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Original Paper

GTPBP4 Promotes Gastric Cancer Progression via Regulating P53 Activity

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Key Words
Gtpbp4 • p53 • gastric cancer • MKN-45 • AGS

Abstract
Background/Aims: gastric cancer is a serious health concern with high morbidity and mortality. Therefore, it is urgent to find novel targets for gastric cancer diagnosis and treatment. ***Methods:*** qRT-PCR and immunohistochemistry assays were used to detect GTPBP4 expression in gastric cancer tissues, and gastric cancer and gastric epithelial cells. Lentivirus infection was used to construct GTPBP4 stable knockdown cells. Annexin V/PI apoptosis, CCK8, EdU incorporation and cell clone formation analysis were performed to evaluate the effects of GTPBP4 on gastric cancer cell proliferation and apoptosis. Further RNA-based high-throughput sequencing and co-IP assays were constructed to explore the related mechanisms contributing to GTPBP4-mediated effects. ***Results:*** GTPBP4 expression was significantly increased in gastric cancer tissues compared with that in adjacent normal tissues, and positively correlated with gastric cancer stages. Meanwhile, GTPBP4 level was markedly upregulated in gastric cancer cells than in gastric epithelial cells. Additionaly, stable knockdown of GTPBP4 inhibited cell proliferation and promoted cell apoptosis. Mechanistically, p53 and its related signaling were significantly activated in GTPBP4 stable knockdown cells. And GTPBP4 interacted with p53 in gastric cancer cells. ***Conclusions:*** our results provide insights into mechanistic regulation and linkage of the GTPBP4-p53 in gastric cancer, and also a valuable potential target for gastric cancer.

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Introduction

Gastric cancer is a kind of tumor with high morbidity and mortality [1]. Although surgical treatment, chemotherapy and radiotherapy could greatly improve the survival of patients with gastric cancer, which could not be cured or recurred within several years [2]. Thus, it is very important for patients with gastric cancer to get early diagnosis and treatment, and it is an urgent need to find new targets for treating or predicting gastric cancer.

P53 has been proved to play important roles in gastric cancer progression. For example, p53 deregulation is closely associated with Epstein-Barr virus-associated gastric cancer [3].

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P53 loss could drive gastric tumorigenesis [4]. On the contrary, activating p53/caspase-3 signaling could suppress gastric cancer progression [5]. Based on the critical roles of p53 in tumor progression, researchers had been investigating the mechanisms regulating p53 expression. NML-mediated rRNA base methylation links ribosomal subunit formation to cell proliferation in a p53-dependent manner [6]. During mitotic catastrophe, chromosomal breaks could trigger gammaH2AX-ATM-p53-mediated apoptosis [7]. However, the detailed mechanisms regulating p53 expression in gastric cancer are not well-defined.

Previous study investigated the protein interaction profiles of Drosophila p53 and uncovered additional nodes of human p53 network via a genome-scale assay [8], in which GTPBP4 (guanosine triphosphate binding protein 4), also known as Nog1, was identified as an interactor and GTPBP4 knockdown induced p53 activation in the absence of nucleolar disruption. Previous study has shown that TOR regulates late steps of ribosome maturation in the nucleoplasm via GTPBP4 in response to nutrients, and GTPBP4 is required for biogenesis of the 60 S ribosomal subunit [9, 10]. Importantly, in wide-type p53 breast tumors, GTPBP4 expression is negatively correlated with patient survival, demonstrating a potential relevance of GTPBP4-p53 interaction in cancers. Recently, Yu, H et al. indicated that upregulation of GTPBP4 is responsible for colorectal tumor metastasis [11]. However, the roles of GTPBP4 and GTPBP4-p53 interaction have not been revealed in gastric cancer progression.

Here, GTPBP4 expression was meaured in gastric cancer and normal adjacent tissues, and we found that GTPBP4 expression was significantly increased in gastric cancer tissues and positively correlated with gastric cancer stages. Further GTPBP4-stable knockdown gastric cancer cells were constructed, and used to evaluate the effects of GTPBP4 in cell proliferation and apoptosis. Additionally, RNA-based high-throughput sequencing and western blot analyses were performed to explore the mechanisms contributing to GTPBP4 roles in gastric cancer.

