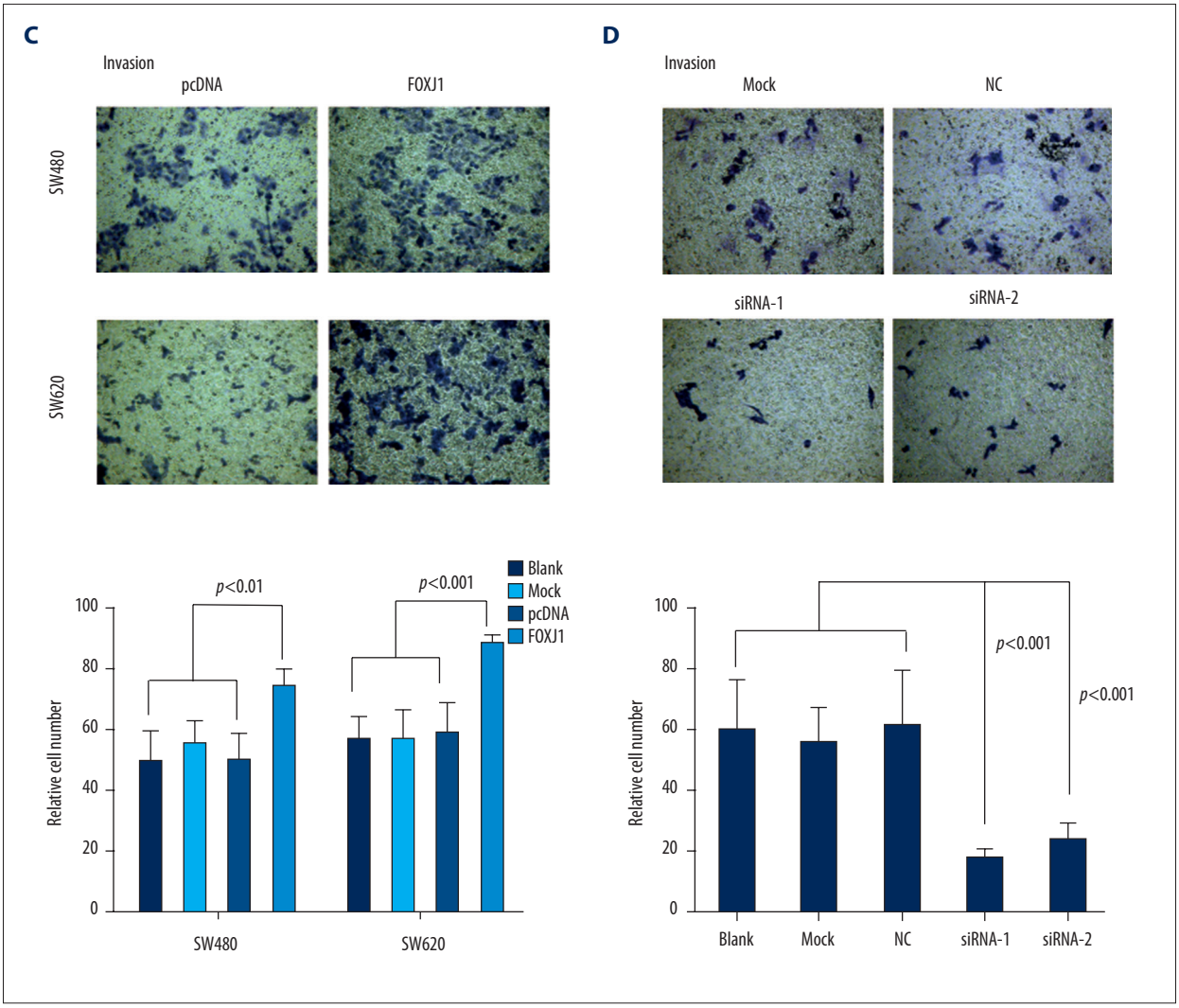

Figure 4. (**A–D**) In Transwell migration assays, up-regulation of FOXJ1 in SW480 and SW620 cells both significantly promoted migration of tumor cells (both p<0.01), while down-regulation of FOXJ1 in HCT116 cells using siRNA-1 and siRNA-2 both significantly inhibited migration of tumor cells (both p<0.001). Likewise, in Transwell invasion assays, up-regulation of FOXJ1 in SW480 and SW620 cells both significantly promoted invasion of tumor cells through matrigel Transwell chambers (p<0.01 and p<0.001 respectively), while down-regulation of FOXJ1 in HCT116 cells using siRNA-1 and siRNA-2 both significantly inhibited invasion of tumor cells through matrigel coated Transwell chambers (both p<0.001). FOXJ1 – transfection of FOXJ1-pcDNA3.1 plasmid; pcDNA – transfection of pcDNA3.1 empty plasmid; siRNA-1 – transfection of siRNA-1; siRNA-2 – transfection of siRNA-2; NC – transfection of siRNA as negative control; Mock – mock transfection with lipofectamine 2000; Blank – no treatment to cells.

