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Original Research

YTHDF1 Promotes Proliferation and Inhibits Apoptosis of Gastric Cancer Cells via Upregulating TCF7 mRNA Translation

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Abstract

Background: N6-methyladenosine (m^6A) modification is one of the most common RNA modifications in mammals. m^6A modification, and associated abnormal gene expression, occur during various biological processes, most notably tumorigenesis. YTH domain-containing family protein 1 (YTHDF1), a m^6A reader, bind to messenger RNAs (mRNAs) containing a m^6A modification and this enhances its interaction with the ribosome and promotes translation. The function of YTHDF1 in gastric cancer (GC) has been the subject of earlier studies; however, the precise mechanism underlying YTHDF1's role in GC has not been fully elucidated. **Methods**: The expression of YTHDF1 was evaluated using quantitative real time polymerase chain reaction (qRT-PCR), immunohistochemistry and western blotting. CCK-8, 5-Ethynyl-2′-deoxyuridine (EdU) and flow cytometry assays were utilized to explore the effect of YTHDF1 on GC cell viability and proliferation. Transcriptome sequencing and RNA immunoprecipitation assays were utilized to explore the underlying mechanisms mediated by YTHDF1. **Results**: We observed that YTHDF1 is upregulated in GC cancer tissues. Knockdown of YTHDF1 in GC cells significantly inhibited proliferation and promoted apoptosis, suggesting that YTHDF1 increases proliferation and blocks apoptosis in GC cells. Mechanistically, data gathered suggest that YTHDF1 promotes the translation of the transcription factor TCF7 and this results in activation of the WNT signaling axis. **Conclusions**: We found that YTHDF1 was upregulated in GC and that YTHDF1 could promote GC progression through modulating the translational efficiency of TCF7. Taken together, these findings may provide a novel therapeutic target for GC.

Keywords: YTHDF1; TCF7; gastric cancer; m^6A modification; proliferation

1. Introduction

Gastric cancer (GC) ranks as the fifth most common and fourth most deadly cancer globally, posing a serious threat to human health [1]. GC is particularly common in China, where the proportion of new cases and deaths accounts for almost half of the world's total case load. Patients with GC are usually diagnosed at a late stage, and have poor treatment options and prognosis [2]. The 5-year overall survival rate of patients with advanced distant metastases remains less than 5% [3]. Therefore, it is urgent to better understand the molecular mechanisms that control GC development and progression to reduce GC morbidity and mortality.

m^6A modification, a prevalent RNA modification in the eukaryotic, is a reversible process involving methylation and demethylation [4]. This dynamic process can affected by specific methyltransferases, termed writers, and demethylases, termed erasers [5]. Methylated RNAs interact with m^6A readers, and this interaction can influence gene expression at multiple levels including transcription and post-transcriptional processes [6].

m^6A modification was reported to affect multiple biological processes including tumorigenesis [6–8]. Recent studies have demonstrated that dysregulation of m^6A methyltransferases (writers), readers, and erasers exert vital roles in tumorigenesis [9–11]. YTHDF1, a member of the YT521-B homologous (YTH) domain protein family, has been reported to function as a m^6A reader [12]. YTH family members bind to methylated RNAs through a C-terminal YTH domain and this property is critical in effecting gene expression [13]. YTHDF1 has proved to be closely related in both the occurrence and development of multiple tumor types [12–14]. At present, the function of YTHDF1 has been initially studied [12,15,16], and the function of YTHDF1 in GC has been preliminarily explored. Chen *et al.* [17] reported that YTHDF1 could facilitate GC tumorigenesis and metastasis by modulating USP14 translation. In addition, YTHDF1 is associated with GC antitumor immunity through recruitment of mature dendritic cells [18]. These findings suggest a role for YTHDF1 in GC. However, there remains much to be discovered regarding the function of YTHDF1 in GC, and the potential mechanism of YTHDF1 regulate GC progression.

This study aims to analyze the expression and prognostic relationship of YTHDF1 in GC and adjacent tissues in clinical samples. We also report the effects of knock-

down and overexpression of YTHDF1 on the phenotype of GC cells. The downstream target genes and pathways of YTHDF1 were also studied by analyzing RNA-seq, RNA immunoprecipitation sequencing (RIP-seq), and m6A-seq data with the hope that these findings may provide a basis for a novel molecular targeted therapy for GC.

2. Materials and Methods

2.1 Patient Samples

A total of 24 paired GC tissues and corresponding adjacent normal tissues were collected from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). Pathological examination was performed to confirm the resected samples were GC tissues. Human ethnics approval was obtained from the Ethics committee of the First Affiliated Hospital of Chongqing Medical University. The informed consents were obtained from all patients.

RNA-seq data was acquired from the Cancer Genome Atlas (TCGA) database. This data included 27 patient-matched tumor and normal samples, 32 unpaired normal samples, and 375 unpaired tumor samples.

2.2 Cell Culture

The GC cell lines MGC803 and AGS were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Beijing, China). All cell lines were validated by STR profiling and tested negative for mycoplasma. Cells were cultured in a humidified incubator at 37 °C with 5% CO_2 using RPMI 1640 medium (#01-100-1ACS, BI, Kibbutz Beit Haemek, Israel) containing 10% certified Fetal Bovine Serum (VivaCell, Shanghai, China).

2.3 RNA Isolation, cDNA Synthesis and Quantitative Real-Time PCR (qRT-PCR)

Total RNA from tissues and cells was isolated using Trizol reagent (#9108, Takara, Osaka, Japan) according to manufacturer's protocol. The concentration and quality of RNA was tested by using a nanodrop (#K5600C, KAIAO, Beijing, China). The RNA was used in cDNA synthesis reactions using PrimeScript RT reagent kit (#RR037A, Takara, Osaka, Japan). Relative RNA levels of YTHDF1 was determined using Ultra SYBR Mixture (#RR42WR, Takara, Osaka, Japan) and GAPDH mRNA was used as internal reference. Primers used in qRT-PCR are listed in **Supplementary Table 1**.

2.4 Construction of Plasmids, Transfection, and Engineering of Stable Cell Lines

Small interfering RNA (siRNA) were used to knockdown gene expression, and siRNA targeting YTHDF1 and TCF7 was purchased from Sangon Biotech (Sangon Biotech, Shanghai, China).

Small hairpin RNA (shRNA) targeting YTHDF1 were ligated into the psi-LVRU6GP plasmid and a full-length YTHDF1 cDNA was cloned into the phosphoglycerate dehydrogenase-Cytomegalovirus (pCDH-CMV) plasmid. All plasmids were transfected into cells using Lipofectamine 2000 (#11668019, Invitrogen, Carlsbad, CA, USA). YTHDF1 overexpression and knockdown plasmids, together with psPAX2, pMD2.G plasmids, were transfected into 293T cells to generate lentiviral particles, and these lentivirus were subsequently used to transduce GC cell lines. Puromycin (4 µg/µL, Beyotime, Shanghai, China) was used to select stably transduced GC cells. Information relevant to siRNA, shRNA, and primers used is shown in **Supplementary Table 2**.