Materials and Methods

Clinical samples and cell culture

30 pairs of gastric cancer and normal adjacent tissue samples were collected from The Affiliated Hospital of Xuzhou Medical University. Written informed consent from all patients and approval of the Hospital Ethic Review Committees were obtained. Gastric cancer cell lines MKN-45、AGS、BGC-823、MGC-803 and normal gastric epithelial cell line GES-1 were purchased from the Chinese Academy of Sciences Cell Bank. SCG7901-DDP (SCG7901 cisplatin resistant cells) and SCG7901 cells were purchased from (KeyGEN BioTECH, Nanjing, China). MKN45 and BGC-823 were cultured in Dulbecco's Minimum Essential Medium (DMEM) medium (Gibco, USA), and AGS, MGC-803, GES-1, SCG7901-DDP and SCG7901 were cultured in 1640 medium (Gibco). All medium were supplemented with 10% FBS (Fetal Bovine Serum) (Gibco), 80 U/ml penicillin and 0.08 mg/ml streptomycin. Cells were cultured at 37°C under humidified atmosphere with 5% CO_2.

Lentivirus plasmids and stable knockdown cell lines construction

We thanked for OBiO Inc. for constructing the knockdown lentivirus vectors of GTPBP4 (LV-GTPBP4-shRNA), ATP1B3 (LV-ATB1B3-shRNA), MLLT11 (LV-MLLT11-shRNA), CHN1 (LV-CHN1-shRNA), CORO1C (LV-CORO1C-shRNA), GAS7 (LV-GAS7-shRNA), KRT6B (LV-KRT6B-shRNA), CDCA7 (LV-CDCA7-shRNA), RFTN1 (LV-RFTN1-shRNA), DNAJB5 (LV-DNAJB5-shRNA), IL8 (LV-IL-8-shRNA), IFITM3 (LV-IFITM3-shRNA), VCL (LV-VCL-shRNA), CAP2 (LV-CAP2-shRNA), MCM8 (LV-MCM8-shRNA), TREM2 (LV-TREM2-shRNA), RERG (LV-RERG-shRNA), MNDA (LV-MNDA-shRNA), GTSF1 (LV-GTSF1-shRNA), CLIP4 (LV-CLIP4-shRNA), HECW2 (LV-HECW2-shRNA) and p53 (LV-p53-shRNA) which were verified by DNA sequencing. An empty virus was utilized as a control group in this work.

High Content Screening (HCS) assay

HCS assay was performed in Cellinsight CX5 High Content Screening Platform with Click-iT™ EdU Alexa Fluor™ 555 HCS Assay (C10353, ThermoFisher Scientific) according to the manufacturer's protocols.

Transcriptome sequencing assay

Total RNA was extracted from MKN45 cells with GTPBP4 knockdown or not, and purified using HiPure kit (REF MB602-50PR, HiMedia, Mumbai, India), according to the manufacturer's instruction, and then subjected to transcriptome sequencing assay. The procedure could be referred to the manufacturer's protocol (Illumina, San Diego, USA).

Cell viability assay

Cell count Kit-8 (CCK-8) (Vazyme Biotech Co.,Ltd, China) was used to evaluate cell proliferation ability. Cells with GTPBP4 stable knockdown or not were seeded in 96-well plates at 4000 cells/well. After 1 - 5 days, cell ability was examined. Triplicate experiments were performed for each assay.

Cell apoptosis assay

Cell apoptosis was measured using flow cytometry analysis with AnnexinV-FITC staining (Vazyme Biotech Co.,Ltd, China). Cells with GTPBP4 stable knockdown or not were harvested and washed with ice-cold PBS, and. then stained with Annexin V-FITC following the manufacturer's recommendation. The flow cytometry analysis was performed by flow cytometry (BD Biosciences).

Cell clone formation assay

Cell clone formation assay was used to examine cell viability. Briefly, 500 cells with GTPBP4 stable knockdown or not were seeded into 6-well plates and cultured for 11 days. Cell clones were fixed with methanol and stained with crystal violet, and then photographed with camera. The represented results were presented in results. Triplicate experiments were performed for each assay.

5-ethynyl-2'-deoxyuridine (EdU) incorporation assay

EdU incorporation assay was used to determine the speed of DNA synthesis with a keyFluor488 Click-iT EdU kit (KeyGEN BioTECH) following the manufacturer's instructions. The detailed procedure was referred to the previous study [12]. Briefly, cells were seeded in confocal dish and 50 mM EdU was added, and cultured for additional 3 h at room temperature. Then cells were fixed with 4% paraformaldehyde for 15 min. After washing with 3% BSA (Bovine Serum Albumin), cells were permeabilized with 0.5% Triton X-100 for 20 min at room temperature. After washing with 3% BSA, 500 ml Click-iT mix was added to each well, and cells were incubated for 30 min, and then cells were subsequently stained with 1 ml Hoechst 33342 for 15 min and visualized under a laser scanning confocal microscope.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells using Trizol reagent (Invitrogen, USA) following the manufacturer's commendation. Then total RNA was reverse transcribed into cDNA using M-MLV (Promega, USA) following manufacturer's protocols. Then, mRNA expression levels were detected according to the protocols of SYBR Green Master Mix (ThermoFisher Scientific, USA), and performed on an ABI Prism 7500 Detection System (Applied Biosystems, Inc., USA). qRT-PCR primers were listed as follows: GTPBP4 forward: 5'- AGTTGCTCTCGAACTCCACG -3', reverse: 5'- TGTCTATCCGCCTCCCCTTT -3'. GAPDH was used as an internal reference. qRT-PCR primers for GAPDH were listed as follows: GAPDH forward: 5'- GCCTCAAGACCTTGGGCTGGGACTG -3', reverse: 5'- CTAAGTCCCTCCTACAAAA -3'. Then, expression of each transcript was calculated using the $2^{-\Delta\Delta ct}$ method.

