Original Research Article

Knockdown of TRIP13 inhibits gastric cancer stemness and cisplatin resistance by regulating FBXW7/ENO1 axis

Jiayun Liu[1], Che Chen[1], Guannan Wu[1], Xuequan Yao[1], Fukun Liu[1], Shenlin Liu[2]*

[1]Department of Digestive Tumor Surgery, [2]Department of Oncology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu 210029, China

*For correspondence: **Email:** liushenlin30207@126.com; **Tel:** +86-13951778559*

Abstract

Purpose: To explore the role of thyroid hormone receptor interactor 13 (TRIP13) in gastric cancer (GC) and the associated mechanism.

Methods: TRIP13 expression in GC was found in The Cancer Genome Atlas (TCGA) database and si-TRIP13 was transfected in GC cells in order to generate cell lines with low TRIP13 expression. Cell proliferation was determined by colony formation assay, while cell migration and invasion were evaluated by scratch test and Transwell assay, respectively. Sphere formation assay was used to assess cell stemness characteristics whereas half-inhibitory concentration (IC_{50}) value was evaluated by Cell Counting Kit-8 (CCK8). F-box and WD repeat domain containing 7 (FBXW7) and enolase 1 (ENO1) expression were determined by western-blot assay.

Results: TRIP13 was significantly expressed in GC based on TCGA database ($p < 0.001$) and high expression predicted poor prognosis in GC patients ($p < 0.05$). Based on the findings of the cell function assay, si-TRIP13 inhibited the proliferation, migration, invasion and stemness of GC cells ($p < 0.001$) and decreased the IC50 value. Mechanically, knockdown of TRIP13 decreased ENO1 expression level by stabilizing FBXW7.

Conclusion: TRIP13 functions as an oncogene in GC and stimulates growth and stemness via FBXW7/ENO1 pathway in GC. Thus, a potential therapeutic target for the treatment of gastric cancer is provided by this study.

Keywords: TRIP13, Gastric cancer, Cell proliferation, Cell migration, Stemness, Cisplatin resistance

INTRODUCTION

The fifth most prevalent type of cancer is gastric cancer (GC), and surgery is typically the first-line method used to treat it, followed by chemotherapy or radiation therapy [1]. Several patients, however, still experience recurrence and metastasis. In addition, when GC is discovered in most patients, the disease is already advanced., which leads to a high mortality rate of patients with gastric cancer. Therefore, finding potential therapeutic targets for GC is crucial for treating gastric cancer [2].

Researchers have discovered that malignancy metastasis, medication resistance, and recurrence are all influenced by cancer stem cells (CSCs).. Consequently, it is important to study tumor stemness [3]. Enolase 1 (ENO1) is an enolase isoenzyme that often functions as an oncogenic factor in a variety of tumors. ENO1 has been shown to support GC growth, metastasis and drug resistance. In addition, ENO1 is highly expressed in GC stem-like cells and can enhance the CSC-like characteristics of GC cells [4]. Therefore, finding targets associated with ENO1 is crucial for GC stemness research. An E3 ubiquitin ligase called F-box and WD repeat domain containing 7 (FBXW7) degrades a variety of substrates by ubiquitinating them. As a tumor suppressor, FBXW7 prevents proliferative growth [5] and stemness [6] in gastric cancer. It has been revealed that ENO1, a novel substrate of FBXW7, is negatively regulated by FBXW7 at the translational level [7]. It is speculated that FBXW7 may participate in the stemness characteristics of gastric cancer by regulating ENO1.

A tumor-promoting factor such as Thyroid Hormone Receptor Interaction Factor 13 (TRIP13) is involved in a variety of cancers. For example, TRIP13 promotes the proliferation, migration, invasion and stemness of cholangiocarcinoma cells [8]. Additionally, it has been demonstrated that TRIP13 promotes glioblastoma progression by directly interacting with the FBXW7 promoter region and repressing the transcription of the E3 ubiquitin ligase FBXW7 [9]. TRIP13 has cancer-promoting effects in other tumors, but its role and related mechanisms in gastric cancer remain unknown. Therefore, this study was aimed to explore the role of TRIP13 in gastric cancer.

