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Integrin Subunit Alpha 5 (ITGA5) Gene Circular RNA Sponges microRNA-107 in Colorectal Carcinoma Cells and Tissues and Regulates the Expression of the Forkhead Box J3 (FOXJ3) Gene

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Circular RNAs (circRNAs) can function as sponges for microRNA (miRNA) in carcinogenesis. This study aimed to investigate the role of the circRNA of the integrin subunit alpha 5 (ITGA5) gene and microRNA-107 (miR-107) in human colorectal carcinoma (CRC) cells *in vitro* and tissue samples from patients with CRC and the expression of forkhead box J3 (FOXJ3) protein.

Material/Methods: Thirty paired CRC tissue samples and adjacent normal colon tissue samples were studied. Human CRC cell lines, including HT29, SW480, LoVo, and HIEC cells, were studied for cell proliferation using the cell counting kit-8 (CCK-8) assay. Cell migration was studied using a transwell assay, and cell apoptosis was determined using flow cytometry. The luciferase reporter assay was used to study the interactions between ITGA5 circRNA, FOXJ3, and miR-107 in human CRC cells. The expression of ITGA5 circRNA and miR-107 was determined using quantitative real-time polymerase chain reaction (qRT-PCR). The protein levels of FOXJ3 were measured by Western blot.

Results: The expression of ITGA5 circRNA was significantly reduced in CRC tissues and CRC cell lines. High ITGA5 circRNA expression inhibited the proliferation and cell migration of CRC cells and promoted the apoptosis of CRC cells. The luciferase reporter assay confirmed that ITGA5 circRNA bound to miR-107, which directly targeted FOXJ3.

Conclusions: ITGA5 circRNA may act as a sponge for miR-107 to upregulate FOXJ3 expression and act as a tumor suppressor in CRC cells.

MeSH Keywords: Colorectal Neoplasms • Genes, Tumor Suppressor • MicroRNAs • RNA, Untranslated

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Background

Colorectal cancer (CRC) is a malignancy that arises from the glandular epithelium of the colorectal mucosa and is the third most common cancer worldwide [1]. Although considerable achievements in surgery and chemotherapy have been made, the overall survival rate of patients with CRC remains low [2]. The occurrence and progression of CRC is a complex process involving the down-regulation of multiple oncogenes and the interactions of several extrinsic factors [3,4]. However, the exact molecular mechanism of CRC remains unknown. It is important to identify new tumor markers and treatment methods to improve the prognosis for patients with CRC.

Circular RNAs (circRNAs) are noncoding RNAs that are characterized by a covalently closed continuous loop without a 5' cap or 3' poly (A) tail, which accounts for its stability and resistance to RNase [5–7]. The high stability and tissue-specific expression of circRNAs in various animal tissues and cells indicate that they may have a potential role as biomarkers and therapeutic targets in cancer [8]. Several studies have shown that circRNAs contain selectively conserved microRNA (miRNA) target sites and function as miRNA sponges, further regulating the expression of miRNA target genes by competing for shared miRNA target sites [9]. The characteristics of circRNAs determine whether they play roles in tumorigenesis. For example, circRNA EPSTI1 regulates BCL11A expression and promote TNBC proliferation by sponging miR-4753/miR-6809 [10]. CircNT5E binds to miR-422a as a sponge to promote the progression of glioblastoma [11]. Chen et al. [12] found that 316 circRNAs were differentially expressed in CRC tissues using circRNA microarrays. Recently, ITGA5 circRNA expression was reported to be reduced in CRC tissues and was significantly associated with lymph node metastasis [13]. However, the function and molecular mechanism of ITGA5 circRNA in CRC remains unknown.

Therefore, this study aimed to investigate the role of the circRNA of the integrin subunit alpha 5 (ITGA5) gene and microRNA-107 (miR-107) in human CRC cells *in vitro* and tissue samples from patients with CRC and the expression of forkhead box J3 (FOXJ3) protein.

Material and Methods

Samples of colorectal carcinoma (CRC) tissue

Thirty paired tissue samples containing colorectal carcinoma (CRC) and corresponding adjacent non-tumor tissues were obtained from the Second Affiliated Hospital of Zhejiang Chinese Medical University and Zhejiang Provincial Peoples' Hospital. Depending on the expression of ITGA5 circRNA, tumor tissue samples were divided into two subgroups and identified as the low ITGA5 circRNA expression group and high ITGA5 circRNA expression group. The patients with CRC included in this study were not treated with radiotherapy or chemotherapy before surgery. This study was approved by the Ethics Committees of the Second Affiliated Hospital of Zhejiang Chinese Medical University and Zhejiang Provincial Peoples' Hospital.

Cell culture and transfection

HT29, SW480, LoVo, and HIEC cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (Gibco, Wellesley Hills, MA, USA) at 37°C with 5% CO_2. For cell transfection, cells were seeded in cell culture plate and cultured to 60–80% confluence before transfection, and siRNA-ITGA5, siRNA-control, miR-107 mimics, inhibitors, or their negative controls (Genechem, Shanghai, China) were transiently transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Plasmid construction

To construct the stable cell lines that overexpressed ITGA5 circRNA, the sequence was firstly cloned into the pLCDH-ciR vector (Geneseed, Guangzhou, China) and confirmed by sequencing. Then, pLCDH- ITGA5 circRNA or pLCDH-ciR empty vector was transfected into 293 T cells by Lipofectamine 2000 to construct the lentivirus. After determining the viral titer, SW480 cells were infected by the lentiviral particles obtained. The overexpression vector for FOXJ3, pCMV3-FOXJ3-GFPSpark, was obtained from Sino Biological (Beijing, China).

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

RNA extraction was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA synthesis and qRT-PCR were performed using HiFiScript cDNA Synthesis Kit and Ultra SYBR mixture (Kangwei Century Biotechnology, Beijing, China). Then, the qRT-PCR data were analyzed by the $2^{-\Delta\Delta CT}$ method. GAPDH was used as an mRNA/circRNA expression standard, and the expression of miRNA was normalized to U6 RNA internal control.