Western blotting

Detailed procedure was described elsewhere [13]. The antibodies against GTPBP4 (ab92342), TNFRSF10B (ab199357), MDM2 (ab16895), p53, CCND1 (ab134175), FAS (ab82419), PTEN (ab79156), CDKN1A (ab109520) and GAPDH (ab9485) were purchased from Abcam. Blots were washed and incubated with a peroxidase-conjugated antibody (R&D Systems), and chemiluminescence was detected using an enhanced chemiluminescence kit (orb90504, biorbyt) followed by exposure with Tanon 5200.

Co-immunoprecipitation (IP) assay

Total cell lysis buffer were prepared in RIPA lysis buffer with protease inhibitor. Proteins were immunoprecipitated with anti-GTPBP4 (1:100), anti-p53 (1:100) and captured by protein G magnetic beads

(Bio-rad, America) at 4°C overnight. Then the beads were washed three times using RIPA. 50 µl loading buffer were added into centrifuge tubes. All samples were analyzed using SDS-PAGE electrophoresis.

Immunohistochemistry assay

Gastric tumor and adjacent normal tissue specimens were fixed in formalin and embedded in paraffin. The detailed procedure was referred to the previous study [14]. Briefly, paraffin sections were cut to a thickness of 4 µm, and the slides were dewaxed in xylene and rehydrated with graded ethanol. A standard Tris-EDTA buffer and high pressure cooking was used for antigen retrieval and then sections were blocked in 3% H_2O_2 in TBS and 5% BSA (bovine serum albumin). Sections were incubated with primary antibody (anti-GTPBP4 (ab92342)) over night at 4°C and then reheated at 37°C for 45 min, blotted with HRP-conjugated secondary antibody at room temperature for 1 h. After washing with PBS for three times, slides were stained with the ABC detection system following the manufacturer's recommendation.

Statistical analysis

All data were presented as mean ± SD (standard deviation). GraphPad Prism software was used to analyze the data. For three independent experiments, two-tailed of the student's t test was used to evaluate the differences between groups and *P < 0.05 or less was considered significant.

Results

GTPBP4 expression is significantly higher in gastric tumor than normal adjacent tissues

Firstly, 30 pairs of gastric tumor and adjacent normal tissues were used to detect the expression of genes. RNA-based transcriptome sequencing assay showed that 20 genes expression (GTPBP4, ATP1B3, MLLT11, CHN1, CORO1C, GAS7, KRT6B, CDCA7, RFTN1, DNAJB5, IL8, IFITM3, VCL, CAP2, MCM8, TREM2, RERG, MNDA, GTSF1, CLIP4, HECW2) were significantly increased in gastric tumors compared with that in normal adjacent tissues (Fig. 1A). Then lentivirus infection was used to knockdown these genes, and followed by further HCS assay to investigate the effects on cell proliferation ability. As shown in Fig. 1B, IL-8 and GTPBP4 knockdown exerted more efficient effects on cell viability than other genes. As the functions of IL-8 were well-known, GTPBP4 was used to further analysis. Further qRT-PCR, western blot and immunohistochemistry assays confirmed the expression of GTPBP4 in gastric tumor and adjacent normal tissues