EXPERIMENTAL

Bioinformatics collection

The Ualcan database (http://ualcan.path.uab.edu/), GEPIA database (https://cistrome.shinyapps.io/timer/) and Timer database (http://gepia.cancer-pku.cn/index.html) were used to investigate the expression of TRIP13 in gastric cancer. Using the Kaplan database, a correlation between TRIP13 expression and the prognosis of patients with stomach cancer was found.

Cell culture and transfection

AGS, HGC27, SNU-1 and MKN45 cell lines as well as stomach epithelial cells GES-1 were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. DMEM medium (Thermo Fisher, USA) containing 10 % fetal bovine serum, 50 U/ml penicillin and 50 µg/ml streptomycin was used to cultivate the cells. In compliance with the manufacturer's instructions, si-TRIP13 (Gene Pharma, China, Shanghai) was transfected into AGS and HGC27 cells using Lipofectamine 2000 (Thermo Fisher) when cell confluence reached approximately 80 %. After transfection, further tests were conducted 48 h later.

The targeting sequences of si-TRIP13#1 and si-TRIP13#2 were 5'-GCUGGUAACCAAGAUGUU UTT-3' and 5'-GGAUGCAUAAUGCCAGCAATT-3'. The targeting sequences of si-FBXW7 was 5'ACAGGACAGUGUUUACAAADTDT3'.

CCK8 assay

Using 96-well plates, the cells were seeded at a density of 2×10^3 cells/well and incubated for 24 h, 48 h and 72 h and then 10 µL of CCK8 solution was added and the mixture was incubated for another 2 h at 37 °C before the absorbance at 450 nm was determined.

Cloning formation assay

In 6-well plates, 1×10^3 cells were seeded and the cells were cultivated for two weeks before being fixed with 4 % paraformaldehyde and stained with crystal violet. The staining solution was then gently rinsed with running water, dried and the clone numbers were counted under a microscope.

Wound healing assay

After the cells were inoculated into 6-well plates, a straight line was made on the plate's surface using a pipette tip when the cell confluence got to about 80 %. The broken cells were removed with PBS, serum-free medium was used to cultivate the cells and then they were incubated at 37 ℃ with 5 % CO_2 and photographs were taken at 0 h and 24 h respectively.

Transwell assay

Cells (2×10^5) were inoculated in the upper compartment containing Matrigel and serum-free medium. In the lower chamber, medium containing 20 % FBS was added. Once the cells were incubated for 24 hours, the cells were fixed with 4 % paraformaldehyde and dyed with crystal

violet and the invaded cells were measured under a microscope.

Sphere-formation assay

Serum-free DMEM/F12 medium containing 1 % B27, 20 ng/mL epidermal growth factor (EGF) and 20 ng/mL basic fibroblast growth factor (bFGF) was used to cultivate the cells and the number of spheroids was counted after 7 days' incubation.

Western blot

The protein concentration was determined using the BCA kit after the cells were lysed using RIPA lysate.. The samples were denatured in a water bath after adding the loading buffer and following centrifugation, the supernatant was collected.. The proteins were separated by 10 % SDS-PAGE gel electrophoresis and the bands were transferred to PVDF membrane. The PVDF membrane was blocked with 5 % skimmed milk for 2 h, washed and incubated with TRIP13 (1:1000, Amyjet , ABP57021), FBXW7 (1:1000, Abcam, ab192328), ENO1 (1:5000, Abcam, ab193111), Sox2 (1:1000, Abcam, ab92494), Oct4 (1:5000, GeneTex, GTX101497) and β-actin (1:1000, GeneTex, GTX109639) antibodies overnight and the PVDF membrane was incubated with a secondary antibody (1:10000, Abcam, ab205718) the next day. The ECL luminescence kit was used to display the proteins.

Statistical analysis

SPSS 21.0 was used for data analysis and the data expressed as mean ± standard deviation (SD). Student's t-test was applied to compare the differences between two groups while one-way ANOVA was used for multiple comparisons between groups. $P < 0.05$ was regarded as statistically significant. significant.

RESULTS

TRIP13 is linked to a bad prognosis and is substantially expressed in GC.

