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Knockdown of Tripartite Motif-Containing Protein 37 (TRIM37) Inhibits the Proliferation and Tumorigenesis in Colorectal Cancer Cells

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Tripartite motif-containing protein 37 (TRIM37), a new member of the RING-B-box-coiled-coil (RBCC) subfamily of zinc finger proteins, was found to be involved in the development and progression of several cancers. However, the expression pattern and biological functions of TRIM37 in colorectal cancer (CRC) remain unknown. Therefore, in the present study, we examined the expression pattern of TRIM37 in CRC and investigated the function of TRIM37 in the progression of CRC. Our results showed that TRIM37 expression was upregulated in CRC cell lines. Knockdown of TRIM37 inhibited CRC cell proliferation and tumor growth in vivo. Furthermore, knockdown of TRIM37 inhibited the migration and invasion in CRC cells. Last, knockdown of TRIM37 inhibited the protein level expression of β-catenin, cyclin D1, and c-Myc in CRC cells. In conclusion, these results demonstrate that TRIM37 may play an important role in the proliferation, invasion, and tumorigenesis of CRC cells. Thus, TRIM37 may be a potential therapeutic target for the treatment of CRC.

Key words: Tripartite motif-containing protein 37 (TRIM37); Colorectal cancer (CRC); Proliferation; Invasion

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the world (1). Despite the fact that various treatments for CRC have been improved recently, most patients are initially diagnosed at an advanced stage, and the 5-year survival rate of patients with distant metastasis is less than 10% (2–4). Therefore, evaluation of the expression of biological markers expressed in CRC cells might contribute to the investigation of novel therapeutic approaches and the identification of novel molecular targets for therapy.

Tripartite motif-containing protein 37 (TRIM37) is a new member of the RING-B-box-coiled-coil (RBCC) subfamily of zinc finger proteins. The *TRIM37* gene is located in the 17q23 chromosomal region and contains a peroxisomal RBCC protein (5). TRIM37 was found to possess E3 ubiquitin ligase activity and to have both peroxisomal and nuclear localization (6,7). In addition, reports have indicated that TRIM37 is overexpressed in several cancers and may be a potential therapeutic strategy for the treatment of different cancers (8–10). Jiang et al. reported that overexpression of TRIM37 promoted the growth and migration of pancreatic cancer cells, while knocking down the expression of TRIM37 suppressed the growth and migration of pancreatic cancer cells (11). However, the expression pattern and biological functions of TRIM37 in CRC remained unknown. Therefore, in the present study, we examined the expression pattern of TRIM37 in CRC and investigated the functions of TRIM37 in the progression of CRC.

MATERIALS AND METHODS

Cell Culture

Healthy human colon mucosa cell line (NCM460) and CRC cell lines (SW480, HT-29, and HCT116) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were maintained in DMEM (Gibco, Rockville, MD, USA), with supplements of 10% (v/v) fetal bovine serum (FBS; Gibco) and 100 U/ml streptomycin and penicillin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a humidified incubator containing 5% CO_2.

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Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from CRC cells using TRIzol reagent and reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using the 7500 Real-Time PCR System (Applied Biosystems, USA) with FastStart Universal SYBR Green Master (Roche, USA). The following primers were used: TRIM37, 5′-AACAGAGCG TGGAGAGCATT-3′ (sense) and 5′-CTTCTGCCCAA CGACAATTT-3′ (antisense); β-actin, 5′-CCGTGAAA AGATGACCCAGATC-3′ (sense) and 5′-CACAGCCT GGATGGCTACGT-3′ (antisense). Results were represented as relative mRNA expression data calculated according to the $2^{-\Delta\Delta Ct}$ method.

Western Blot

Total protein of CRC cells was prepared using RIPA lysis buffer (Beyotime, Nantong, P.R. China) according to the operating instructions. The protein concentration in the lysates was determined using a Bradford protein assay kit (Bio-Rad, Hercules, CA, USA). Protein (30 μg) was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidine difluoride membranes (Millipore, Bedford, MA, USA). Membranes were then blocked with 5% fat-free milk and incubated with primary antibodies (anti-TRIM37, anti-MMP-2, anti-MMP-9, anti-β-catenin, anti-cyclin D1, anti-c-Myc, and anti-GAPDH) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. After washing three times with TBST, the membranes were incubated in horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) for 1 h. The signals were determined using an enhanced chemiluminescence (Gibco).