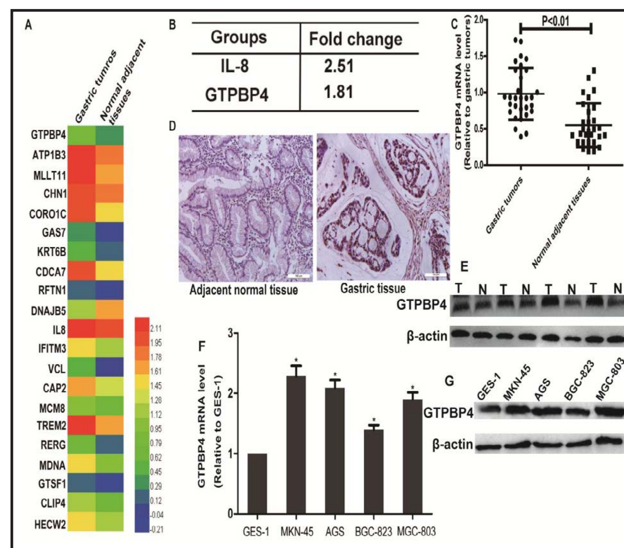

Fig. 1. GTPBP4 expression is significantly increased in gastric tumor tissues compared with that in normal adjacent tissues. (a) Genes expression in gastric tumors and normal adjacent tissues based on RNA-sequencing analysis. (b) The fold change of IL-8 and GTPBP4 on cell viability via HCS assay. (c) GTPBP4 mRNA level was detected in gastric tumor tissues and normal adjacent tissues via qRT-PCR assay. (d and e) GTPBP4 protein expression was examined in gastric tumor tissues and normal adjacent tissues via qRT-PCR (c), immunohistochemistry (d) and western blot (e) assays, represented Fig. was shown, N was indicated as normal, T was indicated as Tumor. (f and g) GTPBP4 mRNA (f) and protein (g) levels were examined in normal gastric epithelial cells (GES-1) and gastric cancer cells (MKN-45, AGS, BGC-823 and MGC-803) via qRT-PCR (f) and western blot (g) assays. Data were presented as mean ± s.d; **P<0.01 vs. Control.

(Fig. 1C - E). Importantly, GTPBP4 expression was positively correlated with gastric cancer stages (Table 1). Additionally, consistent results were obtained in gastric cancer and normal epithelial cells (Fig. 1F and 1G). Therefore, our results indicate that GTPBP4 expression is increased in gastric cancer and GTPBP4 might hold promotive effects on gastric cancer progression.

Table 1. The characteristics of GTPBP4 in clinical gastric tumors

Clinical characteristics	N	Expression of GTPBP4		P value
		Negative	Positive	
Gender				
Male	16	4	12	0.694
Female	14	5	9	
Age				
≥60	14	3	11	0.440
<60	16	6	10	
Differentiation				
Well and moderate	13	6	7	0.123
Poor	17	3	14	
T stage				
T1+T2	6	4	2	0.049*
T3+T4	24	5	19	
N stage				
N0	6	5	1	0.005*
N1+N2	24	4	20	
Cancer stage				
I-II	11	6	5	0.042*
III-IV	19	3	16	

Knockdown of GTPBP4 inhibits cell proliferation and promotes cell apoptosis in gastric cancer cells

Since GTPBP4 expression is higher in MKN45 and AGS cells than other gastric cancer cells, we chose MKN45 and AGS cells for the subsequent studies. GTPBP4 was knockdown via lentivirus infection, knockdown efficiency was confirmed in MKN45 and AGS cells via qRT-PCR and western blot analyses (Fig. 2A and 2B). Then MKN45 and AGS cells with GTPBP4 knockdown or not were subjected to cell proliferation analysis via cell counting and EdU incorporation assays. As shown in Fig. 2C and 2D, knockdown of GTPBP4 significantly inhibited gastric cancer cell proliferation. Identical results were obtained via EdU incorporation assay (Fig. 3A). Additionally, cell apoptosis assay indicated that GTPBP4 knockdown significantly promoted cell apoptosis in gastric cancer cells (Fig. 3B), And increased the expression of apoptotic executors (cleaved caspase3 and cleaved PARP) (Fig. 3C).