The primer sequences included:
ITGA5 circRNA, forward: 5'-CCAGACACCCAGGACTTATT-3';
ITGA5 circRNA, reverse: 5'-ATCTCTCTGCAATCCTCTCG3';
FOXJ3, forward: 5'-AGCCTAACATCTATGGACTGGT-3';
FOXJ3, reverse: 5'-GGTCAAGGAGTGCATTCTTCTTA-3';
GAPDH, forward: 5'-GCACCGTCA AGGCTGAGAAC-3';
GAPDH, reverse: 5'-TGGTGAAGACGCCAGTGGA-3';
miR-107, forward: 5'-AGCAGCATTGTACAGGGCTATCA-3';
miR-107, reverse: 5'-AAGGCGAGACGCACATTCTT-3';

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U6, forward: 5'-CTCGCTTCGGCAGCACA-3';
U6, reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Western blot

Proteins were extracted and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Burlington, MA, USA). After blocking with 1% bovine serum albumin (BSA), the membranes were incubated overnight at 4°C with primary antibodies to FOXJ3 (ab183112; Abcam, Cambridge, MA, USA), Twist (ab49254; Abcam, Cambridge, MA, USA), MMP-2, MMP-9, caspase-3, and Bax (10373-2-AP; 27306-1-AP; 19677-1-AP; 50599-2-Ig) (Proteintech, Manchester, UK), and then incubated with secondary antibody (Proteintech, Manchester, UK). GAPDH was used as a control (10494-1-AP) (Proteintech, Manchester, UK).

Cell proliferation assay

Cell proliferation was measured by the cell counting kit-8 (CCK-8) assay. Briefly, 5×10^3 cells were seeded in 96-well plates and incubated for 48 h. The CCK-8 solution was added to each well at different time points and incubated at 37°C for a further 2 h. The OD_{450} was measured using a SpectraMax M3 microplate reader (Molecular Devices, San Jose, CA, USA).

Cell migration and invasion assay

The cells were seeded in the upper chamber of the inserts (Corning, New York, NY, USA) at a density of 2×10^3/well. DMEM containing 20% FBS was added to the lower chamber of each well. After culturing for 36 h, the cells on the lower surface of the chamber were fixed with 4% paraformaldehyde and stained by 0.1% crystal violet. For the invasion assay, the upper chamber was pre-coated with Matrigel (BD Biosciences, Bedford, MA, USA). The chambers were viewed using a Nikon Eclipse Ti-S microscope (Nikon, Tokyo, Japan).

Cell apoptosis assays

The apoptotic rate of cells was measured by staining with Annexin V- fluorescein isothiocyanate (FITC) and propidium iodide (PI) (Beyotime Biotechnology, Haimen, China). After filtering through 200 μm mesh sieves, all samples were assessed using a BD FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The study was performed in triplicate.

Luciferase reporter assay

ITGA5 circRNA sequences were inserted into psiCHECK2 vector (Promega, Madison, WI, USA), and the 3'-UTR fragment of FOXJ3 containing the putative binding site of miR-107 were cloned into the psiCHECK2, termed ITGA5 circRNA-WT and FOXJ3-WT, respectively. The mutated plasmids were termed ITGA5 circRNA-MT and FOXJ3-MT and were used as controls. Luciferase activity was measured using the Dual-Luciferase Reporter Gene Assay Kit (Beyotime Institute of Biotechnology, Haimen, China).

Statistical analysis

The results were presented as the mean±standard deviation (SD) of experiments performed in triplicate. Student's t-test or one-way analysis of variance (ANOVA) was used to determine statistical significance. Pearson's correlation analysis was used to compare the data between groups. The Kaplan-Meier method and the log-rank test were used to evaluate the patient overall survival (OS). A P-value <0.05 was considered to be statistically significant.

Results

Down-regulation of ITGA5 circular RNA (circRNA) in colorectal cancer (CRC) tissues and cell lines

To explore the function of ITGA5 circRNA in CRC progression, we first compared ITGA5 circRNA expression levels in 30 pairs of CRC tissues and adjacent normal tissues. As shown in Figure 1A, the ITGA5 circRNA level was significantly lower in CRC tissues compared with matched controls. The ITGA5 circRNA expression level was also reduced in HT29, LoVo, and SW480 cells compared with the HIEC cells (Figure 1B). As shown in Table 1, ITGA5 circRNA expression was significantly associated with the tumor stage of CRC (P=0.0217), distant metastasis (P=0.0312), and tumor size (P=0.0332), but not with patient age and gender. To determine whether ITGA5 circRNA could act as a prognostic biomarker, 30 CRC tissue samples were divided into two subgroups depending on the ITGA5 circRNA levels. Further Kaplan-Meier analysis showed that patients with CRC with lower ITGA5 circRNA expression had significantly reduced prognosis (Figure 1C).

ITGA5 circRNA regulated the proliferation, migration, and apoptosis of CRC cells

We constructed ITGA5 circRNA that was stably overexpressed SW480 cells. As shown in Figure 2A, 2C, and 2D, ITGA5 circRNA upregulation reduced cell proliferation and cell migration of SW480 cells. Also, ITGA5 circRNA overexpression significantly increased cell apoptosis in SW480 cells (Figure 2G), and significantly reduced the protein expression levels of Twist, MMP-2, MMP-9, and N-cadherin and increased E-cadherin, Bax, and cleaved caspase-3 in SW480 cells (Figure 2I). The gene knockdown experiments used HT29 cells, which had high ITGA5

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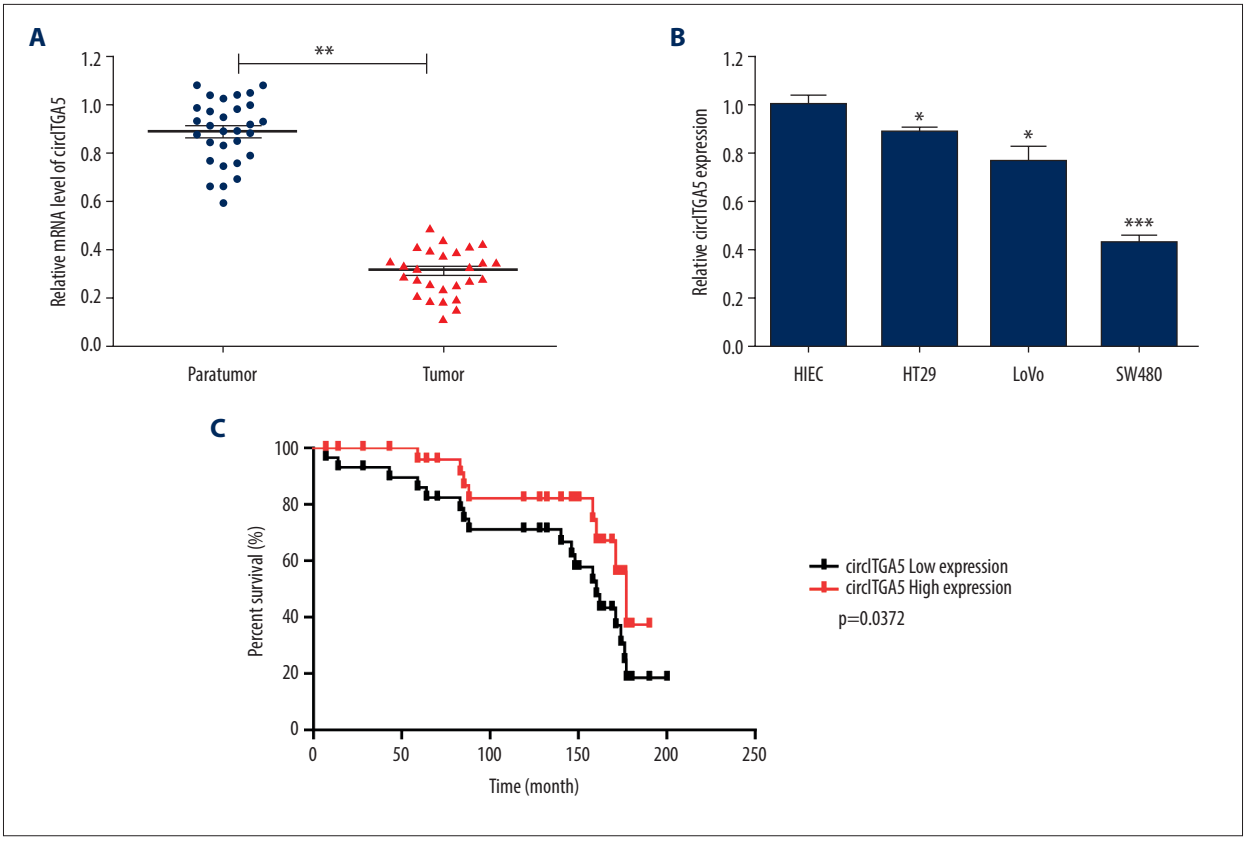