2.5 Flow Cytometry Analysis, Colony Formation Assays, CCK-8 Assays, and 5-Ethynyl-2′-Deoxyuridine (EdU) Staining

Flow cytometry was conducted at Chongqing Medical University, College of Life Sciences and was used to evaluate cell cycle dynamics and apoptosis of GC cells. For CCK-8 assays, cells were seeded in 96-well plates at a density of 2×10^3 cells per well. Adherent cells were treated with 10 uL CCK-8 reagent (#CK04, DOJNDO, Tokyo, Japan) at 0, 24, 48 and 72 hours after seeding. Following a 2-hour incubation with CCK-8 reagent at 37 °C, the absorbance at 450 nm was measured using a spectrophotometer (Synergy2, BioTek, VT, USA). For colony formation assays, GC cells were seeded at 8×10^2 cells/well into 6-well plates and further cultured. After 10 days, cells were fixed using 10% paraformaldehyde (#P0099, Beyotime, Shanghai, China) and stained using 0.1% crystal violet (#C0121, Beyotime, Shanghai, China) prior to counting. For the EdU staining, a total of 3×10^4 GC cells per well were planted into a 96-well plate. After 24 hours, cell proliferation was determined following the manufacturer's protocol (RiboBio, Guangzhou, China). Where noted, cells were imaged using fluorescence microscopy (#Leica DMi8, Leica, Wetzlar, Germany).

2.6 RNA Immunoprecipitation (RIP)

RIP was conducted according to the manufacturer's instructions included in the Magna RIP Kit (Merck Millipore, Billerica, MA, USA). RIP experiments were conducted with anti-YTHDF1 antibody (Proteintech, Wuhan, China). Co-precipitated TCF7 was analyzed using qRT-PCR.

2.7 Immunohistochemistry (IHC) Staining

Tissues from GC patients and xenografts were fixed using 10% paraformaldehyde and subsequently embedded in paraffin. Immunohistochemistry staining was conducted as previously described [19]. Primary antibodies used were anti-YTHDF1 (1:200, Proteintech, Wuhan, China) and anti-Ki-67 (1:500, Cell Signaling Technology, Boston, USA). IHC images were captured with an inverted Leica microscope (#Leica DMI3000B, Leica, Wetzlar, Germany).

2.8 Protein Extraction and Western Blotting

Proteins from cell and tissue samples were extracted using RIPA lysis buffer (1:100) containing PMSF (Beyotime, Shanghai, China). Proteins were subsequently added to SDS-PAGE gels prior to conducting electrophoresis. Proteins in SDS-PAGE gels were electrotransferred to PVDF membranes (#03010040001, Millipore, USA). Then, the PVDF membrane was blocked using 5% skim milk (Beyotime, Shanghai, China) for 1 hours. The primary antibodies used in this study are anti-GAPDH (1:6000, Proteintech, Wuhan, China), anti-YTHDF1 (1:3000, Proteintech, Wuhan, China), anti-MYC (1:8000, Proteintech, Wuhan, China), anti-Cyclin D1 (1:5000, Proteintech, Wuhan, China), anti-MMP7 (1:2500, Proteintech, Wuhan, China), and anti-TCF7 (1:5000, Proteintech, Wuhan, China). These antibodies were incubated with PVDF membranes at 4 °C for 12 hours. The next day, the membrane was incubated with corresponding secondary antibody (1:6000, Proteintech, Wuhan, China) for 2 hours. Proteins were visualized using an enhanced chemiluminescence solution ECL (#K-12049-D50, Advansta, CA, USA).

2.9 Animal Studies

Six female nude mice were bought from the Animal Experimental Laboratory of Chongqing Medical University. MGC803 cells stably overexpressing YTHDF1 and control cells were injected (7×10^6 cells) into the flank of each mouse. Tumor volume was assessed every 7 days using a vernier caliper. After four weeks, mice were anesthetized with pentobarbital sodium and then euthanized by cervical dislocation. Then, the tumors were harvested for immunohistochemistry and western blot analysis. Animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University.

2.10 Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA), SPSS 19.0 (SPSS, Chicago, IL, USA) and R (version 4.1.0, Auckland, New Zealand) software. Data are expressed as mean $\pm$ SD. $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ were considered to have statistical significance.

3. Results

3.1 YTHDF1 is Highly Expressed in Gastric Cancer and Associated with Poor Survival

To explore a potential role for YTHDF1 in GC, we first accessed gene expression and survival data from the TCGA database. We compared the expression level of YTHDF1 in 375 tumor tissues and 32 normal tissues and result indicated that the mRNA level of YTHDF1 is significantly higher in tumor tissues (Fig. 1A). Next, we compared expression of YTHDF1 between 27 paired GC tissues and adjacent normal tissues and similar results showing higher expression in GC tumors was observed in these tissue pairs (Fig. 1B). We then compared overall survival differences between the low YTHDF1 expression group and the high YTHDF1 expression groups. Here, we observed that GC patients with higher YTHDF1 expression have poorer overall survival (Fig. 1C).

We also collected 24 paired GC cancer/normal tissues from our hospital as an independent validation of the differential expression of YTHDF1 observed in the TCGA dataset. Results demonstrated that YTHDF1 mRNA was elevated in most GC tissues compared with adjacent normal tissues (Fig. 1D). In addition, immunohistochemistry and western blotting were used to directly evaluate the level of YTHDF1 protein in these tissues. Results obtained showed that YTHDF1 was upregulated in 20 out of 24 GC tissues in our panel when compared with adjacent normal tissues (Fig. 1E,F).

3.2 YTHDF1 Promotes Proliferation and Inhibits Apoptosis in Gastric Cancer Cells

To further explore the role of YTHDF1 in GC, we constructed GC cell lines stably overexpressing YTHDF1. qRT-PCR and western blot assays were utilized to confirm overexpression (Fig. 2A).

CCK-8 and colony forming assays indicated that proliferative ability of GC cells was increased following YTHDF1 overexpression (Fig. 2B–D). Flow cytometry indicated that YTHDF1 overexpression induced a higher proportion of S phase cells than that seen in normal control (NC) cells (Fig. 2E–G). In addition, EdU staining was utilized to measure cell proliferation in GC cells overexpressing YTHDF1. Consistent with other results, YTHDF1 overexpression significantly enhanced the rates of DNA synthesis in GC cells (Fig. 2H,I).