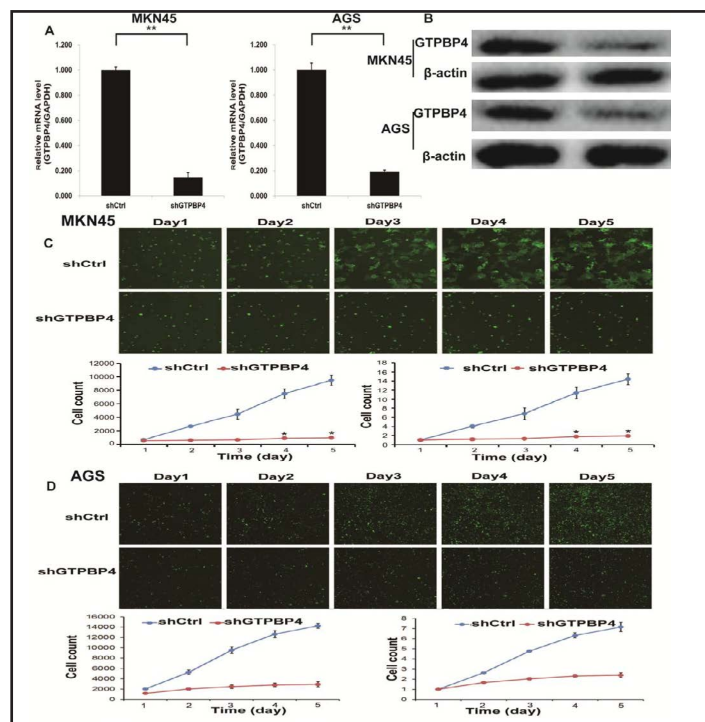

Fig. 2. Knockdown of GTPBP4 inhibits gastric cancer cell proliferation. (a and b) The knockdown efficiency of shGTPBP4 was assessed via qRT-PCR (a) and (b) western blot analyses in MKN45 and AGS cells. (c and d) The cell viability of MKN45 and AGS cells with GTPBP4 knockdown or not was evaluated by CCK8 assay. Data were presented as mean ± s.d; **P<0.01 vs. Control.

Knockdown of GTPBP4 inhibits the cloning ability of gastric cancer cells

As cell clone formation ability could greatly reflect cell viability, MKN45 and AGS cells with GTPBP4 knockdown or not were further subjected to cell clone formation assay. In consistent with cell proliferation assay, GTPBP4 knockdown remarkably suppressed cell clone formation in MKN45 and AGS cells (Fig. 4A and 4B). Thus, these results demonstrate that

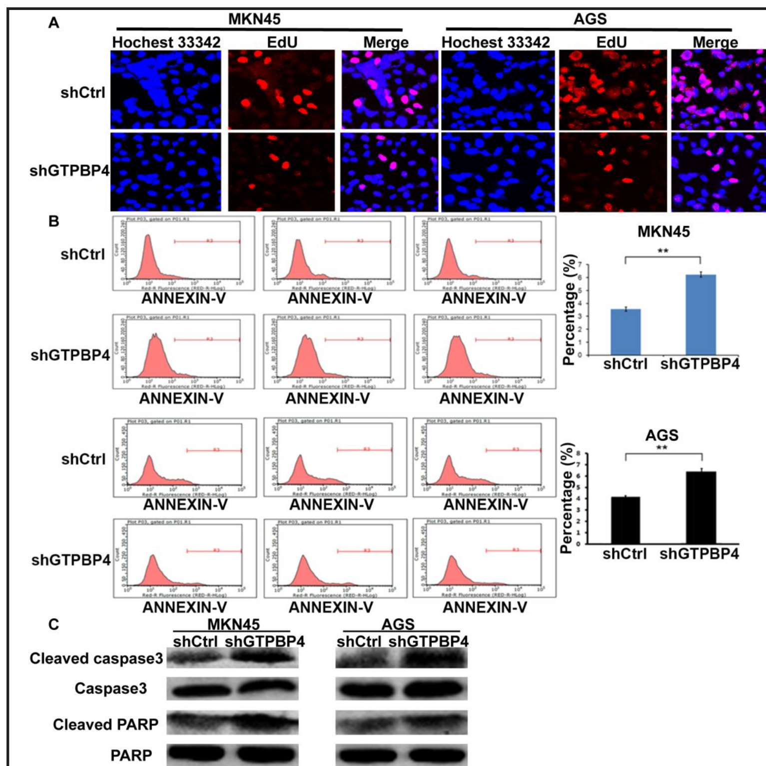

Fig. 3. Knockdown of GTPBP4 promotes cell apoptosis in gastric cancer cells. (a) MKN45 and AGS cells with GTPBP4 knockdown or not were analyzed by EdU incorporation assays. (b) Cell apoptosis was measured in MKN45 and AGS cells with GTPBP4 knockdown or not. (c) Apoptotic executors (cleaved caspase3 and cleaved PARP) were detected in cells depicted in (a). Data were presented as mean ± s.d; **P<0.01 vs. Control.

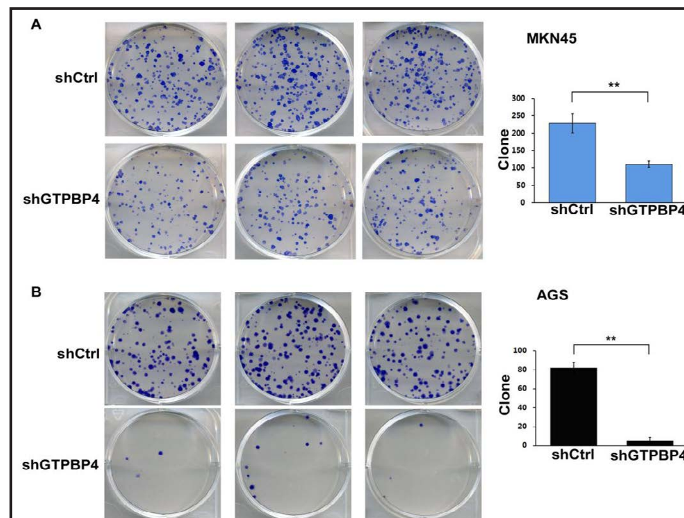

Fig. 4. Knockdown of GTPBP4 inhibits the cloning ability of gastric cancer cells. (a and b) The cloning ability of MKN45 and AGS cells with GTPBP4 knockdown or not was measured by cell clone formation assay. Data were presented as mean ± s.d; **P<0.01 vs. Control.

knockdown of GTPBP4 indeed could suppress gastric cancer cells viability.