FOXJ1 in colon cancer was significantly higher than in normal tissues. In tumor tissues, the expression of FOXJ1 was mostly observed in cell cytoplasm, whereas in normal colon epithelium the expression of FOXJ1 was seen both in cytoplasm and nuclei of epithelial cells (data not shown), consistent with findings in gastric cancer [16] and hepatocellular carcinoma [20]. Further analysis of TMA2 in 206 specimens of colon cancer revealed that FOXJ1 overexpression was significantly correlated with clinicopathological indexes of lymph node metastasis, invasion depth, and TNM stage. These findings indicate that FOXJ1 has a role in promoting the progression of CRC.

Moreover, in the present study, FOXJ1 expression levels in 3 cell lines – SW620, SW480, and HCT116 – were evaluated and FOXJ1 expression was highest in HCT116 cells. Subsequently, to test the effect of FOXJ1 on CRC cells, HCT116 cells were selected for silencing FOXJ1 using siRNAs, and FOXJ1-pcDNA plasmids were transfected into SW620 and SW480 cells for overexpression of FOXJ1. As expected, overexpression of FOXJ1 significantly enhanced the growth, migration, and invasion ability of SW620 cells. Additionally, knockdown of FOXJ1 significantly inhibited the proliferation, migration, and invasion ability of HCT116. Taken together, these findings further demonstrate

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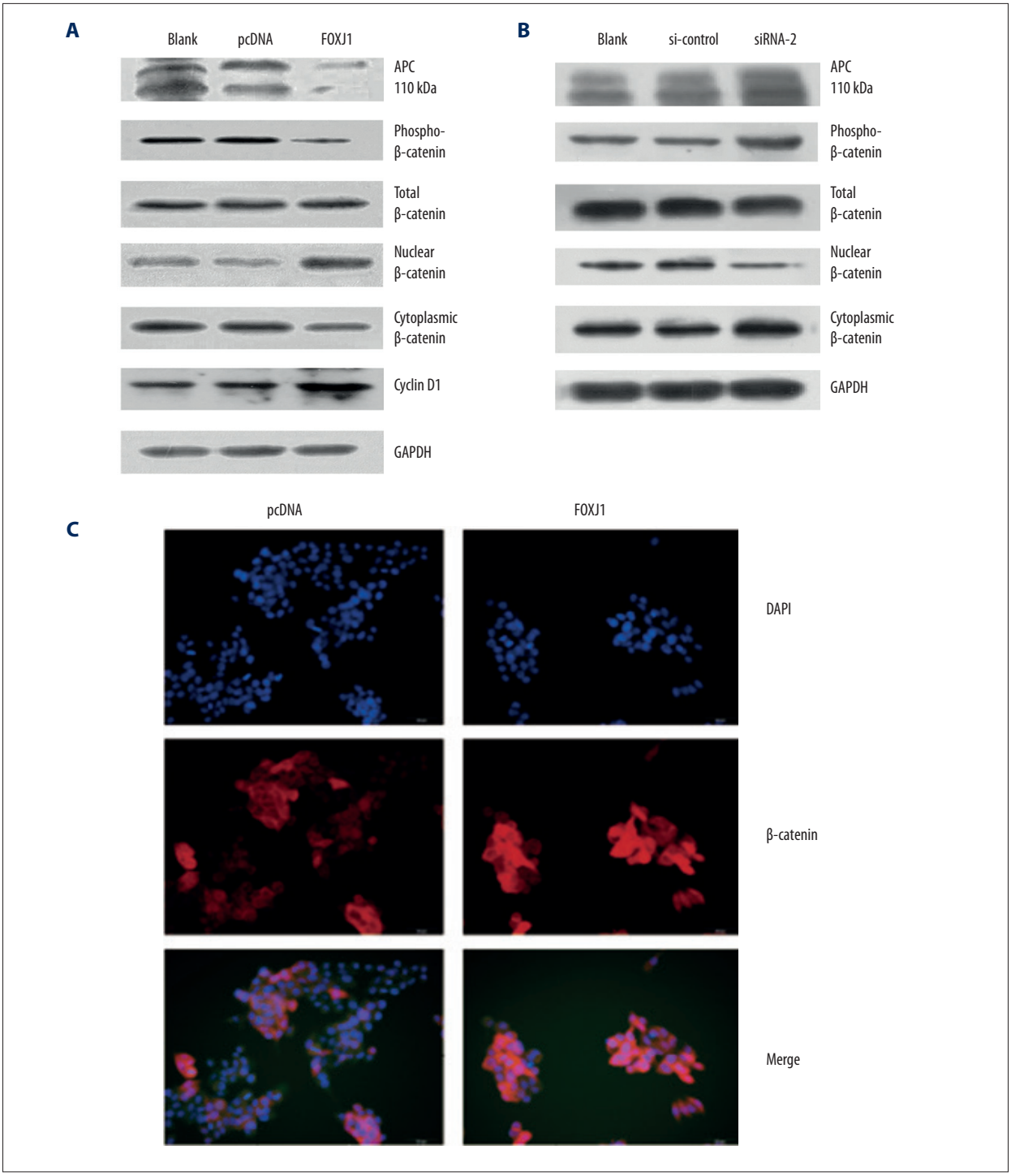

