Original Research Article

TRIM52 promotes proliferation, invasion, and migration of gastric cancer cells by regulating Wnt/β-catenin pathway

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Abstract

Purpose: This study aimed to reveal the role and mechanism of tripartite motif-containing 52 (TRIM52) in gastric cancer (GC) progression.
Methods: The Cancer Genome Atlas (TCGA) database was utilized to analyze TRIM52 expression in GC samples and para-carcinoma tissue samples, and the results were confirmed by quantitative real-time polymerase chain reaction. Cell counting kit-8 and colony formation assays were used to evaluate cell viability. Wound healing assay was utilized to analyze cell migration, while Transwell assay was utilized to evaluate cell invasion. TRIM52, proliferating cell nuclear antigen, matrix metalloproteinase-2, Wnt5a, β-catenin, and c-Myc protein levels were measured by western blot.
Results: TRIM52 was expressed more in GC tissue samples and cells compared to normal tissues and cells (p < 0.001). Overexpression of TRIM52 promoted growth, migration, and invasion of HGC-27 cells, and silencing inhibited growth, migration, and invasion of HGC-27 cells (p < 0.001). In addition, TRIM52 overexpression increased Wnt5a, β-catenin, and c-Myc protein expression, and silencing decreased Wnt5a, β-catenin, and c-Myc protein expression (p < 0.001 or p < 0.01), indicating that TRIM52 activates Wnt/β-catenin signaling pathway.
Conclusion: These findings reveal that TRIM52 facilitates GC cell proliferation, migration and invasion, but activates Wnt/β-catenin signaling.

Keywords: TRIM52, Gastric cancer, Migration, Invasion, Wnt/β-catenin signaling pathway

INTRODUCTION

Gastric cancer (GC) is an aggressive cancer of the digestive system that affects ~1 million patients worldwide [1]. GC is the second most common malignant tumor in China, with the second highest mortality rate of all cancers [2]. Although progress on various cancer treatments for GC have been made in recent years, the therapeutic effects are far from satisfactory [3]. Because the 5-year survival rate for GC is < 25% [4], it is vital to identify new target genes to ameliorate the treatment effects for GC patients.

The tripartite motif-containing (TRIM) protein family includes more than 80 members and has been associated with multiple illnesses [5]. Previous studies have shown that multiple TRIM

family members function in cellular activities, such as cell growth and cell invasion [6]. It has also been reported that TRIM family proteins can mediate cell viability and apoptosis [7]. TRIM52 is a new member of the TRIM protein family that can inhibit Japanese encephalitis virus (JEV) replication [8]. Additionally, TRIM52 associates strongly with various cancers, such as hepatitis B virus-associated hepatocellular carcinoma and colorectal cancer [9]. However, little is known about the role of TRIM52 in the genesis and development of GC.

The Wnt/β-catenin pathway is a well-known signaling pathway that associates strongly with various diseases [10,11]. Upon activation of the Wnt/β-catenin pathway, β/catenin is transferred from the cytoplasm to the nucleus where it binds to TCF/LEF transcription factors and activates transcription of oncogenes, including Cyclin D1 and c-Myc [12]. It has been reported that β/catenin and APC are involved in Wnt-induced GC [13]. In addition, several cancer antagonists have been shown to affect biological functions mediated by Wnt/β-catenin signaling [14]. Therefore, this study investigated the effect of TRIM52 on GC development and revealed the mechanism of TRIM52 regulation of the Wnt/β-catenin pathway.

EXPERIMENTAL

Bioinformatic analysis

Data on TRIM52 expression in 34 normal samples and 415 primary stomach adenocarcinoma (STAD) samples were obtained from The Cancer Genome Atlas (TCGA) website (http://gdac.broadinstitute.org/). The bioconductor package edgeR 3.3.2 was used to determine p values, and p values < 0.001 were regarded as significant.

Cell culture

HGC-27 cells were purchased from Shanghai Huzhen Biotechnology (China) and maintained in RPMI media containing 10 % fetal bovine serum (FBS, BC-SE-FBS01C, Senbeijia Biotechnology, China) at 37 $^{\circ}$C with 5 % CO_2.

Cell counting kit (CCK)-8 assay

HGC-27 cell viability was measured with the CCK-8 kit (HY-K0301, MCE, USA) according to the manufacturer's protocol. Briefly, HGC-27 cells transfected with specific vectors were cultured in a 96-well plate (1×10^3 cells/well). At the indicated time, 10 µL CCK-8 was added into every well and the plate was incubated for

another 2 h. Optical density (OD)$_{450}$ values were determined by microplate reader (EnVision, PerkinElmer, USA).