The TCGA database is dedicated to collecting information about cancer patients. The gene expression data in the Ualcan Timer and GEPIA databases originated from TCGA, so the expression of genes in the tumors in these three databases can be examined. First, the expression of TRIP13 in GC was checked in different online websites including Ualcan (Figure 1 a), GEPIA (Figure 1 b) and Timer (Figure 1 c), and all of the above databases were found to show significantly increased expression of TRIP13 in GC. Next, by querying Kaplan database, it was discovered that the survival rate of patients with elevated TRIP13 expression was greatly reduced (Figure 1 d). In addition, TRIP13 protein expression level in gastric epithelial cells (GES-1) and GC cell lines, when measured, was discovered to be significantly increased in GC cell lines (Figure 1 e). Thus, TRIP13 was substantially expressed in GC and patients with GC have a bad prognosis due to the elevated expression of TRIP13.

Knockdown of TRIP13 inhibits proliferation of GC cells

To investigate how TRIP13 functions in GC cells, the interfering sequence of TRIP13 (si-TRIP13) was first transfected into AGS and HGC27 cells and the expression of TRIP13 protein after transfection was determined by western-blot. The results revealed that both si-TRIP13#1 and si-TRIP13#2 considerably lowered the expression level of TRIP13 (Figure 2 a), which indicate successful transfection. Next, to assess cell viability, the CCK8 test was employed. And it was found that si-TRIP13 significantly reduced cell viability (Figure 2 b). Clone formation assay results demonstrated that si-TRIP13 significantly reduced the number of crystalline violet (Figure 2 c). These findings implied that that reduced TRIP13 expression slowed the growth of GC cells.

Knockdown of TRIP13 prevents GC cells from migrating and invading.

In order to explore the role of si-TRIP13 in GC cell migration and invasion, the cell migration level was determined by wound healing assay and it was discovered that compared with in the si-control group, the scratch width at 24 h in the si-TRIP13 group became wider (Figure 3 a) and the ratio of the scratch width at 24 h to the initial scratch width became larger (Figure 3 b), indicating that the knockdown of TRIP13 inhibits cell migration. Furthermore, the number of crystal violets in the si-TRIP13 group was lower than in the si-control group. (Figure 3 c) and the number of invasive cells in the si-TRIP13 group was significantly reduced (Figure 3 d), indicating that the knockdown of TRIP13 inhibited cell migration.