Short Hairpin RNA and Cell Transfection

TRIM37 short hairpin RNA (shTRIM37) or control shRNA (scramble) was purchased from Shanghai Sangon Co. Ltd. (Shanghai, P.R. China). The shTRIM37 or scramble was transfected into SW480 cells using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. Results were checked by Western blot at 48 h after transfection.

Cell Proliferation Assay

Cell proliferation was evaluated by 3-(4.5-methylthiozol-2yl)-2.5-diphenyltetrazolium bromide (MTT) assay. Briefly, infected cells (1×10^4 cells/well) were seeded into 96-well plates and cultured for 24, 48, 72, and 96 h, respectively. Then 20 μl of 5 mg/ml MTT (Sigma-Aldrich) in DMEM was added, and cells were cultured for an additional 4 h. Formazan was solubilized with 100 μl of dimethyl sulfoxide (DMSO; Sigma-Aldrich) for 10 min. The absorbance [optical density (OD)] was measured with a microplate reader (Bio-Rad) at a wavelength of 570 nm.

Cell Migration and Invasion Assay

The migration assay was performed using modified Boyden chambers with filter inserts for 24-well dishes containing 8-μm pore size polycarbonate membrane (Millipore). Briefly, infected cells were added to the upper compartment of the chamber, and 600 μl of DMEM with 10% FBS was added into the lower compartment. After 24 h of incubation, the cells on the lower surface of the membrane were fixed in 95% ethanol and stained with 0.05% crystal violet in PBS for 15 min. The number of migrated cells was counted under a light microscope (Olympus, Tokyo, Japan).

For invasion assay, the same procedures described above were used, except that the filters were precoated with 100 ml of Matrigel at a 1:4 dilution in DMEM to form a genuine reconstituted basement membrane.

Tumorigenicity Assay

To establish the CRC xenograft model, infected CRC cells (1×10^6) were injected subcutaneously into the right hindlimb of 4-week-old nude mice ($n=5$ per group). Tumor growth was monitored every week and measured in two dimensions. The tumor volume was calculated using the following formula: tumor volume = width$^2\times$length$\times0.5$. After 4 weeks, the mice were sacrificed, and the tumors were dissected out and weighed. All animal studies were carried out with the approval of the ethics committee of The Second Affiliated Hospital of Xi'an Jiaotong University in accordance with the Guide for the Care and Use of Laboratory Animals.

Statistical Analysis

All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., USA). Data are presented as mean ± SD. Statistical analysis involved Student's *t*-test for the comparison of two groups or one-way ANOVA for multiple comparisons. A value of $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

TRIM37 Expression in CRC Cell Lines

First, we detected the endogenous expression of TRIM37 in CRC cell lines by qRT-PCR and Western blot. The expression levels of TRIM37 mRNA were significantly increased in CRC cell lines, compared with the NCM460 cells (Fig. 1A). Consistent protein levels were observed in the Western blot (Fig. 1B). HCT116 displayed a higher expression level of TRIM37. Thus, we