Colony formation assay

HGC-27 cells transfected with specific vectors were cultured in a 6-well plate (300 cells/well) for 14 d. Then, the medium was removed, and 1 % crystal violet reagent (HY-B0324A, MCE, USA) containing paraformaldehyde (158127, Sigma-Aldrich, USA) was added to each well for 30 min to stain the cells. Cell colonies were evaluated using ImageJ software (National Institutes of Health, USA). The areas of the colonies were calculated using the formula:

Area of colonies per plate (100 %) = (number of colonies/number of inoculated cells) × 100 %

Determination of cell invasion

Cell invasion was evaluated by transwell assay using Corning HTS Transwell 24 well permeability supports (CLS3396, Corning, USA). HGC-27 cells (3×10^4 cells) transfected with specific vectors in 150 µL serum-free medium were seeded in the top chamber. The bottom chamber was supplemented with 150 µL fresh medium containing 20 % serum (BC-SE-FBS01C, Senbeijia Biotechnology, China). The plate was incubated for 12 h, and then the medium was removed and the cells were stained with 1 % crystal violet reagent (HY-B0324A, MCE, USA) containing paraformaldehyde (158127, Sigma-Aldrich, USA) for 30 min. An inverted microscope (TP0001268, Olympus Corporation, Japan) was used to count and image cell invasion.

Wound healing assay

HGC-27 cells transfected with specific vectors were cultured in a 6-well plate (1×10^5 cells/well) overnight. A 200 µL sterile pipette tip was used to scratch lines in the wells. Then, old culture medium was removed and serum-free medium preheated to 37 $^{\circ}$C was added gently along the walls of the wells with a 1 mL pipette tip to wash off the exfoliated cells at the scratch. The plates were then incubated and wound widths were measured at the indicated times by microscopy (TP0001268, Olympus Corporation, Japan).

Cell transfection

pcDNA3.1 vectors with or without TRIM52 were purchased from FENGHUISHENGWU (China). The siRNA targeting TRIM52 (siTRIM52, 5'-CCATCTGCTTGGATTACTT-3') and the control

siRNA (siRNA-NC, 5'-CGGAGTAATGATCAGGG CATGTGCT-3') were purchased from BIORN (China). The vectors and siRNAs were transfected into HGC-27 cells using Lipofectamine 3000 Transfection Reagent (L3000075, Invitrogen, USA) according to the manufacturer's protocol.

Western blotting assay

HGC-27 cells transfected with specific vectors were harvested, resuspended in cell lysis buffer (R0278, Sigma-Aldrich, USA), and then a bicinchoninic acid (BCA) kit (PC0020, Solarbio, China) was used to measure protein concentration. The samples were diluted to 2 µg/µL with 5 × loading buffer, heated at 95 °C for 15 min, and then the proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were incubated in Pierce clear milk blocking buffer (37587, Thermo Scientific, USA) for 40 min. Then, the membranes were incubated with primary antibodies to TRIM52 (sc-135589, Santa Cruz Biotechnology, USA, 1:1000), proliferating cell nuclear antigen (PCNA) (sc-56, Santa Cruz Biotechnology, USA, 1:1000), matrix metalloproteinase-2 (MMP2) (10373-2-AP, Proteintech, USA, 1:1000), Wnt5a (ab72583, Abcam, UK, 1:1000), β-catenin (ab32572, Abcam, UK, 1:1000), c-Myc (ab32072, Abcam, UK, 1:1000), and GAPDH (60004-1-Ig, Proteintech, USA, 1:1000) at 4 °C overnight. Then, the membranes were washed with phosphate buffer saline (PBS, 70011069, Gibco, USA) and incubated with secondary antibody (7074, Cell Signaling Technology, USA, 1:2000 or 7076, Cell Signaling Technology, USA, 1:5000) for 2 h. To detect proteins, Super ECL detection reagent (36208ES60, YESEN, China) and gel imaging equipment (JY04S-3D, Beijing Junyi Oriental electrophoresis equipment, China) were used. GAPDH served as the control and relative expression of a specific protein (R) was determined as in Eq 1.

$$R = (D/Dg) \ldots\ldots\ldots (1)$$

where D and Dg are the density of the specific protein and the density of GAPDH, respectively.