Figure 1. The integrin subunit alpha 5 (ITGA5) gene circular RNAs (circRNA) was down-regulated in colorectal carcinoma (CRC) tissue and was associated with reduced prognosis of patients with CRC. (**A**) ITGA5 circRNA expression was analyzed in 30 CRC tissue samples and matched non-tumor tissues. (**B**) ITGA5 circRNA expression was analyzed in normal HIEC control cells and the HT29, LoVo, and SW480 human CRC cell lines. (**C**) Overall survival curves of patients with CRC and high or low ITGA5 circRNA expression. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 1. Correlation between the integrin subunit alpha 5 (ITGA5) gene circular RNA (circRNA) expression and the clinicopathological features in 30 patients with colorectal carcinoma (CRC).

Characteristic		ITGA5 circRNA		P-value
		Low 20	High 10	
Age	≥60	10	6	0.178
	<60	10	4	
Gender	Male	11	6	0.179
	Female	9	4	
T-stage	T1–T2	7	8	0.0217*
	T3–T4	13	2	
Distant metastasis	Yes	15	2	0.0312*
	No	5	8	
Tumor size	≥5 cm	14	2	0.0332*
	<5 cm	6	8	

* $P<0.05$.

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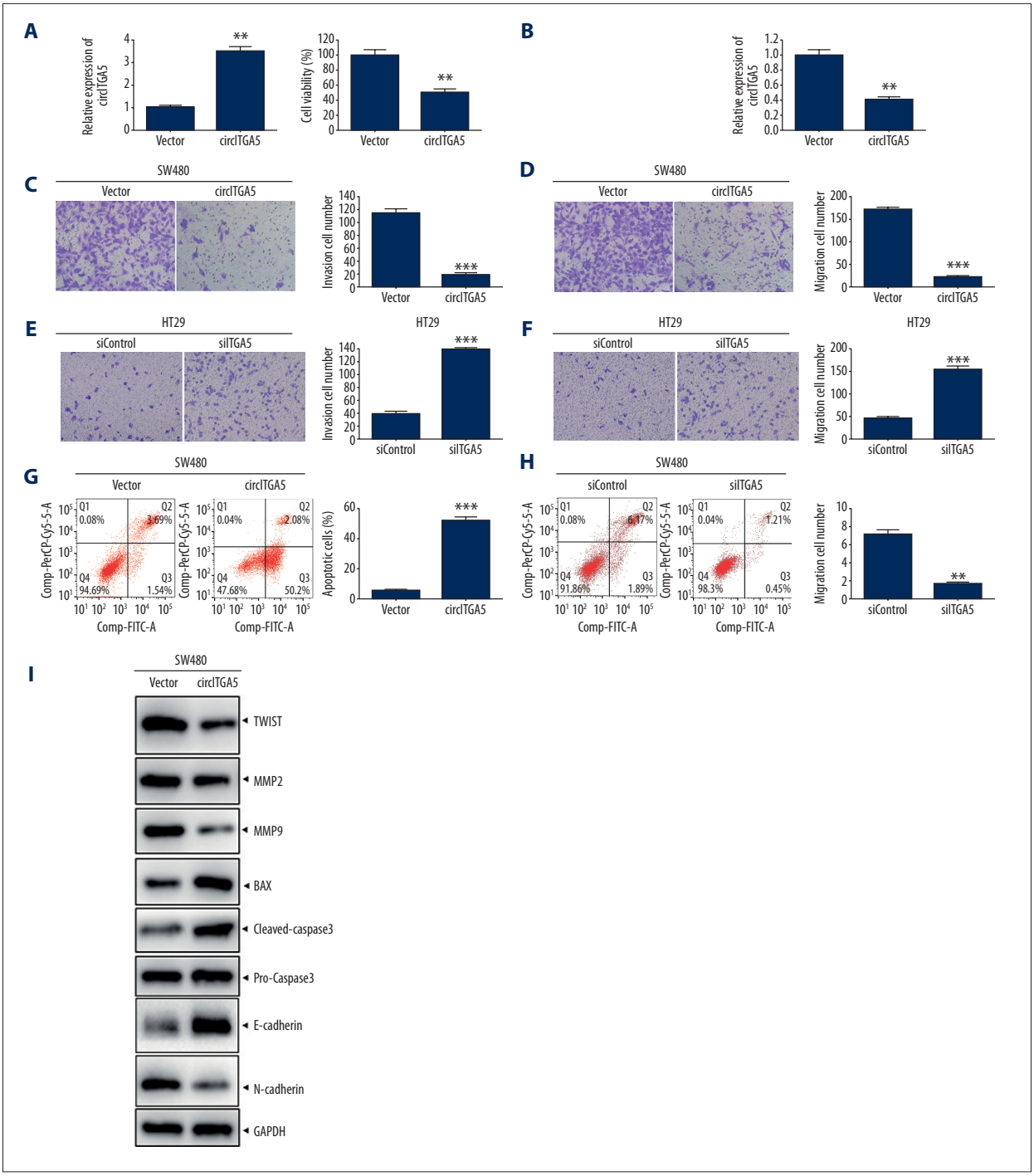