We also examined whether YTHDF1 could affect apoptosis in GC cells. The results suggested that YTHDF1 overexpression partially inhibited GC cell apoptosis (Fig. 2J,K). Taken together, these results suggest that YTHDF1 may act in promoting GC tumor growth.

3.3 YTHDF1 Knockdown Inhibits Proliferation and Promote Apoptosis Abilities of Gastric Cancer Cells

To further examine the potential tumor promoting role of YTHDF1, we designed siRNAs targeting YTHDF1. Knockdown efficiency was measured using qRT-PCR and western blotting (Fig. 3A). CCK-8 and colony formation assays were used to evaluate the proliferative ability of GC cells. We observed that YTHDF1 knockdown significantly inhibited GC cell growth (Fig. 3B–D). Flow cytometry results demonstrated that MGC803 and AGS cells transfected with siRNA specific for YTHDF1 were arrested in the G0/G1 phase of the cell cycle (Fig. 3E–G). Result of EdU assay also showed that proliferation ability of the YTHDF1 knockdown group was significantly inhibited when compared to EdU incorporation than the NC cell group (Fig. 3H,I). Analysis of apoptosis by flow cytometry

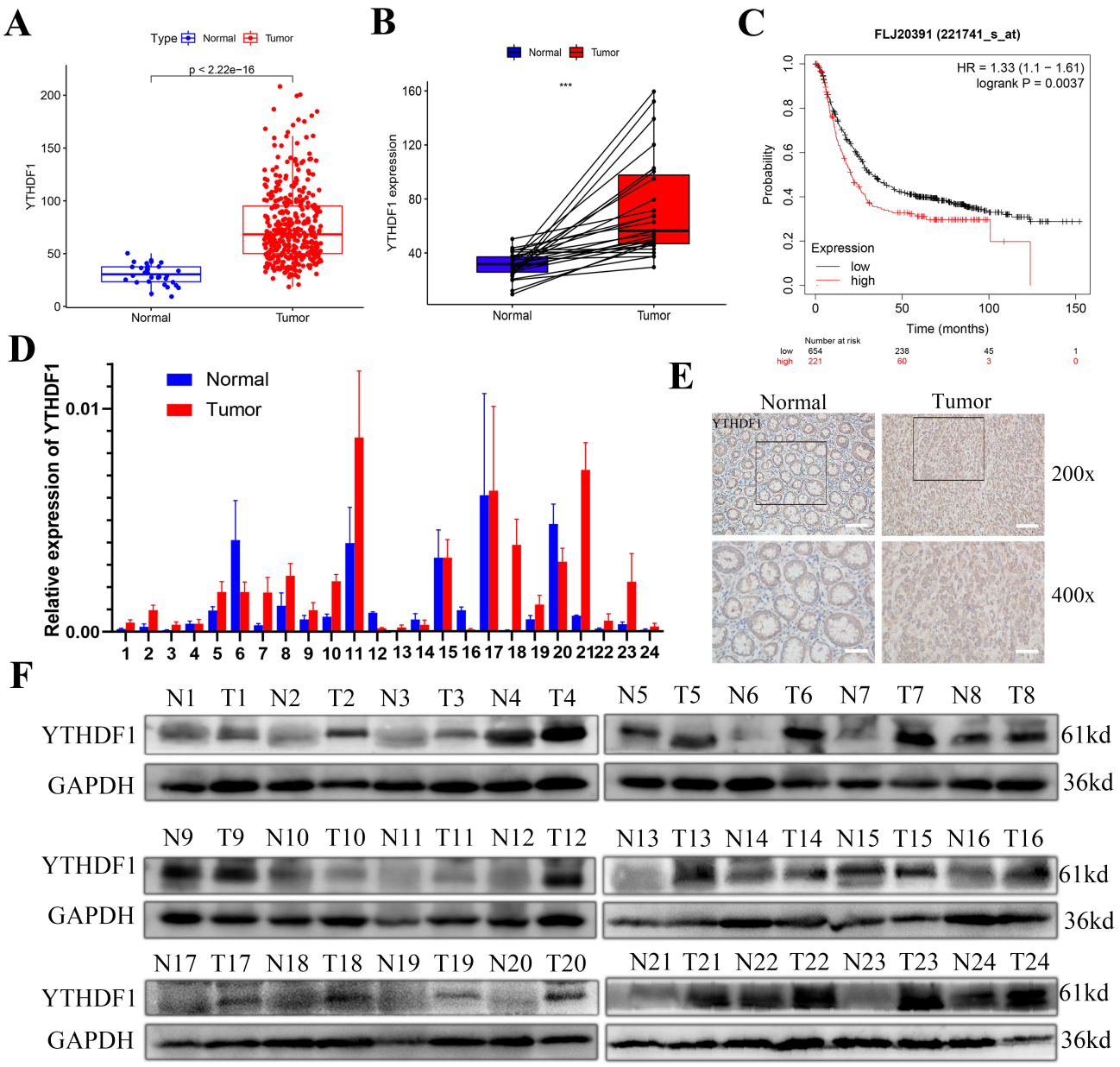

Fig. 1. YTHDF1 is differentially expressed in GC. (A,B) RNA level of YTHDF1 in unpaired and paired GC tissues and adjacent normal tissues in the TCGA database. (C) Survival differences of GC patients with high or low expression of YTHDF1 were analyzed using Kaplan-Meier analyses. (D) RNA expression level of YTHDF1 in 24 patient-matched GC and normal tissues. (E,F) The protein level of YTHDF1 in 24 paired GC tissues and corresponding normal tissues were detected using immunohistochemical and western blot assays (scale bar (200×) = 200 µm, scale bar (400×) = 100 µm). ***, $p < 0.001$. YTHDF1, YTH domain-containing family protein 1; GC, gastric cancer; TCGA, the cancer genome atlas; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

showed that the number of apoptotic GC cells was increased after knockdown of YTHDF1 (Fig. 3J,K). Taken together, these results suggest that YTHDF1 exert a crucial role in establishing/maintaining GC cell malignancy.

3.4 YTHDF1 Promotes GC Cell Proliferation by Upregulating TCF7 Expression

To further explore the underlying mechanism mediated by YTHDF1, we conducted transcriptome sequencing on GC cells with YTHDF1 knockdown (**Supplementary Table 3**). Using the filter condition of ($|\log2FC| > 1$ and $padj < 0.05$), we identified a total of 649 differentially expressed genes, including 394 upregulated genes and 225 downregulated genes. The differentially expressed genes were visualized using a heatmap and volcano plot (Fig. 4A,B).