Figure 5. (**A**) The expression of truncated APC was confirmed in SW620 cells using Western blots. Overexpression of FOXJ1 in SW620 cells significantly inhibited the expression of APC and the extent of phosphorylation of β-catenin. In contrast, the expression of total β-catenin was slightly increased. Further WB of nuclear and cytoplasmic β-catenin after over-expression of FOXJ1 in SW620 cells, significant up-regulation of nuclear β-catenin and down-regulation of cytoplasmic β-catenin were demonstrated. In addition, the expression of cyclin D1 was remarkably increased. (**B**) Silencing of FOXJ1 in SW620 cells elicited the opposite effects. (**C**) In immunofluorescence assays, enhanced nuclear translocation of β-catenin was confirmed. FOXJ1 – transfection of FOXJ1-pcDNA3.1 plasmid; siRNA-2 – transfection of siRNA-2; pcDNA – transfection of pcDNA3.1 empty plasmid; si-control – transfection of control siRNA; Blank – no treatment to cells; DAPI – 4',6-diamidino-2-phenylindole.

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that increased expression of FOXJ1 contributes to the progression of CRC. Interestingly, the basic migration ability of SW480 and SW620 were very similar in the migration assay, although the basic expression level of FOXJ1 in these cells differed, which may suggest that FOXJ1 expression is not a decisive factor in cell migration.

Multiple FOX family transcriptors are intricately involved in interaction with the Wnt/β-catenin pathway, which is an essential signaling pathway for colorectal carcinogenesis [29]. For example, FOXM1 and FOXQ1 are both targets of the Wnt/β-catenin pathway [30,31], and these 2 transcriptors are also both believed to be activators of the Wnt/β-catenin pathway [32,33]. In physiological circumstances, FOXJ1 is also a direct target activated by the Wnt/β-catenin signaling in Kupffer's vesicles of zebrafish [34,35]. However, in lung morphogenesis, FOXJ activates the Wnt/β-catenin antagonist chibby [36]. These controversial findings suggest that the relationship of FOXJ1 with the Wnt/β-catenin pathway may depend on the microenvironment. In the present study, we found that FOXJ1 overexpression in SW620 cells significantly promoted nuclear transport of β-catenin and increased the level of cyclinD1, suggesting that FOXJ1 promotes the Wnt/β-catenin pathway in CRC.

APC is an important tumor suppressor for CRC, and is also an essential component of the Wnt/β-catenin signaling pathway [37]. APC mutations leading to the expression of a truncated APC protein can be found in up to 85% of sporadic colorectal cancers [38]. In normal colon epithelium, APC, axin, glycogen synthase kinase -3β (GSK-3β), and casein kinase 1 (CK1) form the "β-catenin destruction" complex for negatively regulating Wnt/β-catenin pathway signaling, which catalyzes β-catenin phosphorylation on sites Ser45, Thr41, Ser37, and Ser33 in the absence of Wnt signaling, leading to its ubiquitination and degradation [39]. In contrast, the presence of Wnt signaling stimulates the inhibition of β-catenin phosphorylation and degradation by negatively regulating GSK-3β in the destruction complex, leading to its accumulation and entry into the nucleus [39]. APC is also essential in the inhibition of β-catenin phosphorylation and degradation via associating with both β-catenin and axin, as well as transporting β-catenin out of the nucleus [40]. It was previous thought that mutations of APC prevent β-catenin phosphorylation and degradation in colon cancer cells. However, a recent study elegantly showed that CRC-related truncated APC is not a null mutant in terms of Wnt/β-catenin regulation, remaining at least partially able to promote β-catenin phosphorylation activity and to promote export of β-catenin from the nucleus [40,41]. In the present study, we demonstrated that the level of APC mRNA and truncated APC protein was remarkably decreased by overexpression of FOXJ1 in SW620 cells and increased by silencing of FOXJ1 using siRNA, and the phosphorylation activity of β-catenin was also significantly inhibited or promoted. Hence, we speculate that FOXJ1 inhibits the phosphorylation of β-catenin via decreasing the level of truncated APC. However, Li et al. reported that overexpression of APC in lung cancer cells increased the expression of FOXJ1 [42], suggesting that more studies are needed to clarify the complex interaction of FOXJ1 with APC and Wnt/β-catenin signaling in cancer.

Conclusions

In conclusion, in the present study we demonstrated that the overexpression of FOXJ1 contributes to the progression of CRC. FOXJ1 may be a novel biomarker for CRC and an attractive target in CRC therapy. Further studies are necessary to elucidate the tumor promoter role of FOXJ1 in CRC.

Conflict of Interest

None.

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