Knockdown of TRIP13 inhibits stemness of GC cells

As is well known, tumor cells can acquire properties of stem cells that contribute to tumor growth and metastasis.
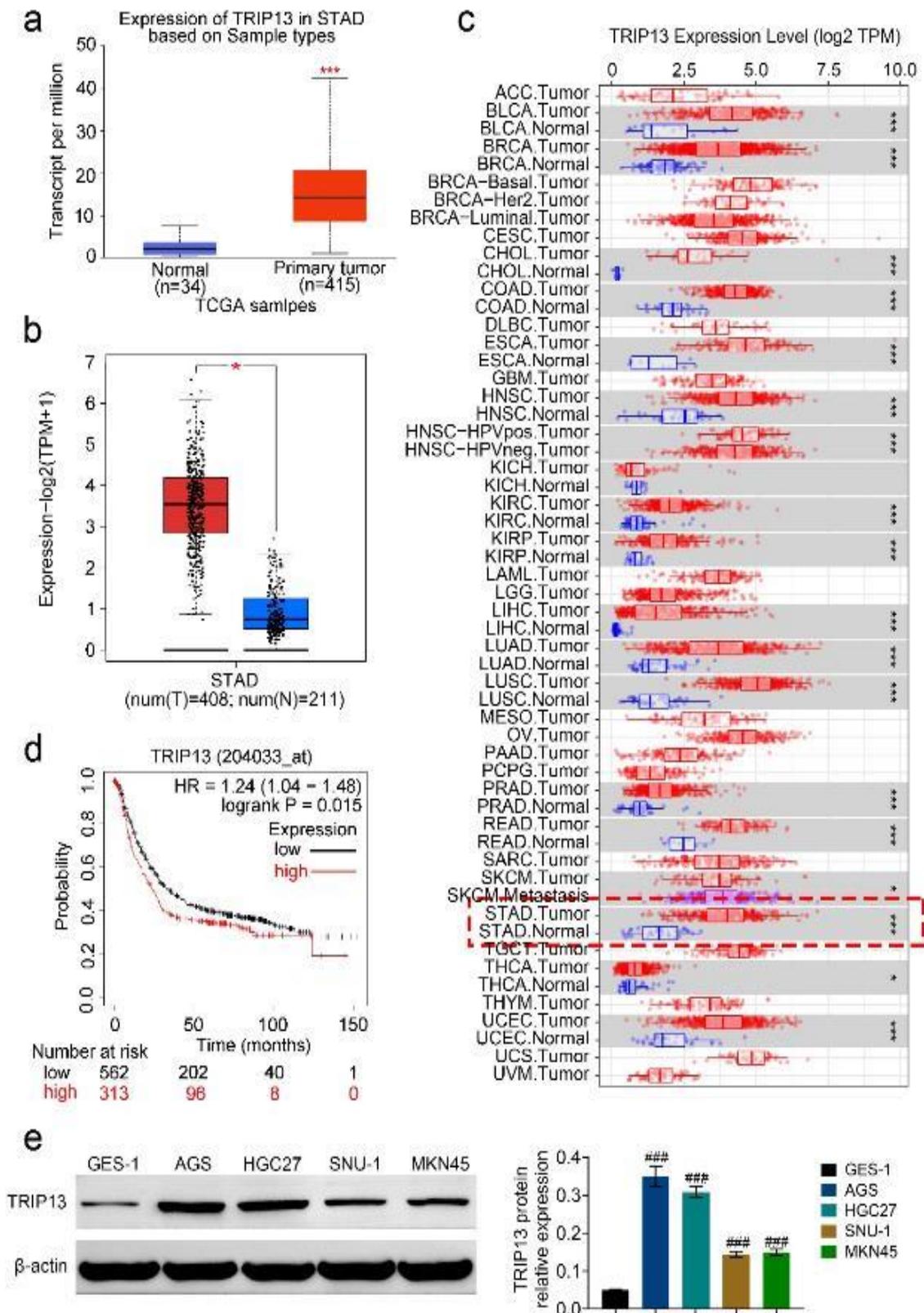
Figure 1: TRIP13 is highly expressed in GC and is associated with poor prognosis. (a) Expression of TRIP13 in gastric cancer tissues from the Ualcan database; (b) Expression of TRIP13 in gastric cancer tissues from the GEPIA database; (c) Expression of TRIP13 in gastric cancer tissues from the Timer database; (d) Relationship between