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was extracted from GC and normal cells with Trizol kit (T9424, Sigma-Aldrich, USA) according to the manufacturer's protocol. The total RNA concentration was determined using the Nano Drop Lite Printer Accessory (714-071400, Thermo Scientific, USA). Next,

complementary DNA (cDNA) was synthesized from 1 µg of total RNA with TaqMan reverse transcription reagent (4304134, Invitrogen, USA) according to the manufacturer's instructions. The QuantStudio 7 Pro Real-Time PCR System (A43162, Applied Biosystems, USA) was used for the qRT-PCR analysis. The TRIM52 primers were 5'-GCCATCTGCTTGGATTACTTC-3' (forward) and 5'-TTCATCTTCCTCCTCGTTCTG-3' (reverse). The GAPDH primers were 5'-GAATGGGCAGCCGTTAGGAA-3' (forward) and 5'-AAAAGCATCACCCGGAGGAG-3' (reverse). GAPDH served as the control and TRIM52 mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were performed with SPSS 21.0 (USA) and GraphPad Prism 6.0 (USA) and data were presented as mean ± SD. The *t*-test was used to analyze differences, and *p* values < 0.05 were considered significant.

RESULTS

TRIM52 was highly expressed in GC tissue samples and GC cells

Analysis of samples in the TCGA database revealed that TRIM52 expression was dramatically higher in GC tissue samples than in normal tissue samples (Figure 1 A). Subsequently, TRIM52 mRNA expression was measured in GC cell lines (HGC-27, AGS, and NCI-N87) and normal epithelial cells of the human gastric mucosa (GES-1) by qRT-PCR. TRIM52 mRNA expression was higher in HGC-27, AGS, and NCI-N87 cells than in GES-1 cells, and TRIM52 mRNA expression was highest in HGC-27 cells (Figure 1 B). Western blotting also showed higher TRIM52 protein expression in the GC cell lines than in the normal cell line (Figures 1 C and D). These findings suggest that TRIM52 plays a role in GC development.

TRIM52 enhanced HGC-27 cell growth

To determine the effects of TRIM52 on GC cells, vectors that overexpress TRIM52 (pcDNA3.1/TRM52) or silence TRM52 (si-TRM52) were introduced into HGC-27 cells. As expected, the TRIM52 protein level increased upon transfection with pcDNA3.1/TRM52 and decreased upon transfection with si-TRIM52 (Figure 2 A). The CCK-8 assay showed that overexpression of TRIM52 facilitated HGC-27 cell proliferation, whereas knockdown of TRIM52 inhibited HGC-27 cell proliferation (Figure 2 B). Moreover, TRIM52-silenced cells exhibited

reduced colony formation, whereas TRIM52 overexpression exhibited increased colony formation (Figure 2 C and D). In addition, expression of PCNA, which is related to cell growth, was upregulated upon overexpression of TRIM52 and downregulated upon silencing of TRIM52 (Figure 2 E). These results showed that TRIM52 accelerates HGC-27 cell growth.
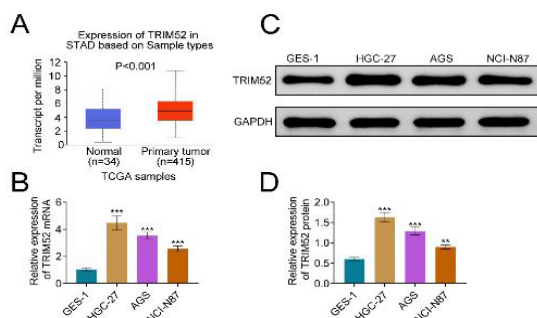

Figure 1: TRIM52 was expressed highly in GC tissue samples and GC cells. (A) Analysis of TRIM52 expression in samples from the TCGA database. (B) TRIM52 mRNA expression in GC cell lines and GES-1 cells. (C) TRIM52 protein expression in GC cells and GES-1 cells. (D) Quantitative analysis of TRIM52 protein expression in GC cell lines and GES-1 cells. ***P < 0.001, ***p < 0.001 vs GES-1
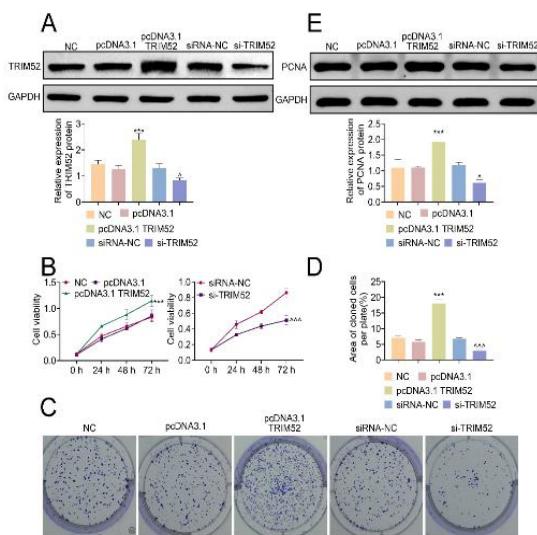