P53-related signaling is engaged in GTPBP4 functions in gastric cancer

To further explore the detailed mechanisms contributing to GTPBP4 functions in gastric cancer, MKN45 cells with GTPBP4 knockdown or not were used for transcriptome sequencing assay. We found that p53 expression was markedly increased in GTPBP4 knockdown cells (Fig. 5A), while the downstream negative effectors of p53 (PTEN, CDKN1A, TNFRSF10B) were downregulated with GTPBP4 knockdown. Further western blot assay confirmed these results (Fig. 5B). Additionally, to further reveal the GTPBP4-p53 interaction in gastric cancer cells, co-IP analysis was performed with anti-GTPBP4 and anti-p53. As shown in Fig. 5C, GTPBP4 could bind to p53, while p53 could also bind to GTPBP4 in gastric cancer cells (Fig. 5D). Importantly, GTPBP4 expression was negatively correlated with p53 and its targets (TNFRSF10B, PTEN, CDKN1A) expression (Fig. 5E - H) in gastric tumor tissues. Thus, we speculate that p53-related signaling might be involved in GTPBP4 functions.

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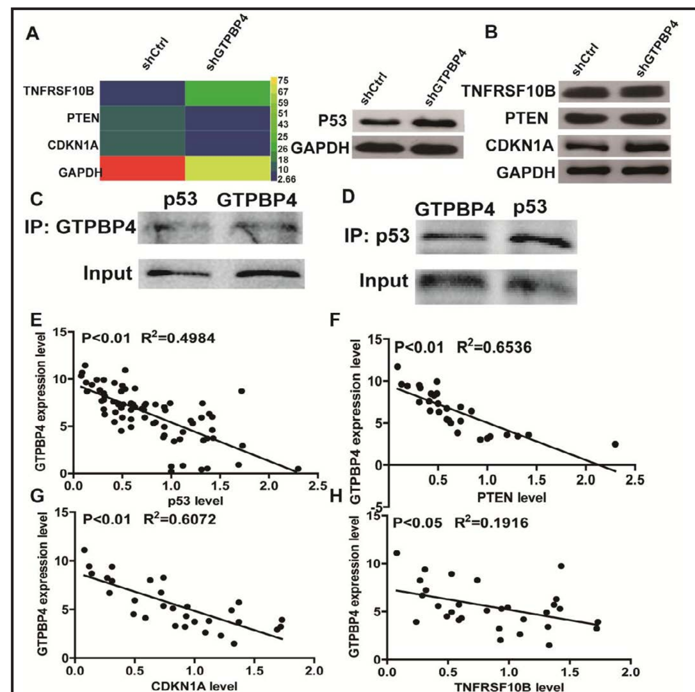

Fig. 5. P53-related signaling is involved in GTPBP4 functions in gastric cancer. (a) Genes expression in MKN45 cells with GTPBP4 knockdown or not was analyzed based on RNA-sequencing analysis. (b) The protein level of PTEN and its downstream negative effectors (PTEN, CDKN1A, TNFRSF10B) was detected in MKN45 cells with GTPBP4 knockdown or not via western blot analysis. (c andd) Inputs for co-IP were also subjected to western blot analysis. (e - h) Correlation between the levels of GTPBP4 and p53, PTEN, CDKN1A or TNFRSF10B was analyzed by qRT-PCR assay.