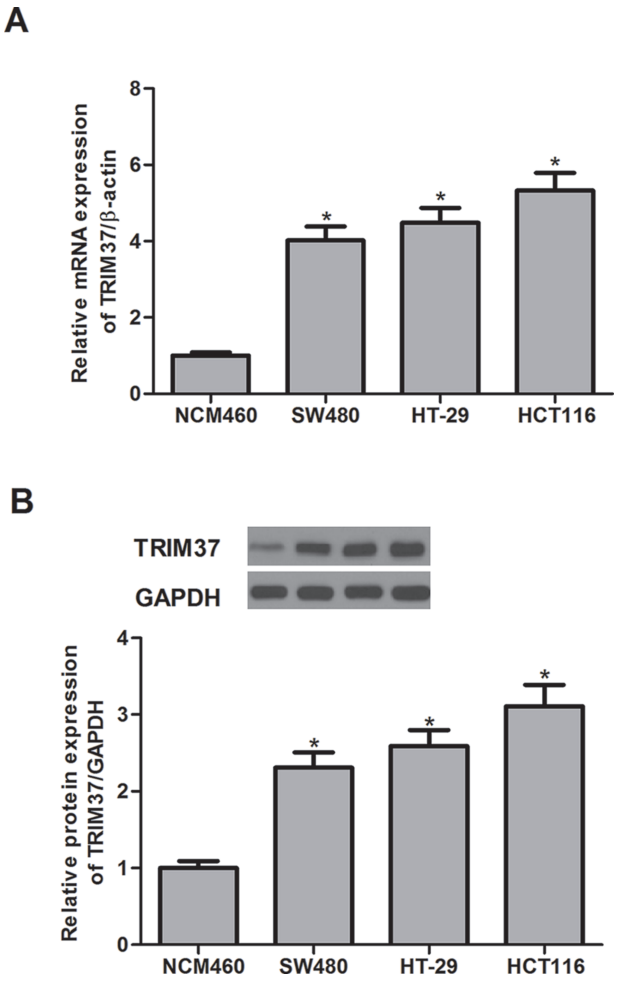

Figure 1. TRIM37 is highly expressed in CRC cell lines. (A) The mRNA expression levels of TRIM37 in CRC cell lines (SW480, HT-29, and HCT116) were analyzed by qRT-PCR. (B) Protein expression levels of TRIM37 were detected by Western blot analysis. Data are mean ± SD from three independent experiments. *$p < 0.05$ versus NCM460 group.

used HCT116 cells as a model to investigate the effect of TRIM37 on cell proliferation and invasion.

Knockdown of TRIM37 Inhibits CRC Cell Proliferation and Tumor Growth In Vivo

To examine the role of TRIM37 in the progression of CRC, stable shTRIM37-expressing HCT116 cells were prepared using loss-of-function study. Transfection efficiency was confirmed by qRT-PCR (Fig. 2A) and Western blot (Fig. 2B) in HCT116 cells. Furthermore, cell proliferation was measured using the MTT assay, and the results revealed that cell proliferation was significantly repressed in HCT116 cells after shTRIM37 transfection compared with scramble transfection (Fig. 2C).

To further investigate the role of TRIM37 in vivo, we performed a nude mouse xenograft assay by injecting HCT116 cells stably transfected with shTRIM37 or scramble into nude mice. Results showed that the size of subcutaneous tumors derived from TRIM37-silencing cells was dramatically reduced, compared with scramble-transfected cells (Fig. 3A). In addition, the average tumor weight of the scramble group was 2.46-fold higher than that of the shTRIM37 group after 28 days postinoculation (Fig. 3B).

Knockdown of TRIM37 Inhibits CRC Cell Migration/Invasion

We investigated the effect of TRIM37 on CRC cell migration using the Transwell assay. As shown in Figure 4A, the number of migrated HCT116 cells was significantly fewer in the shTRIM37-transfected group compared with that in the scramble group. Moreover, a Matrigel invasion assay was performed to validate the effect of TRIM37 on cell invasion. Knockdown of TRIM37 significantly inhibited the invasion of HCT116 cells compared with the control (Fig. 4B). Because tumor cell invasion on a Matrigel-coated surface is partially dependent on the secretion of MMP-2 and MMP-9, we examined the effect of TRIM37 on their protein levels. The results of the Western blot analysis indicated that knockdown of TRIM37 remarkably suppressed the expression of both MMP-2 and MMP-9 in HCT116 cells (Fig. 4C).

Knockdown of TRIM37 Inhibits the Activation of Wnt/β-Catenin Signaling Pathway in CRC Cells

To further explore the molecular mechanism responsible for the function of TRIM37 in CRC, we used Western blot to evaluate the expression levels of β-catenin, cyclin D1, and c-Myc in HCT116 cells transfected with shTRIM37 or scramble. As shown in Figure 5, compared with the scramble group, knockdown of TRIM37 significantly suppressed the protein expression levels of β-catenin, cyclin D1, and c-Myc in HCT116 cells.

DISCUSSION

The main findings of the present study can be summarized as follows: (1) TRIM37 expression is upregulated in CRC cell lines; (2) knockdown of TRIM37 inhibits CRC cell proliferation and tumor growth in vivo; (3) knockdown of TRIM37 inhibits the migration and invasion in CRC cells; (4) knockdown of TRIM37 inhibits the protein level expression of β-catenin, cyclin D1, and c-Myc in CRC cells.