Figure 2: TRIM52 facilitated HGC-27 cell growth. (A) Western blot and quantitative analysis of TRIM52 protein expression. (B) HGC-27 cell viability represented by the CCK-8 assay and the OD$_{450}$. (C) The colony formation assay. (D) The area of the colonies. (E) Western blot and quantitative analysis of PCNA protein expression. ***P < 0.001, ^^^p < 0.001, ^p < 0.05. ***$p < 0.001$ vs pcDNA3.1. ^^^p < 0.001 and ^p < 0.001 vs siRNA-NC

TRIM52 promoted HGC-27 cell migration and invasion

To explore the effects of TRIM52 on HGC-27 cell migration and invasion, wound healing and transwell assays were conducted, respectively. For cells transfected with pcDNA/TRIM52, the wound width decreased after 24 h, whereas for cells transfected with si-TRIM52, the wound width increased (Figures 3 A and B) indicating that TRIM52 overexpression facilitated HGC-27 cell migration. In addition, overexpression of TRIM52 facilitated cell invasion, and knockdown of TRIM52 inhibited cell invasion (Figures 3 C and D). Expression of MMP2 was evaluated to confirm the effect of TRIM52 on HGC-27 cell migration and invasion. Overexpression of TRIM52 upregulated MMP2 expression level and knockdown of TRIM52 downregulated MMP2 expression (Figure 3 E). These findings show that TRIM52 expression accelerates HGC-27 cell migration and invasion.
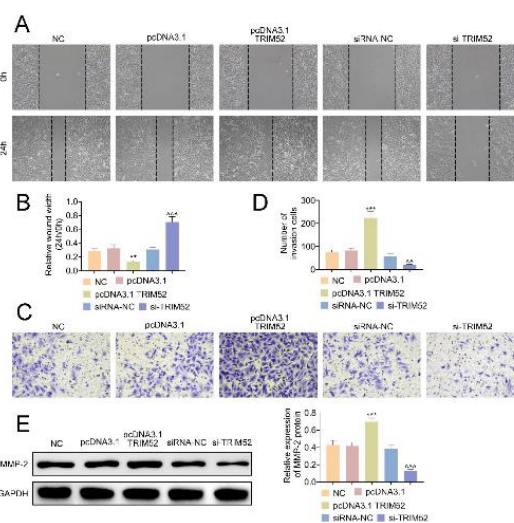

Figure 3: TRIM52 promoted migration and invasion of HGC-27 cells. (A) Images of the wound healing assay at 0 and 24 h. (B) Quantitative analysis of wound width, an indicator of cell migration, in the wound healing assay. (C) Images of the cell invasion assay. (D) Quantitative analysis of cell invasion. (E) Western blot and quantitative analysis of MMP2 protein expression with GAPDH as the control. ***p < 0.001, **p < 0.01, ^^p < 0.001, ^^p < 0.01. ***p < 0.001 and **p < 0.01 vs pcDNA3. ^^^p < 0.001 and ^^p < 0.01 vs siRNA-NC

TRIM52 activated the Wnt/β-catenin signaling pathway

Next, we explored whether TRIM52 regulates the Wnt/β-catenin signaling pathway in HGC-27 cells by measuring expression of key proteins in the Wnt/β-catenin pathway, Wnt5a, β-catenin, and c-Myc. TRIM52 overexpression upregulated

Wnt5a, β-catenin, and c-Myc protein expression, and knockdown of TRIM52 downregulated Wnt5a, β-catenin, and c-Myc protein expression (Figure 4). These results revealed that TRIM52 may exert its effects on HGC-27 cells by triggering the Wnt/β-catenin signaling pathway.
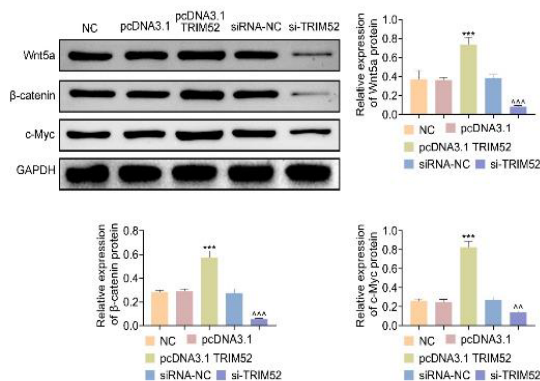