Next, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on these dif-

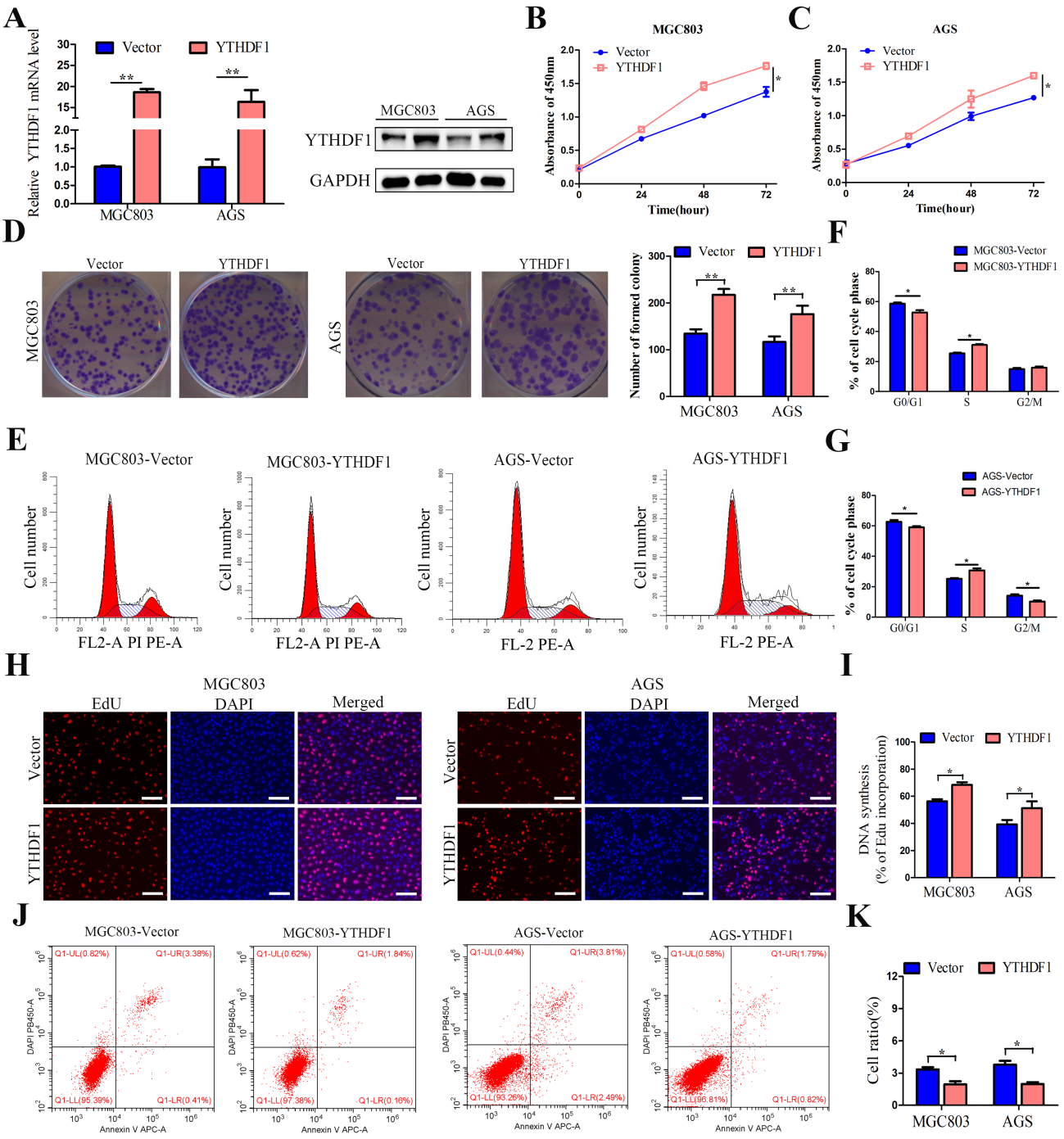

Fig. 2. Overexpression of YTHDF1 promotes proliferation and inhibits apoptosis of GC Cells. (A) qRT-PCR and western blot assays was conducted to evaluate the over expression efficiency of YTHDF1 in GC cells. (B,C) CCK-8 results proved that YTHDF1 promote the proliferation ability of GC cells. (D,E) Colony formation assays were used to evaluate the effect of YTHDF1 on proliferation ability of GC cells. (F,G) Flow cytometry was utilized to assess the cell cycle of GC cells after over expression of YTHDF1. (H,I) EdU staining was performed to evaluate the DNA synthesis abilities of GC cells after over expression of YTHDF1 (scale bar = 120 μm). (J,K) Flow cytometry was conducted to determine whether YTHDF1 effects the apoptotic population in cultured GC cells. *, $p < 0.05$; **, $p < 0.01$. qRT-PCR, quantitative real time polymerase chain reaction; CCK-8, cell counting kit-8; EdU, 5-Ethynyl-2′-deoxyuridine.

ferentially expressed genes. We found that several tumor-associated signaling pathways such as MAPK signaling, Hippo signaling, WNT signaling, and TGF-β signaling pathways were significantly enriched (Fig. 4C). This finding suggests that the tumor promoting function of YTHDF1 is possibly associated with one or more of these pathways.