Emerging studies have indicated that TRIM37 is overexpressed in several types of cancer and has been associated with tumor progression and development. Jiang et al. reported that TRIM37 expression was notably upregulated

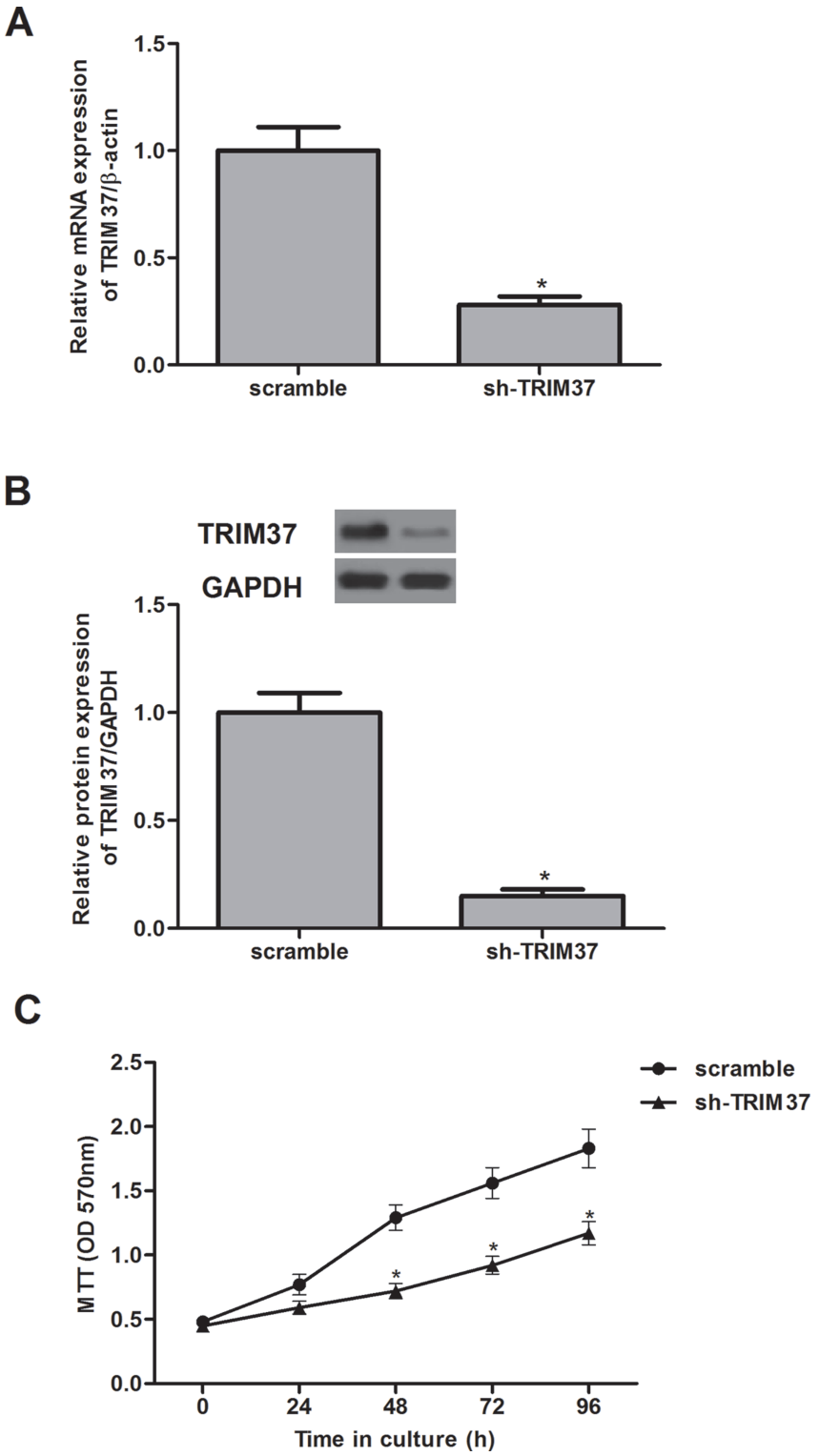

Figure 2. Knockdown of TRIM37 inhibits CRC cell proliferation. HCT116 cells were infected with shTRIM37 or scramble for 48 h. (A) qRT-PCR analysis to detect the mRNA expression levels of TRIM37. (B) Western blot analysis to detect the protein expression levels of TRIM37. Data are mean ± SD. (C) Cell proliferation was measured by the MTT assay. Data are mean ± SD from three independent experiments. *$p < 0.05$ versus scramble group.