Figure 2. The integrin subunit alpha 5 (ITGA5) gene circular RNAs (circRNA) regulated the proliferation, migration, invasion, and apoptosis of human colorectal carcinoma (CRC) cells *in vitro*. (**A**) ITGA5 circRNA expression was increased in SW480 cells following stable transduction with pLCDH- ITGA5 circRNA. The cell counting kit-8 (CCK-8) assay showed that ITGA5 circRNA overexpression reduced SW480 cell proliferation. (**B**) ITGA5 circRNA expression was lower in HT29 cells following transduction with siRNA- ITGA5 circRNA. The CCK8 assay showed that ITGA5 circRNA knockdown increased HT29 cell proliferation. The overexpression of ITGA5 circRNA suppressed cell invasion (**C**) and migration (**D**) of SW480 cells. The knockdown of ITGA5 circRNA promoted invasion (**E**) and migration (**F**) of HT29 cells. (**G**) The overexpression of ITGA5 circRNA promoted apoptosis of SW480 cells. (**H**) The knockdown of ITGA5 circRNA inhibited apoptosis of HT29 cells. (**I**) Western blot identified the protein expression of Twist, MMP-2, MMP-9, Bax, E-cadherin, N-cadherin, and caspase-3 in SW480 cells. ** $P<0.01$, *** $P<0.001$.

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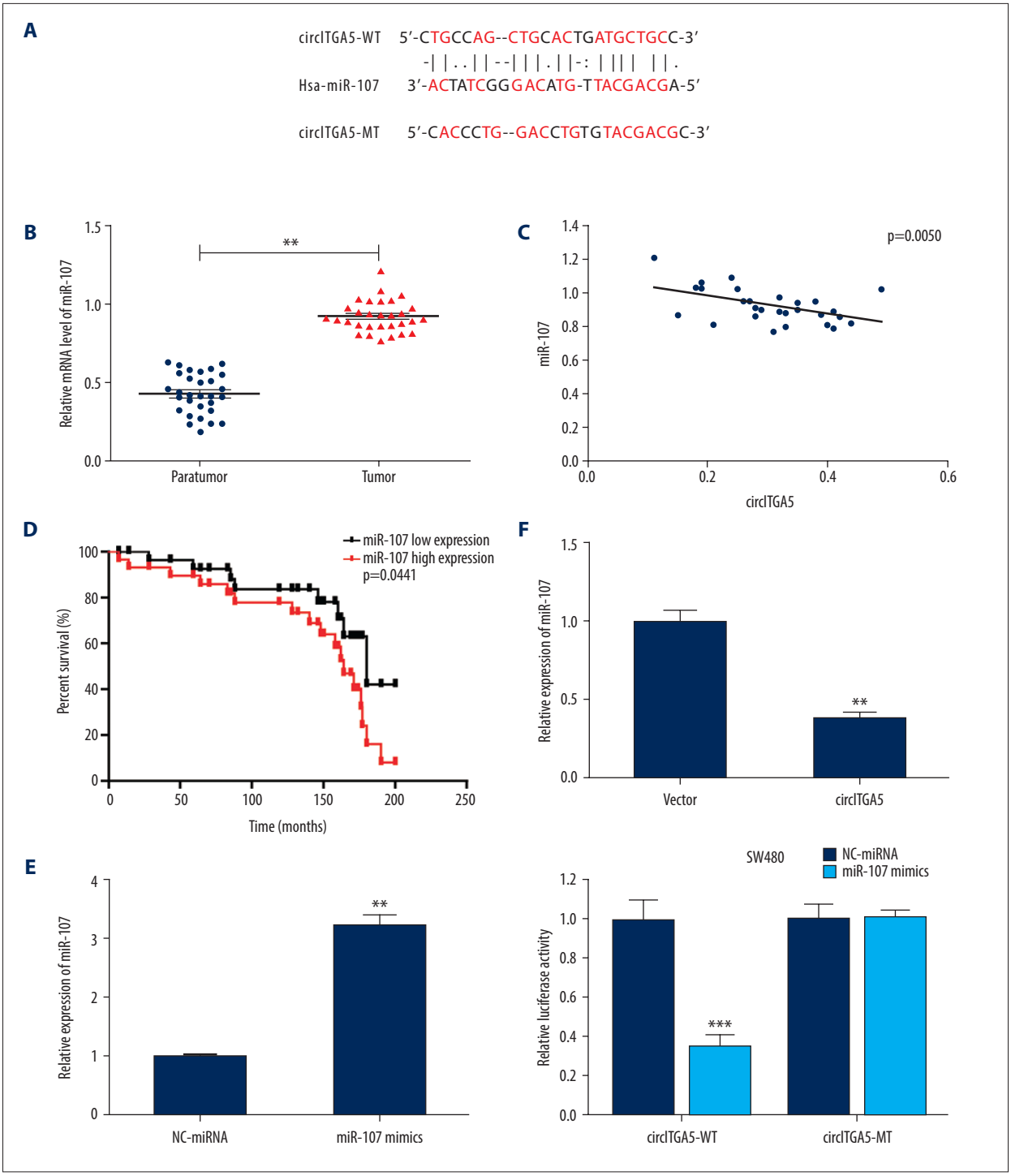

Figure 3. The integrin subunit alpha 5 (ITGA5) gene circular RNAs (circRNA) was a sponge for microRNA-107 (miR-107) in human colorectal carcinoma (CRC). (**A**) The putative binding sites of miR-107 on ITGA5 circRNA. (**B**) miR-107 levels were analyzed in 30 CRC tissues and adjacent non-tumor tissues. (**C**) Pearson's correlation analysis of ITGA5 circRNA and miR-107 expression in 30 CRC tissues. (**D**) Overall survival (OS) curves of patients with CRC and high or low miR-107 expression. (**E**) The luciferase reporter assay showed that miR-107 overexpression inhibited the activity of ITGA5 circRNA-WT but not that of the ITGA5 circRNA reporter. (**F**) ITGA5 circRNA overexpression significantly inhibited miR-107 expression in SW480 cells. ** $P<0.01$, *** $P<0.001$.

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Table 2. Correlation between microRNA-107 (miR-107) expression and the clinicopathological features in 30 patients with colorectal carcinoma (CRC).

Characteristic		miR-107		P-value
		Low 13	High 17	
Age	≥60	6	7	0.447
	<60	7	10	
Gender	Male	7	7	0.427
	Female	6	10	
T-stage	T1–T2	8	7	0.0984
	T3–T4	5	10	
Distant metastasis	Yes	3	13	0.0118*
	No	10	4	
Tumor size	≥5 cm	5	9	0.0578
	<5 cm	8	8	

* $P<0.05$.

circRNA expression levels (Figure 2B). The results showed that ITGA5 circRNA down-regulation significantly increased the proliferative, motility, and cell migration of HT29 cells and reduced cell apoptosis in HT29 cells (Figure 2B, 2E, 2F, 2H). The results of this study supported that ITGA5 circRNA functioned as a tumor suppressor gene in CRC cells.