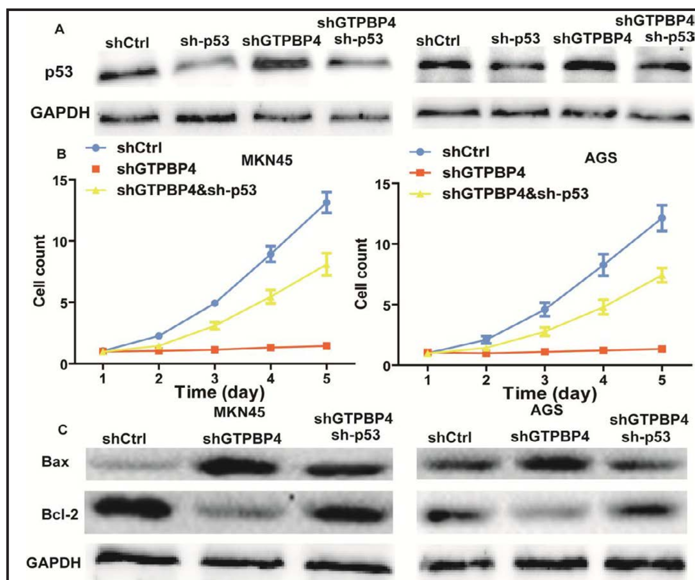

Fig. 6. GTPBP4 promotes gastric cancer cell proliferation and apoptosis dependent on p53 expression. (a) p53 protein level was examined in gastric cancer cells with GTPBP4 knockdown plus p53 knockdown or not via western blot analysis. (b) Cell viability was assessed in cells depicted in (a) via CCK8 assay. (c) The protein levels of apoptotic markers (Bax and Bcl-2) were detected in cells depicted in (a). Data were presented as mean ± s.d; **P<0.01 vs. Control.

GTPBP4 promotes gastric cancer cell proliferation and inhibits cell apoptosis dependent on p53 expression

We sought to explore whether the GTPBP4-p53 interaction contributes to GTPBP4 functions in gastric cancer, rescue experiments were adopted to validate that p53 expression is imperative in GTPBP4-mediated promotion. P53 knockdown lentivirus vector (LV-p53-shRNA) was co-infected with LV-GTPBP4-shRNA in MKN45 and AGS cells. Western blot results showed that infection of LV-p53-shRNA significantly decreased p53 expression, and also attenuated GTPBP4 knockdown-induced increase of p53 expression in MKN45 and AGS cells (Fig. 6A). As expected, decreased cell viability caused by GTPBP4 knockdown was attenuated or even reversed by p53 knockdown (Fig. 6B). Moreover, p53 knockdown significantly restored the promotion of GTPBP4 knockdown on cell apoptosis (Fig. 6C). Therefore, our results suggest that GTPBP4 promotes gastric cancer progression via binding to p53 and thus inhibiting p53-related signaling.

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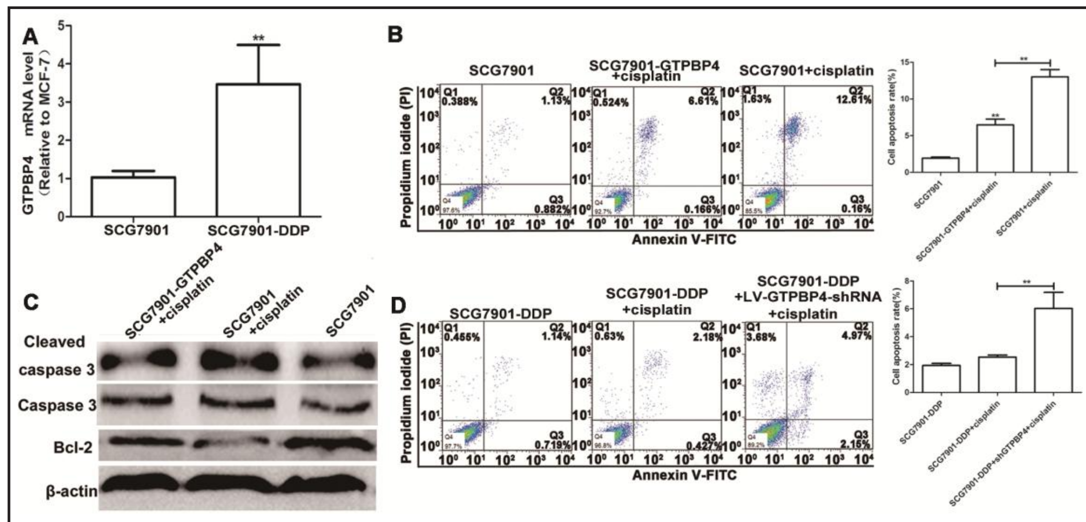

Fig. 7. GTPBP4 decreased cisplatin sensitivity in gastric cancer cells. (A) mRNA level of GTPBP4 was detected in SCG7901 and SCG7901-DDP cells. (B and C) Overexpression of GTPBP4 attenuated cisplatin-induced apoptosis characterized as decreased apoptotic rate (B) and decreased Cleaved-caspase-3, and increased Bcl-2 expression (C). (D) Knockdown of GTPBP4 enhanced cisplatin sensitivity in SCG7901-DDP cells via increasing cell apoptosis. Data were presented as the mean ± s.d; **P<0.01 vs. Control.