TRIP13 expression and survival of gastric cancer patients; (e) TRIP13 protein expression in gastric epithelial cells and gastric cancer cell lines. $^{###}P < 0.001$ vs. GES-1
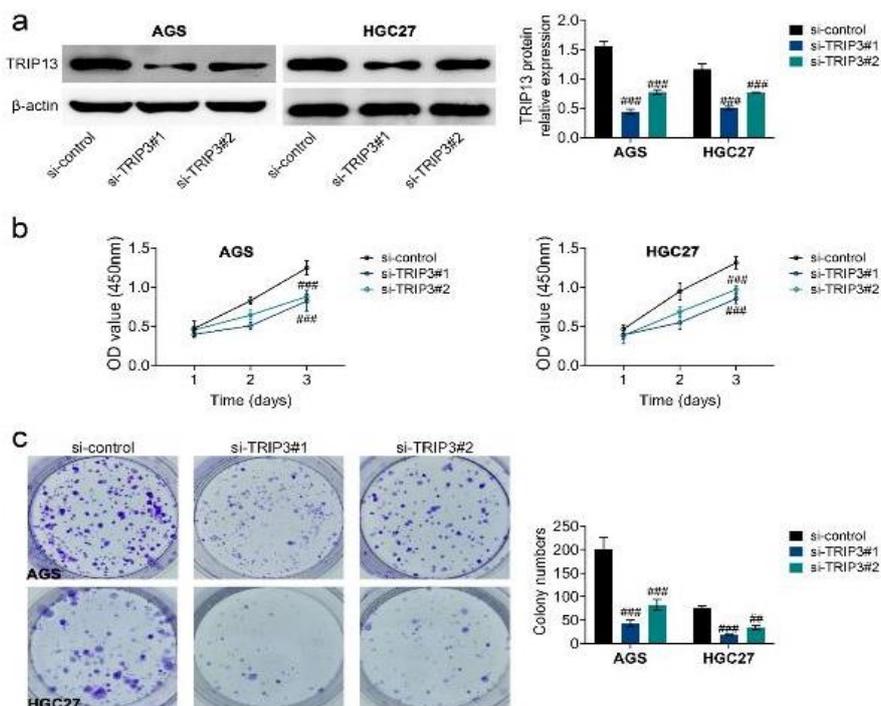
Figure 2: Knockdown of TRIP13 inhibits proliferation of GC cells. (a) Relative quantification of TRIP13 after knockdown of TRIP13 in AGS and HGC27 cells; (b) Cell viability at 24, 48 and 72 h; (c) Cell proliferation. $^{\#\#}p < 0.01$, $^{\#\#\#}P < 0.001$ vs. si-control; n = 3
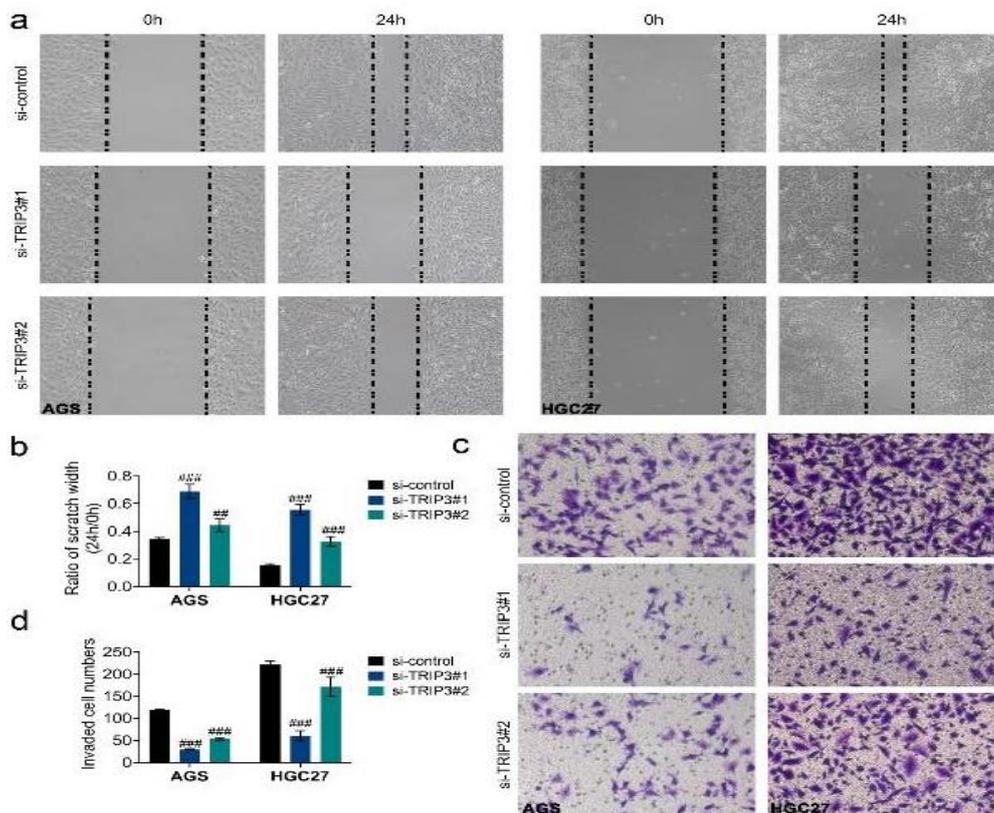