ITGA5 circRNA was a sponge for miR-107

Because previous studies showed that circRNAs act as miRNA sponges to bind miRNAs and liberate mRNA transcripts which were targeted by miRNAs, we first predicted the miRNAs that may bind to ITGA5 circRNA using online bioinformatics databases that included starBase 2.0, miRDB, and TargetScan. ITGA5 circRNA was shown to target miR-107 (Figure 3A).

Previous studies showed that miR-107 stimulates metastasis and inhibits CRC cell apoptosis [14, 15]. The findings from the present study showed that miR-107 expression was increased in CRC tissues compared with the non-tumor tissues and was negatively correlated with ITGA5 circRNA expression (Figure 3B, 3C). Also, the miR-107 level was significantly associated with distant metastasis of CRC (Table 2). Kaplan-Meier analysis showed that patients with CRC with higher miR-107 expression had poorer prognosis (Figure 3D).

We transfected miR-107 mimics with ITGA5 circRNA-WT or ITGA5 circRNA-MT into SW480 cells. The luciferase activity of the ITGA5 circRNA-WT reporter was significantly reduced when compared with ITGA5 circRNA-MT in a miR-107 mimic-dependent manner (Figure 3E). Also, the effect of ITGA5 circRNA overexpression on the miR-107 level in SW480 cells showed that miR-107 expression was significantly inhibited in CRC cells that overexpressed ITGA5 circRNA (Figure 3F). These findings showed that ITGA5 circRNA directly sponged miR-107 in CRC cells.

miR-107 directly targeted FOXJ3 in CRC cells

Bioinformatic analysis showed that the 3'-UTR of FOXJ3 shared miR-107 binding sites matched in ITGA5 circRNA (Figure 4A). Luciferase reporter assays verified that miR-107 mimics reduced the luciferase activity of the FOXJ3-WT vector, which contained the FOXJ3-WT 3'-UTR, but not the FOXJ3 vector, which contained the FOXJ3 mutant 3'-UTR (Figure 4B). As shown in Figure 4C–4E, FOXJ3 expression was down-regulated in CRC tissues compared with the non-tumor tissues and was negatively correlated with FOXJ3 expression and negatively associated with distant metastasis and tumor size (Table 3). Kaplan-Meier analysis showed that patients with CRC with lower FOXJ3 expression had poorer prognosis (Figure 4F). We further investigated whether miR-107 could influence FOXJ3 expression in CRC cells. As shown in Figure 4G and 4H, miR-107 inhibitors significantly promoted FOXJ3 expression in CRC cells compared with the inhibitor NC-transfected group. These results suggest that FOXJ3 is a direct target of miR-107 in CRC.

miR-107 promoted CRC progression by down-regulating FOXJ3 expression

FOXJ3 has previously been shown to inhibit the proliferation and migration of human tumors, including CRC [16–18]. Because FOXJ3 is a direct target of miR-107, miR-107 was chosen for this study for its potential effects on the proliferative, motile, and invasive capacity of CRC cells by regulating FOXJ3 expression. We further explored the biological functions of miR-107 and FOXJ3 in CRC cells. As shown in Figure 5A–5C,

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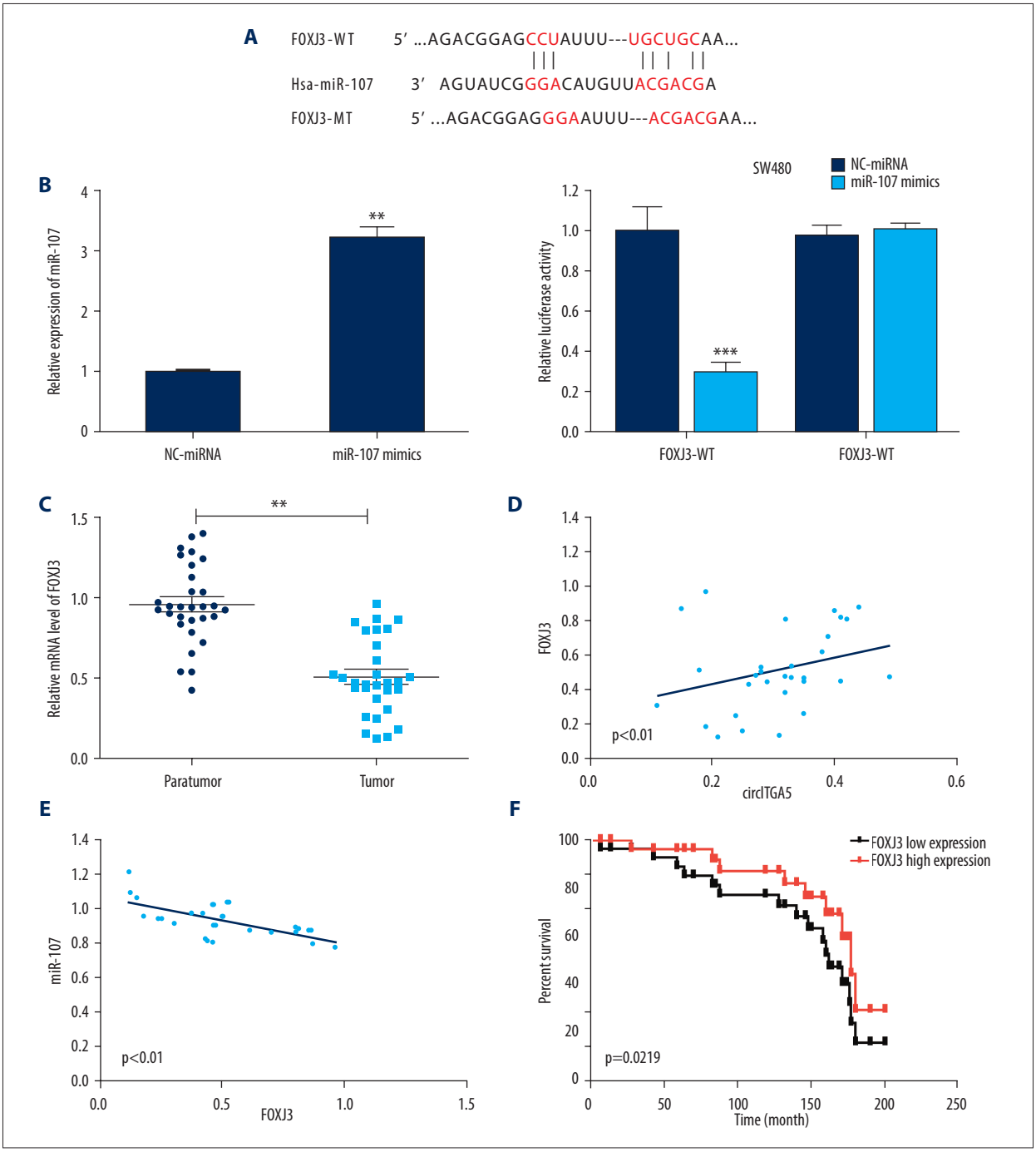
FOXJ3 overexpression significantly inhibited CRC cell proliferation and migration, while miR-107 mimics rescued the inhibitory effects of FOXJ3. Also, FOXJ3 overexpression induced CRC cell apoptosis, while the apoptotic rate of cells was reduced by miR-107 mimics (Figure 5D). Western blot showed that FOXJ3 overexpression inhibited the expression of Twist, MMP-2, MMP-9, and N-cadherin and stimulated the expression of E-cadherin, Bax, and caspase-3 in CRC cells. Transfection of miR-107 mimics into CRC cells that overexpressed FOXJ3 rescued the levels of Twist, MMP-2, MMP-9, and N-cadherin, but reduced the expression of E-cadherin, Bax, and cleaved caspase-3 (Figure 5E). These findings showed that miR-107 functioned as a potential oncogene by down-regulating FOXJ3.