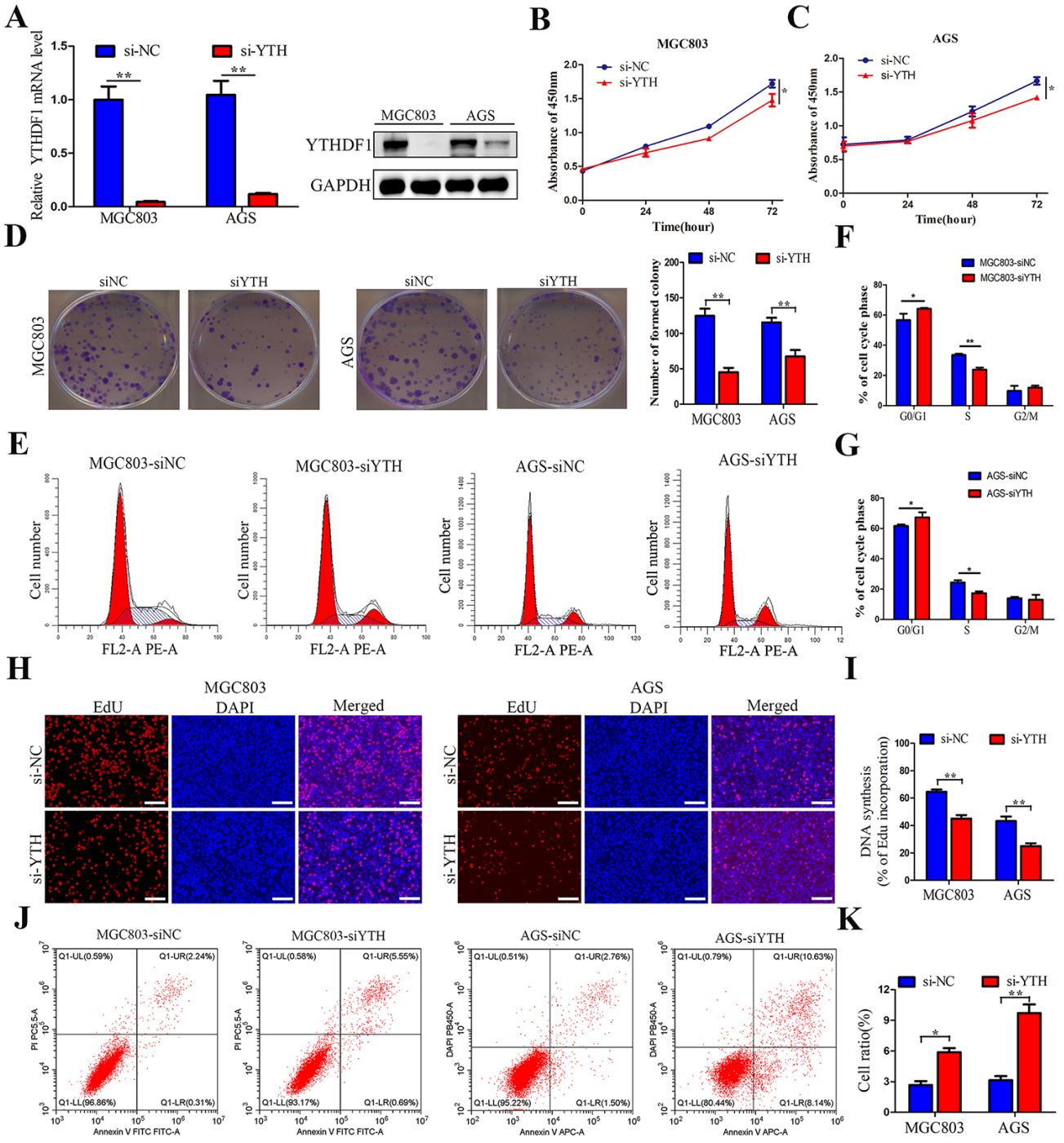

Fig. 3. Knock down of YTHDF1 inhibits proliferation and promotes apoptosis of GC Cells. (A) Knockdown efficiency of YTHDF1 in GC cells was measured using qRT-PCR and western blot analysis. (B,C) CCK-8 assays indicate that knockdown of YTHDF1 inhibits the proliferation of GC cells. (D,E) Colony formation assays were used to evaluate the effect of YTHDF1 knockdown on GC cell colony formation ability. (F,G) Flow cytometry was utilized to assess the effect of YTHDF1 knockdown on the cell cycle of GC cells. (H,I) EdU staining was performed to evaluate the proliferation status of GC cells after knockdown of YTHDF1 (scale bar = 120 μm). (J,K) Flow cytometry was conducted to determine the effects of YTHDF1 knockdown on GC cell apoptosis. *, $p < 0.05$; **, $p < 0.01$. siRNA, small interfering RNA; NC, normal control.

Considering that YTHDF1 was reported to enhance the translational efficiency of its target genes, we speculated that YTHDF1 might promote target gene translation and thereby affect these tumor related pathways. To identify YTHDF1 direct target genes, we acquired the meRIP-seq, RNA-seq and RIP-seq datasets obtained from the anal-

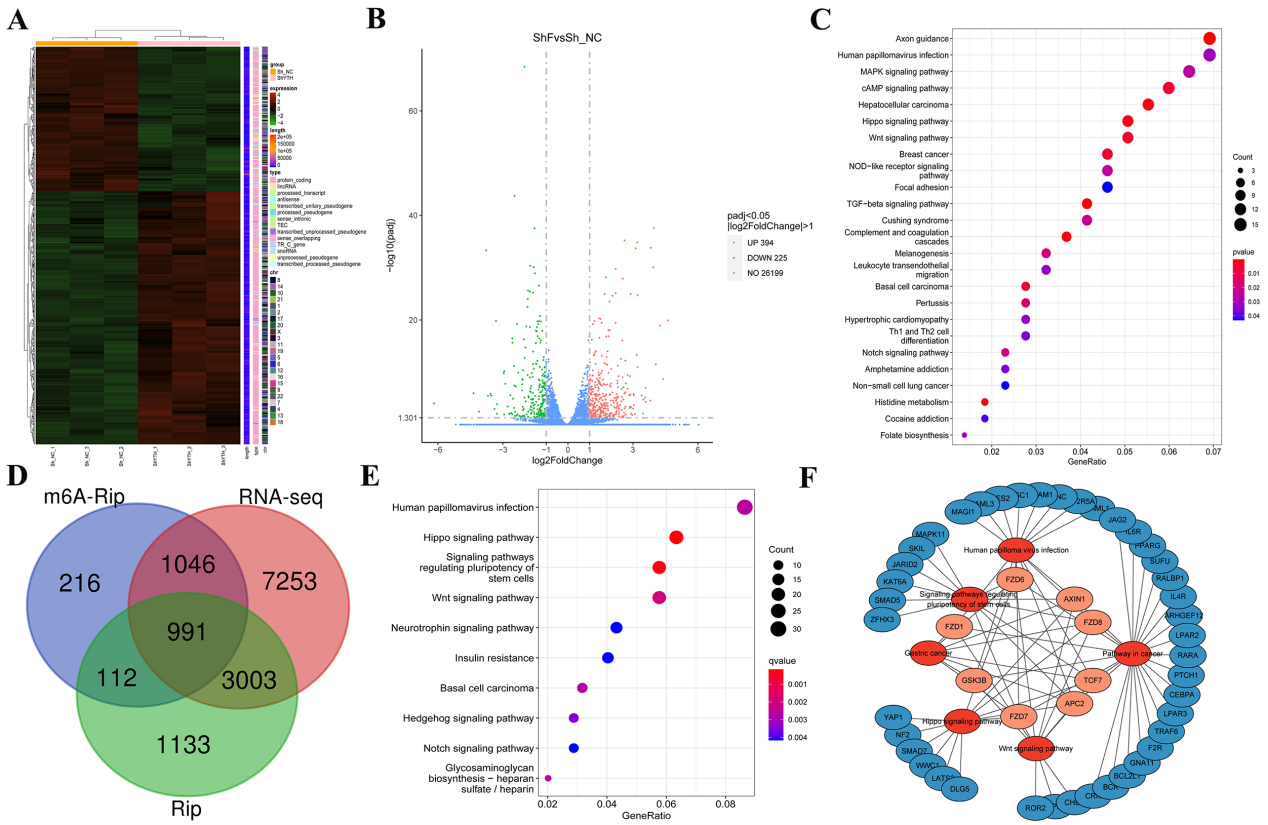

Fig. 4. Identification of potential target pathways regulated by YTHDF1. (A,B) Transcriptome sequencing was conducted after YTHDF1 knockdown. (C) mRNA expression differences between NC and YTHDF knockdown groups were visualized using a heatmap and a volcano map. GO and KEGG enrichment analyses were conducted on the differently expressed genes between the NC group and the YTHDF knock down group. (D) meRIP-seq, RNA-seq and RIP-seq data collected on MGC803 cells in a previously published study were acquired and interogated to obtain the potential target genes of YTHDF1. (E) GO and KEGG enrichment results of 991 YTHDF1 potential target genes from the above analyses. (F) Cytoscape software was utilized to visualize core genes involved in top four signal pathways and two signal pathways correlated with GC. mRNA, messenger RNA; NC, normal control; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; meRIP-seq, methylated RNA immunoprecipitation sequencing.