GTPBP4 overexpression decreasescisplatin sensitivity in gastric cancer cells

We continued exploring whether GTPBP4 is involved in cisplatin sensitivity in gastric cancer cells. Firstly, we found that GTPBP4 level was significantly higher in SCG7901-DDP cells than that in SCG7901 cells (Fig. 7A). And cisplatin-induced pro-apoptotic effect was attenuated by GTPBP4 overexpression (Fig. 7B). Cisplatin-induced upregulation of cleaved-caspase3 and downregulation of Bcl-2 were attenuated by GTPBP4 overexpression (Fig. 7C). Additionally, GTPBP4 knockdown weakened cisplatin resistance in SCG7901-DDP cells (Fig. 7D). Therefore, our results demonstrate that GTPBP4 overexpression could confer cisplatin resistance in gastric cancer cells.

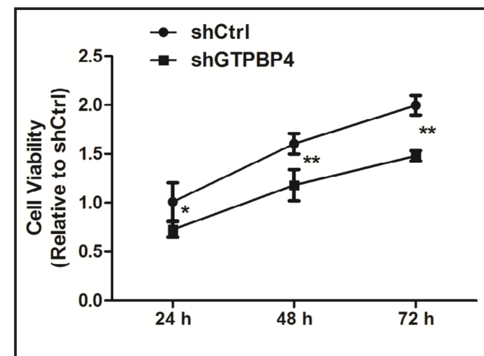

Fig. 8. GTPBP4 knockdown decreased cell viability of SG-7901 cells. SGC-7901 cells with GTPBP4 knockdown or not were subjected to CCK8 analysis. Data were presented as the mean ± s.d; **P<0.01 vs. Control.

Discussion

GTPBPs could act as molecular switches to regulate various cellular process, like cell proliferation and cell cycle [15, 16]. GTPBP4, one of GTPBPs, is a GTPase that could be activated upon binding to GTP [17]. Previous studies have shown that GTPBP4 contributes to 60S ribosome biogenesis by its monomeric GTPase activity [10]. Furthermore, GTPBP4 could inhibit tumorigenic schwanomma cells proliferation via regulating merlin expression [17]. However, recent study has indicated that upregulation of GTPBP4 could promote colorectal cancer metastasis [11]. These results imply complex roles of GTPBP4 in tumor progression. And since the roles of GTPBP4 have not been revealed in gastric cancer, this study aims to explore the roles and related mechanisms of GTPBP4 in gastric cancer.

Here, we found that GTPBP4 expression was significantly increased in gastric tumor tissues compared with that in adjacent normal tissues, and positively correlated with gastric

cancer stages, indicating that GTPBP4 could promote gastric tumor progression. Importantly, we found that GTPBP4 could interact with p53 and thus regulate the downstream negative effectors, which is in line with the previous study indicating that the GTPBP4-p53 interaction exists in lymphoblastoid cell lines and contributes to thiopurine resistance [8, 18]. However, the promotion of GTPBP4 on gastric cancer proliferation could not be abrogated, which means that other targets may be regulated by GTPBP4. As GTPBP4 exerts different functions in different cells, we conclude that the downstream signaling triggered by GTPBP4 might depend on the cell/tissue types. Importantly, it should be further explored whether the GTPBP4-p53 interaction is an universal phenomenon. Moreover, as both MKN45 and AGS cells have wild-type p53, we also examined the effect of GTPBP4 on the cell proliferation ability of SGC-7901 cells which has mutant p53, and consistent result was obtained (Fig. 8), this result suggest that GTPBP4 might promote gastric cancer progression via another signaling independent p53-related pathway. The Rho family members RhoA and CDC42 are small GTPases that could modulate cell migration and actin cytoskeleton reorganization [19, 20]. Furthermore, inactivating Rho GTPase could prevent cell migration and cancer cells stemness [21, 22]. Notably, recent research has shown that Up-regulation of GTPBP4 in colorectal carcinoma is responsible for tumor metastasis, and GTPBP4 may disorganize actin cytoskeleton through repressing RhoA signaling [11]. Thus, the other functions and specific mechanisms underlying the effects of GTPBP4 on RhoA activity should be explored in the future. Notably, in the present study, our data just indicate the roles of GTPBP4 and its downstream potential effectors, further investigations could focus on revealing the upstream regulators of GTPBP4. Also, it must be noted that further *in vivo* experiments should be performed.

Previous study has shown that GTPBP4 expression could distinguish potential endocrine-disrupting compounds in environmental samples [23]. To the best of our knowledge, this is the first study to investigate the roles of GTPBP4 in gastric cancer. Thus, GTPBP4 may be a novel therapeutic target for gastric patients.

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Disclosure Statement

The authors declare that they have no Disclosure Statements.

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