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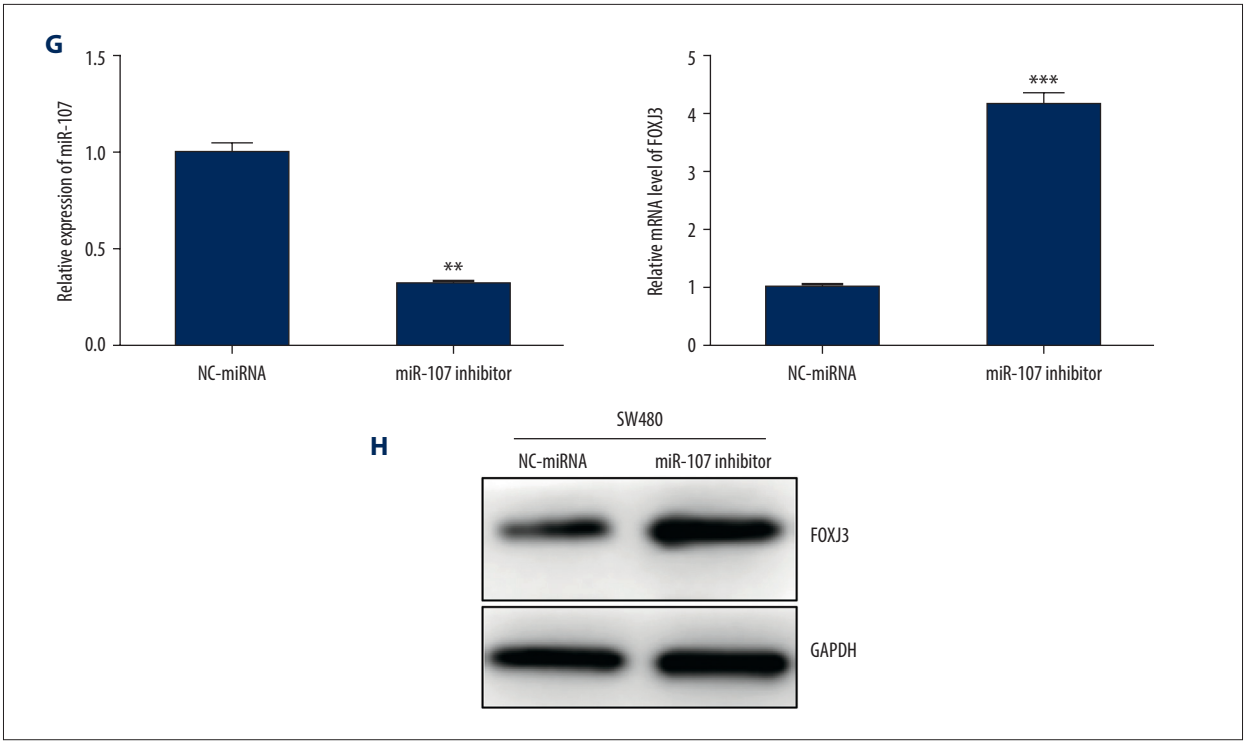

Figure 4. MicroRNA-107 (miR-107) directly targeted FOXJ3 in colorectal carcinoma (CRC) cells. (**A**) The putative binding sites of miR-107 in the 3' UTR of FOXJ3. (**B**) MiR-107 overexpression inhibited the activity of FOXJ3-WT but not that of the FOXJ3-MT reporter. (**C**) FOXJ3 expression was analyzed in 30 CRC tissues and adjacent non-tumor tissues. The correlation between ITGA5 circRNA and FOXJ3 (**D**) miR-107 and FOXJ3 (**E**) in 30 CRC tissues were determined by Pearson's correlation analysis. (**F**) The overall survival (OS) curves of patients with CRC and high or low FOXJ3 expression. Inhibitors of miR-107 promoted both FOXJ3 mRNA (**G**) and protein levels (**H**) in SW480 cells. ** $P<0.01$, *** $P<0.001$.

Table 3. Correlation between FOXJ3 expression and the clinicopathological features in 30 patients with colorectal carcinoma (CRC).

Characteristic		FOXJ3		P-value
		Low 18	High 12	
Age	≥60	10	7	0.334
	<60	8	5	
Gender	Male	9	6	0.415
	Female	9	6	
T-stage	T1–T2	6	7	0.0414
	T3–T4	12	5	
Distant metastasis	Yes	2	2	0.0113*
	No	16	10	
Tumor size	≥5 cm	13	4	0.0224*
	<5 cm	5	8	

* $P<0.05$.