ysis of MGC803 cells from a previously published study [20]. Our analysis of these data sets uncovered 991 genes that are potential direct targets of YTHDF1 (Fig. 4D). Furthermore, we found that these genes were also enriched in Hippo signaling, WNT signaling, and in cells responding to human papillomavirus (HPV) infection (Fig. 4E). To acquire the core genes mediated by YTHDF1, we queried the genes involved in the top six signaling pathways associated with GC. These efforts identified eight (8) genes as core components within these pathways (Fig. 4F).

Among these core genes, we noted that the transcription factor TCF7 was associated with all six signaling pathways and, thus, TCF7 was selected for subsequent analyses. We found that TCF7 has potential m^{6}A methylation site (Fig. 5A,B). In addition, m^{6}A peak enrichment was noted in TCF7 mRNA (Fig. 5C). This led us to speculate that YTHDF1 can bind to mRNA of TCF7 through its m^{6}A modification, thereby affecting TCF7 expression. To further explore the regulatory mechanism of YTHDF1-mediated YCF7 control we conducted a RNA immunoprecipitation (RIP) assay. RIP assay revealed that YTHDF1 co-precipitates with TCF7 mRNA (Fig. 5D).

Further, we also observed that the mRNA level of TCF7 was not altered after YTHDF1 knockdown (Fig. 5E), and the TCF7 protein level was elevated after overexpression of recombinant YTHDF1 as judged by western blotting (Fig. 5F). Of note, we observed TCF7 protein level down-regulation following YTHDF1 knockdown (Fig. 5G).

We also examined the effect of TCF7 on the WNT pathway. After knockdown of TCF7 expression, the mRNA expression levels of WNT downstream target genes, including c-myc, Cyclin D1, Survivin, MMP7 and CD44, were decreased (Fig. 5H). Consistent with these findings, YTHDF1 knockdown in MGC803 and AGS cells resulted in decreased expression of TCF7 protein as were the levels of Cyclin D1, MMP7, and MYC (Fig. 5G). Taken together, these results suggest that YTHDF1 activates the WNT pathway by upregulating TCF7, and that this molecular event likely promotes GC development and/or progression.