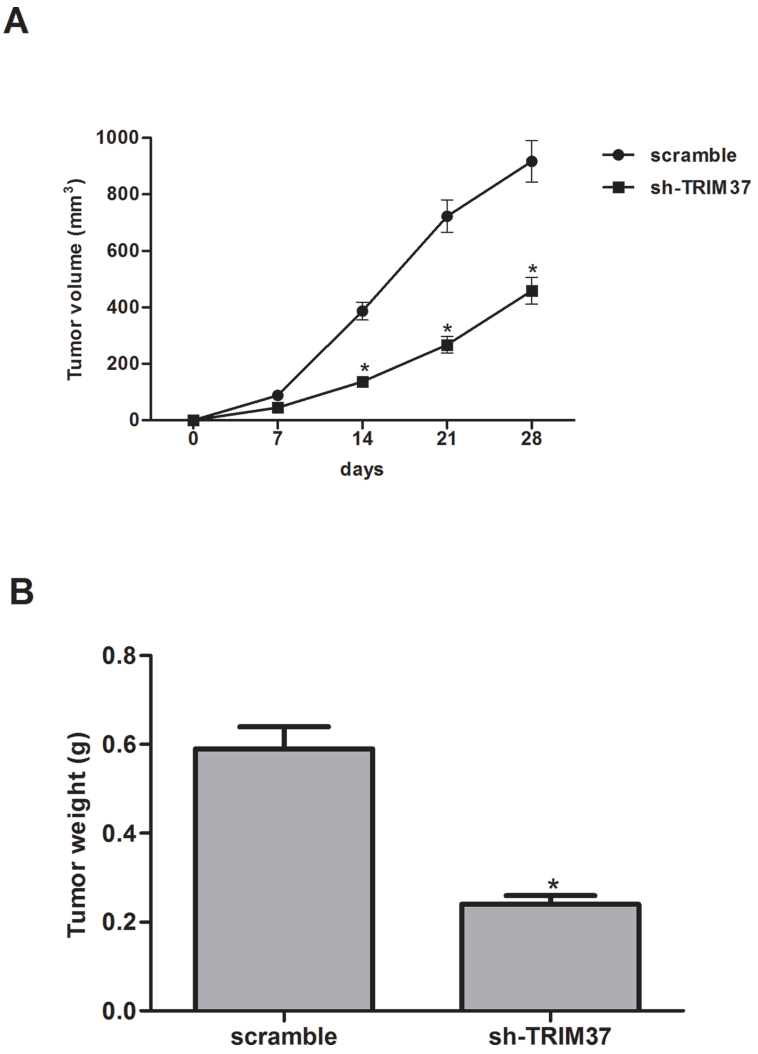

Figure 3. Knockdown of TRIM37 inhibits tumor growth in vivo. Infected HCT116 cells (1×10^6) were injected subcutaneously into the right hindlimb of 4-week-old nude mice. (A) The tumor volume was monitored every week and measured. (B) After 4 weeks, the mice were sacrificed, and the tumors were dissected out and weighed. Data are mean ± SD from three independent experiments. *$p<0.05$ versus scramble group.

in hepatocellular carcinoma samples and was associated with advanced stage and tumor volume (10). Another study demonstrated that TRIM37 is upregulated in human breast cancer cell lines, and ectopic expression of TRIM37 substantially renders tumor growth in mouse xenografts (9). Consistent with these findings, herein we found that TRIM37 was highly expressed in CRC cell lines, and knockdown of TRIM37 significantly inhibited CRC cell proliferation and tumor growth in vivo. Collectively, these results obtained from both in vivo and in vitro experiments strongly suggest that TRIM37 may act as an oncogene in the development and progression of CRC.

Cancer cell migration and invasion are critical steps in tumor progression and metastasis. In CRC, metastasis accounts for about 90% of patient deaths, representing the most lethal event during the course of the disease (12). MMPs play important roles in the matrix degradation required for tumor growth and invasion (13,14). It was reported that MMP-9 was overexpressed in CRC tissues compared with normal tissues, and MMP-9 was correlated with depth of invasion, TNM staging, and lymph node metastasis (15). In this study, we found that overexpression of TRIM37 significantly inhibited CRC cell migration and invasion and decreased the protein expression levels of MMP-2 and MMP-9 in CRC cells. Thus, these data suggest that TRIM37 may promote the invasion and metastasis of CRC cells partially through regulation of the expression of MMP-2 and MMP-9.