Figure 3: Knockdown of TRIP13 inhibits migration and invasion of GC cells. (a) After transfection of si-TRIP13 in AGS and HGC27 cells, cell scratch width at 0 and 24 h; (b) Ratio of scratch width of cells at 24 and 0; (c) Cell invasion; (d) Cell invasion numbers. $^{\#\#}P < 0.01$, $^{\#\#\#}p < 0.001$ vs. si-control; n = 3

Spheroid formation assay is an important method used to assess tumor stemness, and *Sox2* and *Oct4* are representative stemness transcription factors. Therefore, the effect of si-TRIP13 on the stemness of GC cells was explored. The findings confirmed that the sphere formation of GC cells was significantly reduced after transfection with si-TRIP13 (Figure 4 a), and the stemness-related markers including *Sox2* and *Oct4* in the si-TRIP13 group had considerably lower levels of protein expression (Figure 4 b), which indicates that si-TRIP13 inhibits the stemness characteristics of GC cells.

Knockdown of TRIP13 enhances the sensitivity of tumor cells to cisplatin

To investigate the impact of TRIP13 on medication resistance in gastric cancer, AGS and HGC27 cisplatin-resistant cells were transfected with si-TRIP13 to reduce the endogenous expression of TRIP13. CCK8 experiments showed that the knockdown of TRIP13 reduced the IC_{50} values of AGS and HGC27 cisplatin-resistant cells (Figure 5), indicating that the gastric cancer cells were more sensitive to cisplatin after TRIP13 knockdown.
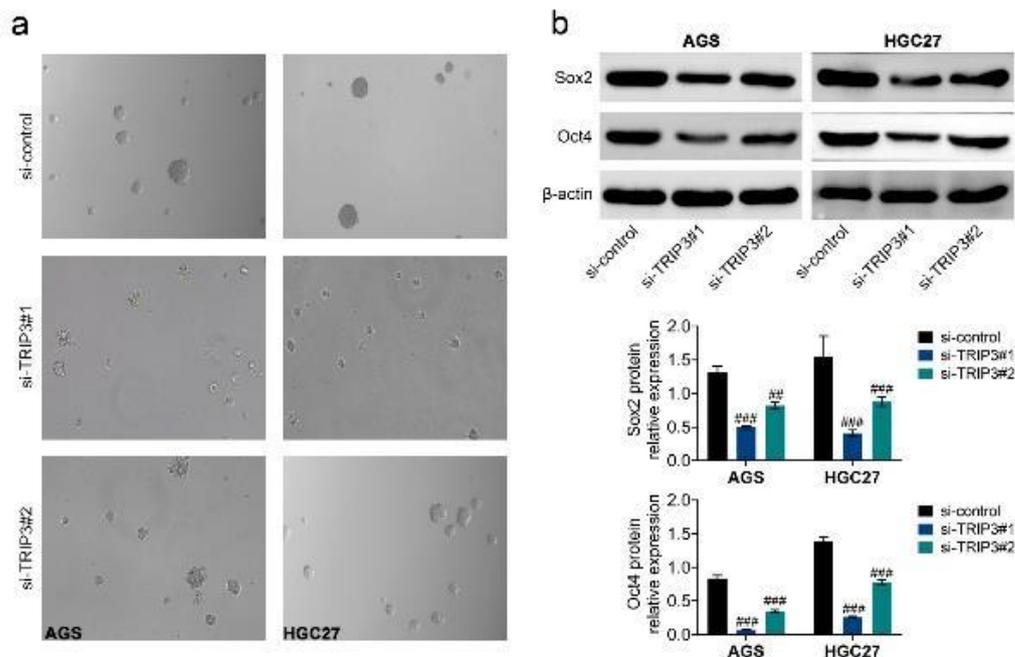

Figure 4: Knockdown of TRIP13 inhibits stemness of GC cells. (a) Cell stemness in AGS and HGC27 cells; (b) Stemness markers (Sox2 and Oct4) after transfection of si-TRIP13 in AGS and HGC27 cells. $^{##}P < 0.01$, $^{###}p < 0.001$ vs. si-control; n = 3
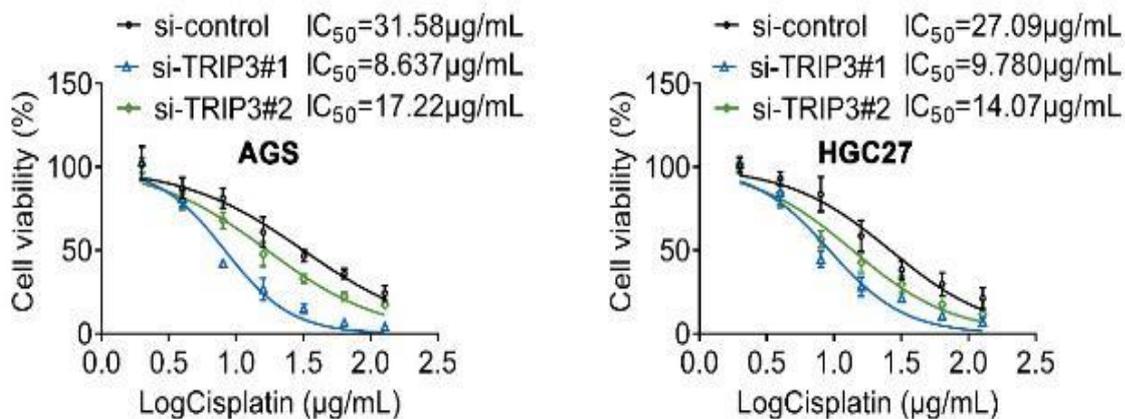