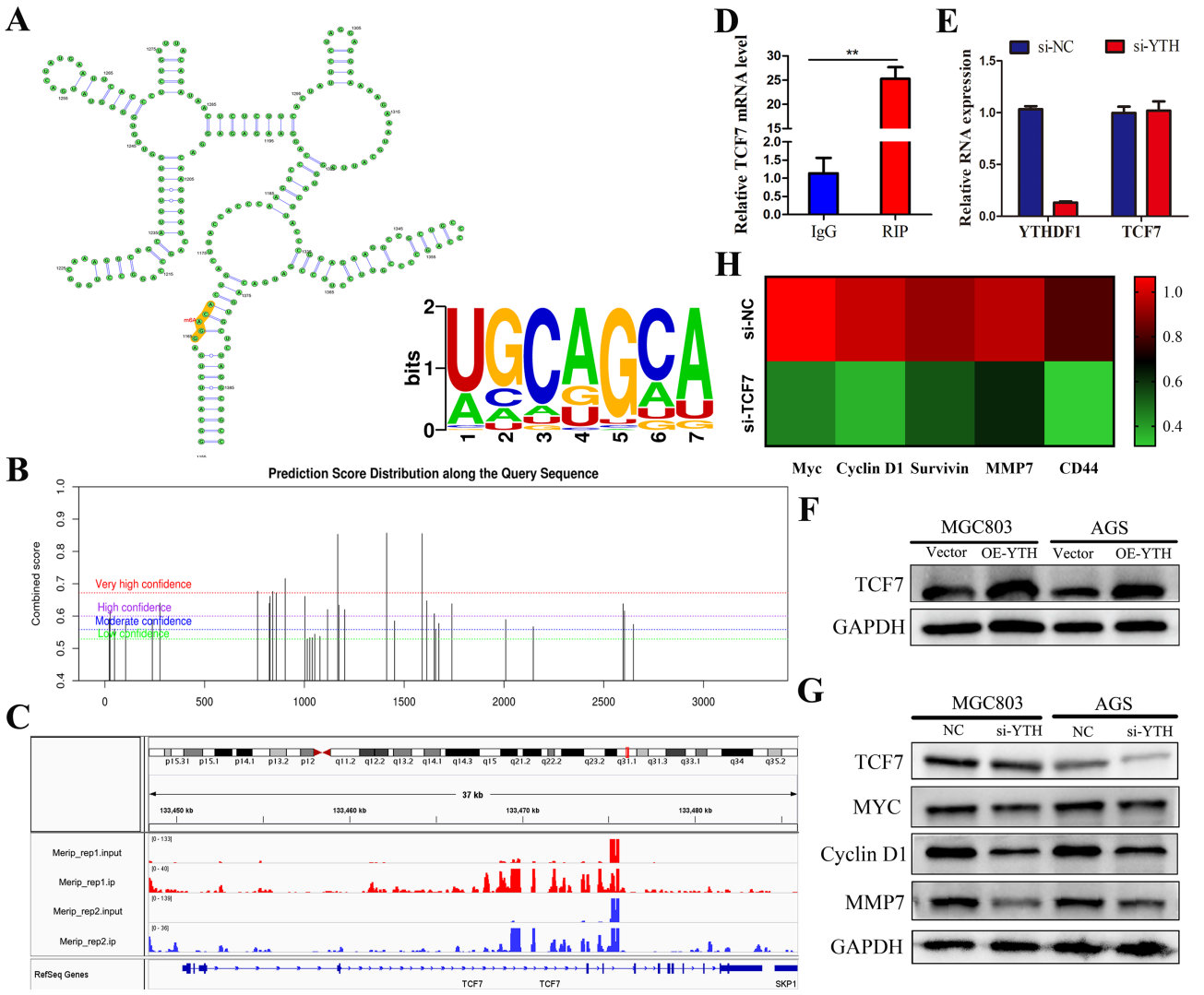

Fig. 5. YTHDF1 regulate the translation efficiency of TCF7, thereby affecting WNT pathway. (A) An online database (https://www.cuilab.cn/) was used to visualize the predicted m^{6}A modification sites within the TCF7 mRNA and the motif of m^{6}A modification. (B) The combined scores between YTHDF1 and potential m^{6}A modification site of TCF7 mRNA was predicted using SRAMP (https://www.cuilab.cn/sramp). (C) m^{6}A peak enrichment of TCF7 mRNA. (D) RIP assay was conducted to prove that YTHDF1 binds to TCF7 mRNA. (E) The level of TCF7 mRNA was determined after YTHDF1 knockdown using siRNA. (F) A heatmap was used to display the changes in TCF7 target gene mRNA levels after TCF7 knockdown. (G) Effect of YTHDF1 knockdown on TCF7 protein level in GC cells. (H) The expression of TCF7 and its target genes after YTHDF1 knockdown was determined using western blot analysis. **, $p <$ 0.01. TCF7, transcription factor 7; MYC, MYC proto-oncogene bHLH transcription factor; MMP7, matrix metalloproteinase 7.

3.5 Depletion of YTHDF1 Inhibits the GC Tumor Growth in Vivo

To directly verify that YTHDF1 serves in a tumor promoting role *in vivo*, MGC803 cells with stable knockdown of YTHDF1 were injected into 4-weeks female nude mice. These MGC803 knockdown cells were engineered by transducing cells with lentivirus encoding a YTHDF1-specific shRNA or normal control (NC) shRNA sequences. Tumor volume was measured every 7 days and after 28 days, we found that the tumor size in the YTHDF1 knockdown tumors was significantly smaller than those in the NC group (Fig. 6A) and that this was observed in four measurements (Fig. 6B). Tumor weight in the YTHDF1 knockdown group was also markedly lower compared with the NC group (Fig. 6C). In addition, compared with the NC group, expression of YTHDF1 and the proliferation marker Ki-67 were significantly lower in the YTHDF1 knockdown group as indicated by IHC staining (Fig. 6D). The protein level of YTHDF1, as well as its target gene TCF7, were also found to be lower in the YTHDF1 knockdown group than that of NC group as measured by western blotting (Fig. 6E). Taken together, these results strongly suggest that YTHDF1 contributes to GC malignancy both *in vitro* and *in vivo*.

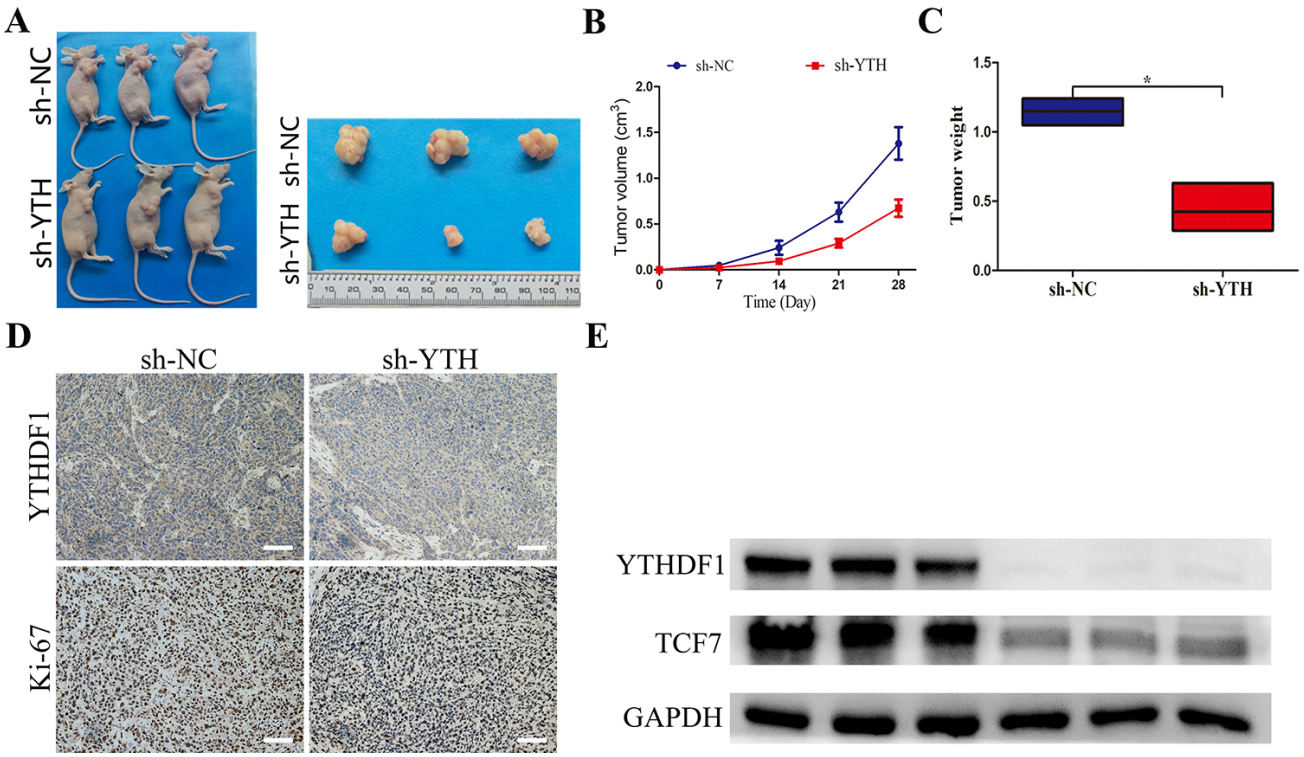

Fig. 6. YTHDF1 promote GC growth both *in vivo*. (A) Knockdown of YTHDF1 effectively inhibits GC tumor growth *in vivo*. (B,C) Tumor volume and tumor weight was decreased in the YTHDF1 knockdown group when compared to the normal control group. (D) Expression of YTHDF1 and Ki-67 in NC and YTHDF1 knockdown tumors was determined by IHC staining (scale bar = 200 μm). (E) Western blotting was performed to evaluate the protein level of YTHDF1 and TCF7 after YTHDF1 knockdown. *, $p < 0.05$. IHC, immunohistochemistry; shRNA, small hairpin RNA.