A growing body of evidence suggests that the Wnt/β-catenin signaling pathway plays a critical role in

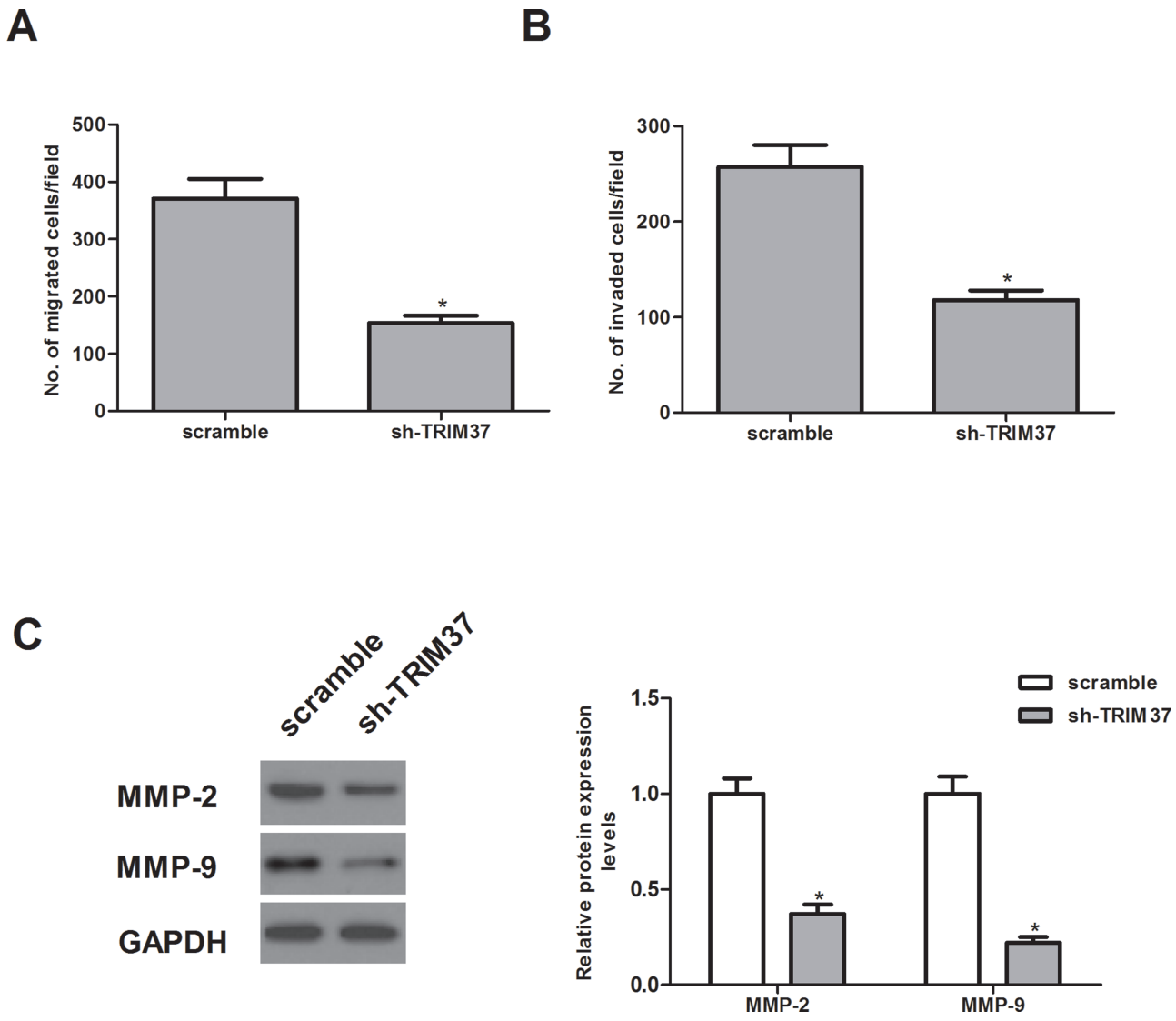

Figure 4. Knockdown of TRIM37 inhibits CRC cell migration/invasion. HCT116 cells were infected with shTRIM37 or scramble for 48 h. (A) Cell migration was measured by Transwell migration assay. (B) Cell invasion was assessed by the Matrigel invasion assay. (C) The protein expression levels of MMP-2 and MMP-9 were evaluated by Western blot. Data are mean ± SD from three independent experiments. *$p < 0.05$ versus scramble group.