Figure 5: Knockdown of TRIP13 enhances the sensitivity of tumor cells to cisplatin. Transfection of si-TRIP13 reduces IC_{50} value of cisplatin-resistant cells
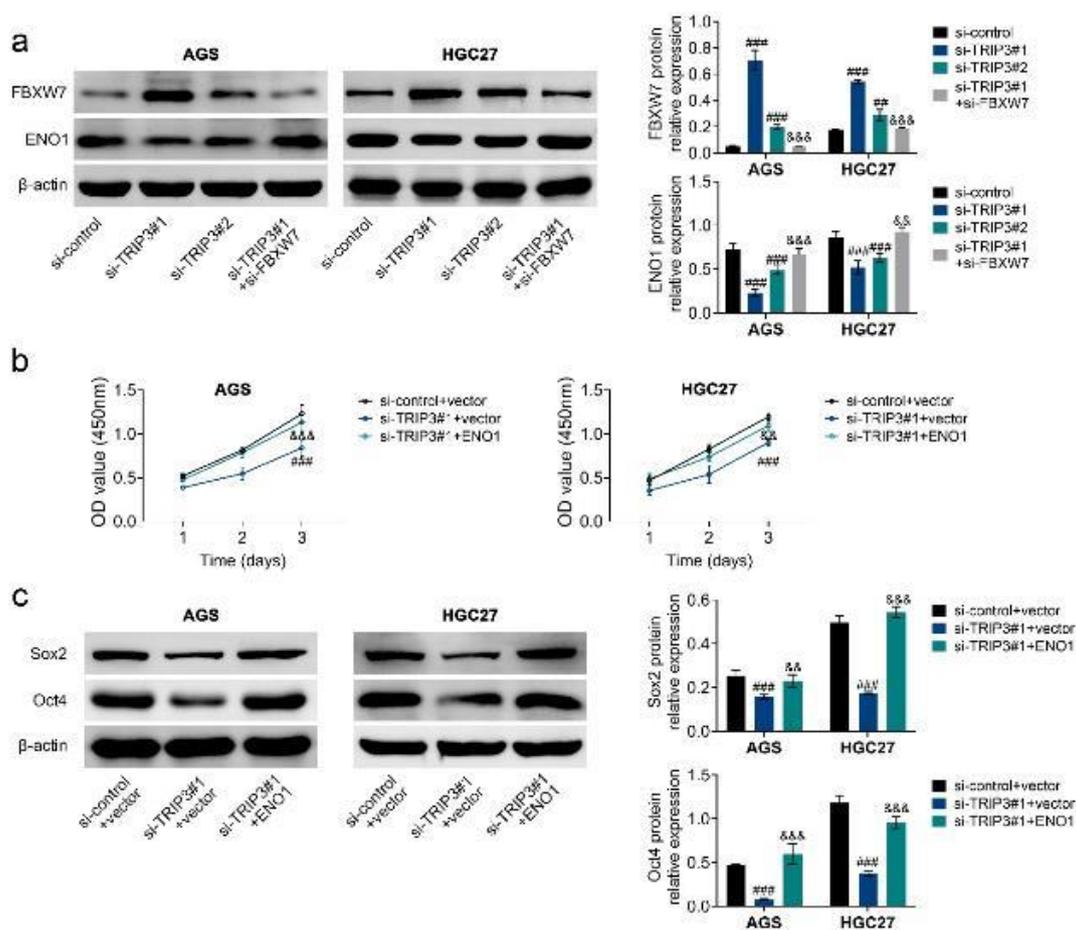
Figure 6: Knockdown of TRIP13 inhibits gastric cancer cell growth and stemness by regulating ENO1 expression. .(a) Protein expression of FBXW7 and ENO1 after transfection. $^{\#\#}P < 0.01$, $^{\#\#\#}p < 0.001$ vs. si-control; $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. si-TRIP13#1; (b) Inhibitory effect of si-TRIP13 on cell viability was reversed after transfection with si-TRIP13+ENO1; (c) Inhibitory effect of si-TRIP13 on stemness markers (Sox2 and Oct4) was reversed by transfection of si-TRIP13+ENO1. $^{\#\#\#}P < 0.001$ vs. si-control + vector; $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs. si-TRIP13#1+vector; n = 3