Figure 4: TRIM52 activated the Wnt/β-catenin signaling pathway. Western blot and quantitative analysis of protein expression of the Wnt/β-catenin signaling pathway proteins Wnt5a, β-catenin, and c-Myc with GAPDH as the control. **$P < 0.001$, ^^^$p < 0.001$, ^^$p < 0.01$. ***$p < 0.001$ vs pcDNA3.1. ^^^$p < 0.001$ and ^^$p < 0.01$ vs siRNA-NC

DISCUSSION

This study showed that TRIM52 is upregulated in GC tissues and cells. In addition, overexpression of TRIM52 promoted cell growth, migration, and invasion of GC cells, but knockdown of TRIM52 inhibited cell growth, migration, and invasion of GC cells. As for the molecular mechanism, TRIM52 overexpression increased expression of Wnt5a, β-catenin and c-Myc, and knockdown of TRIM52 decreased expression of Wnt5a, β-catenin and c-Myc. These data suggest that TRIM52 activates the Wnt/β-catenin signaling pathway in GC cells.

TRIM52 has been implicated in various biological processes. For example, TRIM52 accelerated fibrosis of LX-2 cells by regulating the Smad2/3 pathway [15]. Silencing of TRIM52 improved lipopolysaccharide-induced injury in human periodontal ligament cells via the TLR4/NF-κB axis [16]. TRIM52 suppressed JEV infection by reducing NS2A [8]. TRIM52 has also been shown to function as an oncogene in various malignancies [17,18]. In line with previous reports, this study revealed that TRIM52 was expressed highly in GC tissue samples and cells. In addition, overexpression of TRIM52 increased HGC-27 cell viability and colony formation, and silencing of TRIM52 inhibited HGC-27 cell viability and colony formation. These results

suggest that TRIM52 facilitates GC cell progression.

Increasing evidence has shown that TRIM52 promotes tumorigenesis by enhancing cancer cell migration and invasion [7]. Therefore, wound healing and transwell assays were conducted to determine the effect of TRIM52 on GC cell migration and invasion. TRIM52 overexpression visibly reduced the wound width and increased the number of invading cells, and silencing of TRIM52 increased the wound width and decreased the number of invading cells. This study confirmed that TRIM52 increased the migratory and invasive capabilities of GC cells. Moreover, MMP2, a well-known gelatinase, contributes to tumor metastasis by decreasing collagen and facilitating tumor proliferation [19]. This investigation revealed that overexpression of TRIM52 increased MMP2 protein expression, and silencing of TRIM52 reduced MMP2 protein expression in HGC-27 cells. These data suggest that TRIM52 may promote HGC-27 cell migration and invasion by modulating MMP2 expression.

Emerging evidence has shown that the Wnt/β-catenin pathway is involved in the development of GC [13]. Once activated, β-catenin in the cytoplasm is transferred into the nucleus to form a complex that activates expression of Wnts and their target proteins, such as c-Myc [12]. c-Myc is commonly regarded as an oncogene and aberrant expression of c-Myc correlates strongly with tumorigenesis in a variety of cancers, including GC [20]. Therefore, involvement of the Wnt/β-catenin pathway in the mechanism of TRIM52 in GC was investigated. Overexpression of TRIM52 increased Wnt5a, β-catenin, and c-Myc protein expression, and knockdown of TRIM52 inhibited Wnt5a, β-catenin, and c-Myc protein expression in HGC-27 cells. These results suggest that TRIM52 contributes to GC progression by activating Wnt/β-catenin signaling in HGC-27 cells.

Study limitations

There are several deficiencies in this study. Clinical samples should have been collected to determine whether TRIM52 affects GC development. In addition, more experiments are required to determine whether TRIM52 promotes GC cancer cell invasion and migration by increasing MMP2 expression. Furthermore, an inhibitor of the Wnt/β-catenin pathway should be utilized to confirm that TRIM52 exerts its effects on GC cells by regulating Wnt/β-catenin signaling.

CONCLUSION

This investigation has shown that TRIM52 overexpression may promote proliferation, migration, and invasion of GC cells by regulating Wnt/β-catenin signaling pathway.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Beiyi Zheng and Jianhua Wang designed and carried out the study. Haozhen Wang supervised the data collection and analyzed and interpreted the data. Wenling Wang and Yude Jin prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript for publication.

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