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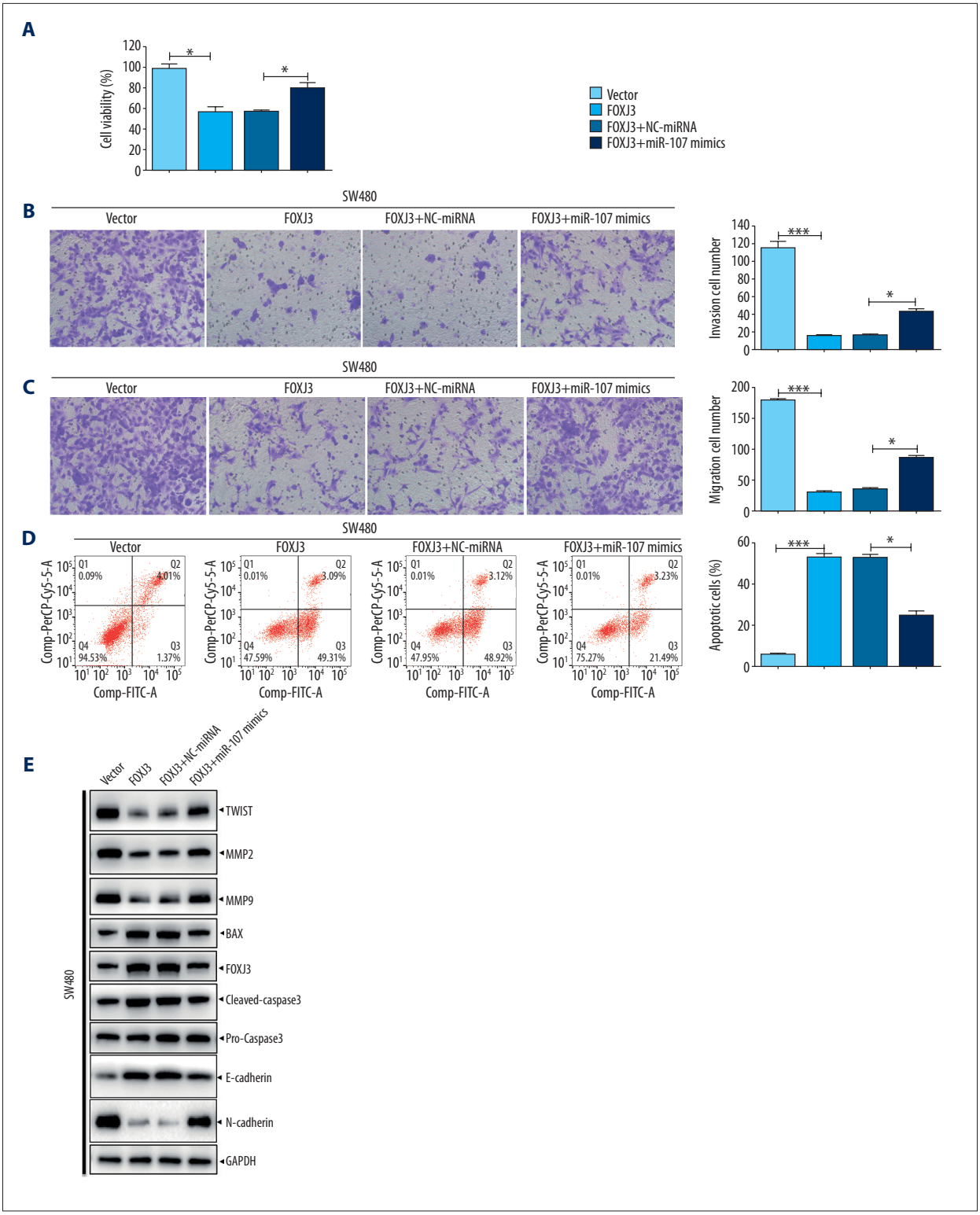

Figure 5. MicroRNA-107 (miR-107) promoted the progression of colorectal carcinoma (CRC) by down-regulating FOXJ3 expression. MiR-107 mimics reversed FOXJ3-induced inhibition of cell proliferation (**A**), cell invasion (**B**), and cell migration (**C**) of SW480 cells and reduced the apoptotic rate of SW480 cells induced by FOXJ3 (**D**). (**E**) Western blot analyzed the protein expression levels of Twist, MMP-2, MMP-9, Bax, E-cadherin, N-cadherin, and caspase-3 in cells transfected with the indicated vectors and/or miRNA mimics. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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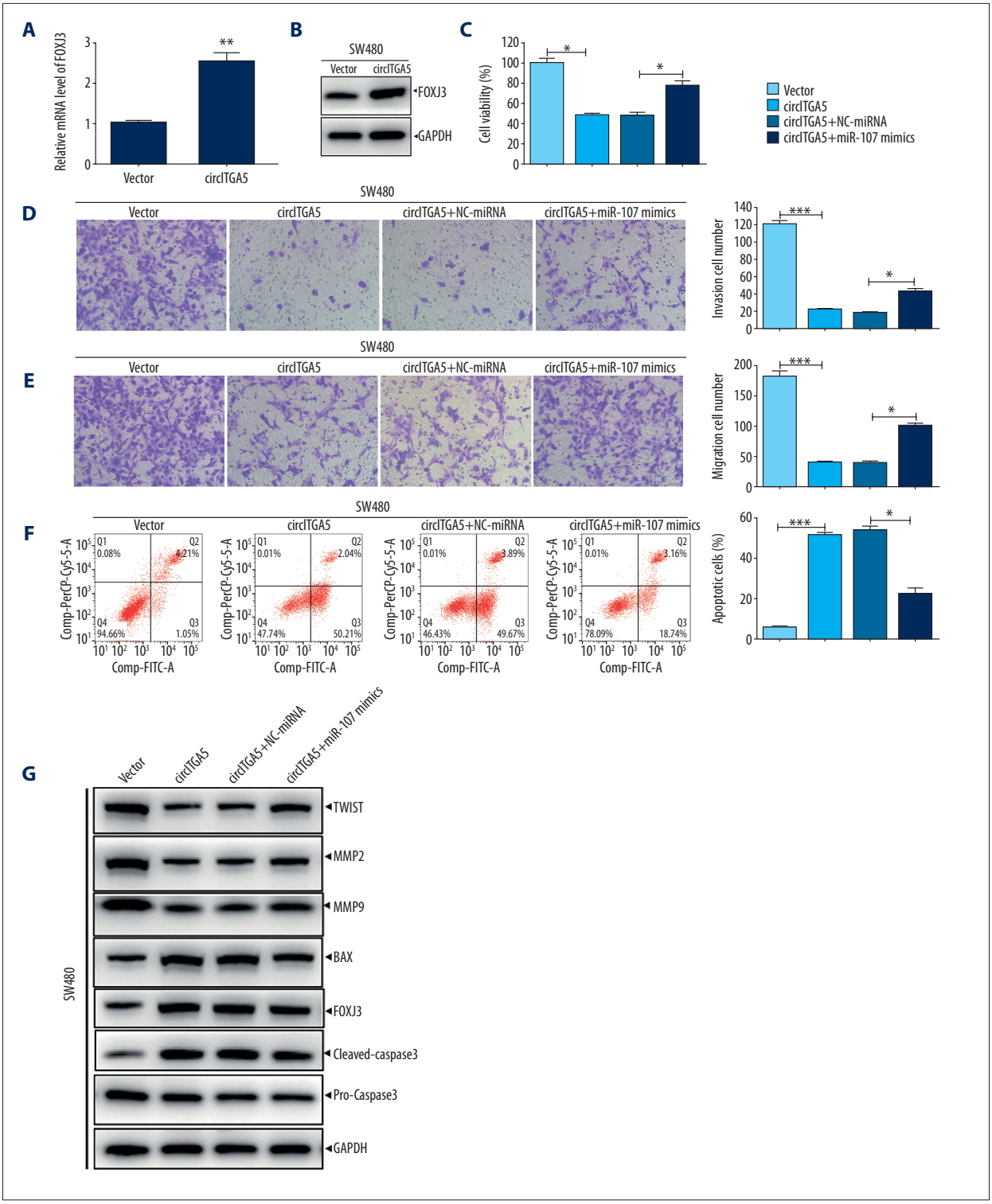