Knockdown of TRIP13 inhibits gastric cancer cell growth and stemness by regulating ENO1 expression

To investigate the mechanism of TRIP13 in GC cells, the expressions of FBXW7 and ENO1 were determined by western blotting and it was found that si-TRIP13 could increase the expression of FBXW7 and decrease the expression of ENO1. However, the inhibitory effect of si-TRIP13 on ENO1 was reversed by the concurrent transfection of si-FBXW7 and si-TRIP13 (Figure 6 a), which indicates that si-TRIP13 inhibits ENO1 expression through FBXW7. To investigate whether the effect of si-TRIP13 on GC cell growth and stemness depends on ENO1, rescue experiments were conducted and it was discovered that the inhibitory effect of si-TRIP13 on GC cell viability (Figure 6 b) and stemness (Figure 6 c) was reversed by ENO1, suggesting that si-TRIP13 promotes GC cell growth and stemness by regulating ENO1.

DISCUSSION

TRIP13 has been found to perform an oncogenic function in a variety of cancers, according to earlier research. TRIP13 promotes lung adenocarcinoma cell growth and migration by activating AKT/mTORC1/c-myc pathway [10]. The growth of ovarian cancer cells is slowed down by silencing TRIP13, which also reduces cell invasion and migration and induces apoptosis [11]. TRIP13 inhibitors inhibits cell proliferation, stops the cell cycle and enhances apoptosis in colorectal cancer cells [12]. In this study, it was confirmed that TRIP13 was significantly expressed in gastric cancer and was crucial to the malignant progression of gastric cancer. The knockdown of TRPI13 significantly inhibits the proliferation, migration, invasion, stemness and cisplatin resistance of gastric cancer cells.

FBWX7 is a substrate recognition component of SCF (SKP1, CUL1 and F-box protein complex) type ubiquitin ligases. FBXW7 acts as a tumor suppressor that degrades some genes that play a pro-oncogenic role in tumors. For example, FBXW7 enhances bladder cancer growth and stemness through the ubiquitinated degradation of ZMYND8 [13]. FBXW7 degrades GFI1 and consequently inhibits the proliferation of gastric cancer cells [14]. Prior investigations have shown that FBXW7 encourages the ubiquitination and degradation of ENO1 [15], which is an oncogene that promotes malignant tumor progression. The knockdown of ENO1 inhibits breast cancer cell growth and migration [16] and the downregulation of ENO1 inhibits lung cancer stem cell growth, migration and self-renewal [17]. Previous studies have shown that ENO1 specifically localizes to the invasive surface of an important subpopulation of gastric cancer stem cells [18]. The findings of this investigation shown that si-TRIP13 could both upregulate FBXW7 expression and downregulate ENO1 expression. Rescue experiments confirmed that si-TRIP3 was able to decrease ENO1 expression through the regulation of FBXW7 expression, thereby inhibiting gastric cancer cell growth, migration, invasion and stemness characteristics.

CONCLUSION

This study presents the first evidence that TRIP13 functions as a pro-oncogenic factor in gastric cancer and that TRIP13 regulates ENO1 expression through FBXW7, thereby affecting gastric cancer cell growth, migration, invasion, stemness and cisplatin resistance. These findings provide a promising target for gastric cancer treatment, but still leaves a gap in content. For some reasons, gastric cancer tissue was not collected to determine the expression levels of TRIP13, nor has it been verified whether the *in vivo* function of TRIP13 is consistent with the *in vitro* function. It is planned that these gaps will be addressed in future studies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them. Conceptualization, methodology and writing - Original draft was prepared by Jiayun Liu, Che Chen; formal analysis, resources and investigation were prepared by Jiayun Liu, Che Chen, Guannan Wu, Xuequan Yao and Fukun Liu; Formal analysis, visualization and data curation were undertaken by Jiayun Liu, Che Chen, Guannan Wu, Xuequan Yao and Fukun Liu; Project administration, supervision and validation were carried out by Jiayun Liu, Che Chen, Guannan Wu, Xuequan Yao and Fukun Liu; Validation, supervision and writing - Review and editing was done by Jiayun Liu, Che Chen and Shenlin Liu. All authors read and approved the final manuscript. Jiayun Liu and Che Chen contributed equally to the work and should be considered co-first authors.

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