4. Discussion

Gastric cancer is one of the most common digestive tract malignancies and exhibits high morbidity and mortality on a worldwide basis [1,21]. Despite great improvements in both the diagnostic and treatment strategies for GC developed in recent years, the mortality of this cancer type remains high. Mounting evidence indicates that epigenetic alteration, genetic mutation, and aberrant activation, or inactivation, of tumor-related signaling pathways are closely associated with gastric carcinogenesis and progression [21,22]. Thus, exploring underlying mechanisms that contribute to gastric carcinogenesis and progression is of great significance as such knowledge may lead to new, effective prognostic biomarkers or potential therapeutic targets for GC patients.

In recent years, mounting evidence implies that m^6A modification is integrally involved in tumorigenesis and progression of various tumor types [23–25]. m^6A modification is a dynamic process that includes both the methylation and demethylation of adenine residues within RNA transcripts, and these processes are directly effected by specific methyltransferase writers and demethylase erasers, respectively [5]. m^6A readers bind to the methylated RNA to affect gene expression at multiple levels, including at the transcriptional and post-transcriptional steps, as well as mRNA translation [6]. Most m^6A readers belong to the YT521-B homologous (YTH) domain protein family, and various YTH domain family members exert different effects in various tumor types. A recent study suggested that YTHDF2 modulates mRNA degradation through METTL3-mediated m^6A modification [26]. Another study indicated that YTHDF3 promotes target gene translation [27]. In addition, YTHDC1 and YTHDC2 were both reported to modulate mRNA splicing and stability [28,29], respectively.

Beyond these various YTH family members, YTHDF1 was found to be involved in the progression of multiple tumors including GC [15–17]. Most studies suggested that YTHDF1 promotes the translation of its target genes, thereby affecting tumor progression [15–17]. Although the function of YTHDF1 has been preliminary explored [17,18], there were still much unknown in regard to the function of YTHDF1 in GC.

In this study, we initially explored the expression of YTHDF1 in GC using the TCGA-STAD data portal. We found that YTHDF1 was aberrantly overexpressed in GC compared to normal tissue. Subsequently, elevated expression of YTHDF1 was validated in 24 patient-matched GC/normal tissue samples. In addition, survival analysis indicated that patients with higher YTHDF1 levels exhibit poorer survival compared to those patients who do not have

elevated YTHDF1 expression. Based on these results, we speculated that YTHDF1 might be involved in GC carcinogenesis or progression.

We overexpressed YTHDF1 in cultured GC cells and determined the effect of YTHDF1 on proliferation and apoptosis. We found that YTHDF1 could promote the proliferation and inhibit the apoptosis of GC cells. Conversely, knockdown of YTHDF1 was found to significantly inhibit the proliferation and promote apoptosis in these cells. These results suggested that YTHDF1 serves in an oncogene role in GC consistent with a previous study [30]. Another study found that YTHDF1 modulates transcription of the deubiquitinating enzyme USP14 and that this promoted GC tumorigenesis and metastasis [17].

While m^6A modification widely exists in RNA, it remains unknown as to whether YTHDF1 could bind and regulate the translation of mRNAs. Transcriptome sequencing of GC cells after YTHDF1 knockdown indicated that alterations in the expression of numerous genes that regulate key signaling pathways, such as MAPK signaling, Hippo signaling, WNT signaling, and TGF-beta signaling were significantly enriched. To further identify the direct target genes of YTHDF1, we queried meRIP-seq, RNA-seq, and RIP-seq data obtained from MGC803 cells in a previously published study [20]. The intersection of genes obtained from these various unbiased molecular screens indicated that identified genes were largely enriched in pathways closely correlated with GC and that these genes are potential direct targets of YTHDF1.

KEGG pathway analysis revealed that these genes were associated with several tumor-related pathways. Among these pathways, Wnt signaling has been reported to control multiple fundamental cell functions, including proliferation, migration, differentiation, and stemness [31]. Underscoring its importance in GC, nearly 46% of GC tumors exhibit dysregulation of the WNT/β-catenin pathway [32]. In the context of GC initiation, the Hippo signaling pathway, another signaling axis uncovered in our study, plays a pivotal role. Hippo signaling has been significantly implicated in various aspects of GC progression, encompassing cell proliferation, evasion of immune responses, and the establishment of tumor innervation [33]. Moreover, numerous genes within the Hippo pathway have emerged as potential therapeutic targets for GC [34].

Another signaling axis that was revealed in our studies were signaling pathways that regulate pluripotency of stem cells. The stemness of GC cells confer upon tumors the capability for self-renewal and the generation of heterogeneous tumor cells [35]. Cancer stem cells play a pivotal role in cancer metastasis, recurrence, and resistance to chemotherapy [36]. It is evident that these pathways play crucial roles in the onset and progression of gastric cancer.

We further visualized the correlation of these GC-associated pathways and core genes using cytoscape. The results identified eight genes, including FZD1, GSK3B, FZD8, AXIN1, FZD6, FZD7, APC2 and TCF7, as the core genes of these pathways. Interestingly, these genes are largely components of the WNT pathway [37]. However, the transcription factor TCF7 is associated with all six signaling pathways leading us to further explore the relationship between YTHDF1 and TCF7. Results indicated that YTHDF1 could modulate the translational efficiency of TCF7. Moreover, m^6A modification was previously reported to be associated with altered WNT/PI3K-AKT signaling in GC [38] and that TCF7 could regulate activities of WNT signaling [39]. Here, we document that knockdown of YTHDF1 significantly reduced the expression of downstream components of the WNT pathway. Based on these results, we conclude that YTHDF1 is an indirect activator of the WNT pathway by upregulating the expression of TCF7 and, thereby promoting the progression of GC.

5. Conclusions

In summary, we identified that YTHDF1 was highly expressed in GC tissues. YTHDF1 could promote the proliferation ability and inhibit the apoptosis of GC cells *in vitro*. In addition, YTHDF1 promotes GC tumor growth *in vivo*. Mechanistically, YTHDF1 activates WNT signaling via upregulation of TCF7 translation. Our results support a novel regulatory mechanism of YTHDF in GC, which might shed light on GC development and progression.

Availability of Data and Materials

GC expression and survival data used in this study can be found in TCGA (https://portal.gdc.cancer.gov/). The project ID is "STAD". All the datasets generated/or analyzed in our study are available from the corresponding author on reasonable request.

Author Contributions

YQL, XLL and ZWW designed this study. YQL and XLL conducted the cell experiments. YQL and XG conducted the animal experiments. YQL wrote the draft. ZWW reviewed the article. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The informed consents were obtained from all patients. The human study was carried out in accordance with the declaration of Helsinki. The animal study was conducted in accordance with the ARRIVE guidelines. The animal study was carried out in accordance with relevant guidelines and regulations of the Ethics committee of Chongqing Medical University. Human study and animal study was approved by the Ethics committee of the First Affiliated Hospital of Chongqing Medical University. The approval number is 2021-30.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2903117.

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