Figure 6. The integrin subunit alpha 5 (ITGA5) gene circular RNAs (circRNA)/microRNA-107 (miR-107)/FOXJ3 pathway inhibited the progression of colorectal carcinoma (CRC). (**A, B**) Overexpression of ITGA5 circRNA promoted FOXJ3 expression in SW480 cells. MiR-107 mimics reversed ITGA5 circRNA-induced inhibition of cell proliferation (**C**), invasion (**D**), and migration (**E**) of SW480 cells and reduced the apoptotic rate of SW480 cells induced by ITGA5 circRNA (**F**). (**G**) Western blot analyzed the protein expression levels of Twist, MMP-2, MMP-9, Bax, E-cadherin, N-cadherin, and caspase-3 in cells transfected with the indicated vectors and/or miRNA mimics. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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The ITGA5 circRNA/miR-107/FOXJ3 pathway inhibited CRC progression

To determine whether circITGA7 acted as a miR-107 sponge to regulate FOXJ3 expression in CRC cells, the effects of ITGA5 circRNA on the expression of FOXJ3 were studied. The results showed that ITGA5 circRNA overexpression increased both mRNA and protein levels of FOXJ3 (Figure 6A, 6B), indicating that ITGA5 circRNA protected CRC cells from FOXJ3 degradation by sponging miR-107.

The role of the ITGA5 circRNA/miR-107/FOXJ3 pathway in CRC progression was investigated. Cell proliferation and transwell assays showed that ITGA5 circRNA overexpression reduced the proliferation and metastasis of CRC cells. However, the inhibitory effects of ITGA5 circRNA were rescued by miR-107 mimics (Figure 6C–6E). The promotional effect of ITGA5 circRNA on CRC cell apoptosis was also reduced by miR-107 mimics (Figure 6F). Western blot analysis showed that transfecting miR-107 mimics into cells overexpressing ITGA5 circRNA reduced the inhibitory effect of ITGA5 circRNA on CRC progression (Figure 6G). These findings suggest that ITGA5 circRNA inhibited CRC progression by acting as a miR-107 sponge to prevent FOXJ3 degradation.

Discussion

Previous studies have shown that the noncoding portion of the genome, including microRNAs (miRNAs), circular RNAs (circRNAs), and long noncoding RNAs (lncRNAs) regulate gene expression in human disease [19–21]. Because of the cell, tissue, and developmental stage specificity of the expression of circRNAs, they are potential cancer biomarkers. However, currently, the biological function of most circRNAs remains unclear.

The findings from the present study showed that the integrin subunit alpha 5 (ITGA5) gene circRNA (hsa_circ_0026735), which is 2268 base-pair in length and located on chromosome 12, was significantly down-regulated in CRC tissues compared with the controls. The corresponding sense mRNA of ITGA5 is located on the human chromosome 12q13.13 and has been reported to be upregulated in human cancer, including CRC [13,22,23]. Inhibition of the expression of the ITGA5 gene may suppress CRC tumorigenesis. The findings from the present study showed that ITGA5 circRNA overexpression inhibited cell proliferation and migration and promoted apoptosis of CRC cells, which suggests that ITGA5 circRNA may have roles in the progression of CRC.

The competing endogenous RNA (ceRNA) hypothesis suggests that circRNAs show crosstalk with mRNAs, lncRNAs, or pseudogenes and regulate their co-expression by competing for shared miRNA target sites [24]. Recently emerging circRNAs are associated with CRC by regulating miRNA target genes [25–27]. Therefore, we hypothesized that ITGA5 circRNA might target miRNAs in CRC cells. Bioinformatics techniques and luciferase reporter assays confirmed that miR-107 directly bound to ITGA5 circRNA, and quantitative real-time polymerase chain reaction (qRT-PCR) showed that ITGA5 circRNA overexpression inhibited miR-107 expression. Also, the findings showed that the miR-107 level was significantly increased in CRC tissues and negatively correlated with the ITGA5 circRNA level. Consistent with these findings, it has previously been reported that miR-107 was significantly upregulated in CRC tissues and cell lines and was associated with poor prognosis in patients with CRC [14]. These findings support that ITGA5 circRNA acted as a ceRNA to sponge miR-107 and showed the importance of interactions between ITGA5 circRNA and miR-107 in CRC tumorigenesis.

FOXJ3, an important transcription factor controlling mitochondrial biogenesis, may regulate zinc finger protein expression related to the control of the cell cycle [28,29]. Despite the known functions of FOXJ3 in the regulation of skeletal muscle development and regeneration, its effects in CRC were unknown. This study showed that the 3'-UTR of FOXJ3 shared miR-107 binding sites that matched with ITGA5 circRNA using bioinformatics, and the results of luciferase reporter assays supported this finding. Also, miR-107 inhibitors reduced the FOXJ3 level in CRC cells.

Co-transfection of FOXJ3 overexpression plasmids and miR-107 mimics identified the role of miR-107 and FOXJ3 in CRC progression. Also, miR-107 overexpression reversed the inhibition of cell proliferation and migration, and promoted cell apoptosis induced by FOXJ3, which supported that FOXJ3 might mediate the role of ITGA5 circRNA and miR-107 in CRC. Therefore, we further investigated whether ITGA5 circRNA could promote FOXJ3 expression in CRC cells. The results showed that ITGA5 circRNA overexpression significantly increased FOXJ3 mRNA and protein levels, and the effect of ITGA5 circRNA overexpression on CRC cells could be reversed by co-transfection of miR-107 mimics. These results showed that ITGA5 circRNA inhibited CRC progression by serving as a miR-107 sponge to prevent FOXJ3 degradation. Also, high ITGA5 circRNA levels were correlated with poor prognosis in patients with CRC.

Conclusions

This study aimed to investigate the role of the circular RNS (circRNA) of the integrin subunit alpha 5 (ITGA5) gene and microRNA-107 (miR-107) in human colorectal carcinoma (CRC) cells *in vitro* and in tissue samples from patients with CRC and the expression of forkhead box J3 (FOXJ3) protein. The ITGA5

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circRNA, miR-107, and FOXJ3 pathway were involved in the progression of CRC *in vitro* and in tissue samples. ITGA5 circRNA may act as a sponge for miR-107 to upregulate FOXJ3 expression and act as a tumor suppressor in CRC cells. Further studies should be performed to investigate the role of ITGA5 circRNA as a potential diagnostic biomarker and possible therapeutic target in CRC.

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Conflict of interest

None.

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