promoting tumor progression (16–18). It is frequently activated in a number of cancer types, particularly CRC (19,20). Zhen et al. reported that it is overexpressed in eight samples of fresh CRC tissues compared with their respective adjacent nontumor colorectal mucosa tissues (21). β-Catenin is a main downstream effector of the canonical Wnt signaling pathway, and accumulation of β-catenin is a hallmark of Wnt signaling activation (22). In addition, abnormal β-catenin expression upregulated the expression of cyclin D1, c-Myc, and MMPs, leading to uncontrolled cell proliferation and invasion (23). Thus, inhibition of the Wnt/β-catenin signaling pathway may represent a promising approach to the treatment of CRC (24–26). It was reported that knockdown of β-catenin inhibits invasion and migration of CRC cells in vitro (27). In this study, we found that knockdown of TRIM37 markedly downregulated the expression of β-catenin, cyclin D1, and c-Myc in CRC cells. These results suggest that knockdown of TRIM37 inhibits proliferation and tumorigenesis by the inactivation of Wnt/β-catenin signaling in CRC cells.

In conclusion, these results demonstrate that TRIM37 may play an important role in the proliferation, invasion, and tumorigenesis in CRC cells. Thus, TRIM37 may be a potential therapeutic target for the treatment of CRC.

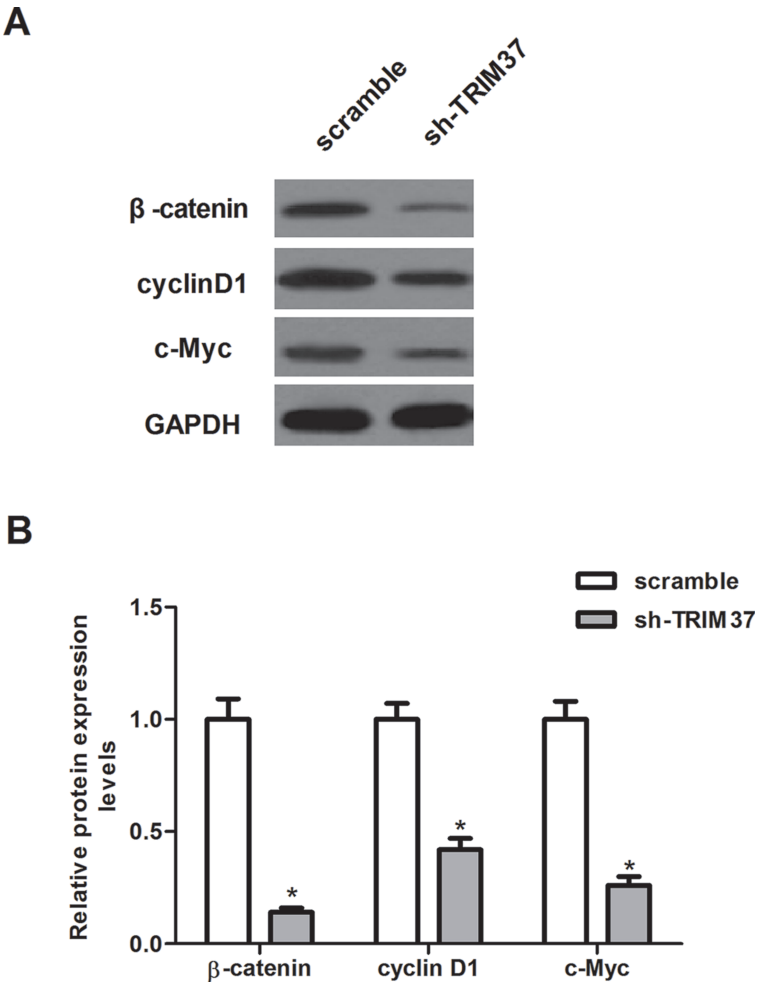

Figure 5. Knockdown of TRIM37 inhibits the activation of Wnt/β-catenin signaling pathway in CRC cells. HCT116 cells were infected with shTRIM37 or scramble for 48 h. (A) The protein expression levels of β-catenin, cyclin D1, and c-Myc were evaluated by Western blot. (B) The relative protein levels were quantified using the Image-Pro Plus 6.0 software. Data are mean ± SD from three independent experiments. *$p < 0.05$ versus scramble group.

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