Tac2-N serves an oncogenic role and promotes drug resistance in human gastric cancer cells

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Abstract. Gastric cancer is one of the most common types of malignancy worldwide. Tac2-N (TC2N) has been reported to serve as either an oncogene or tumor suppressor in numerous different types of cancer; however, the role of TC2N in gastric cancer remains poorly understood. The present study aimed to investigate the role of TC2N in gastric cancer and reveal its regulatory mechanism. A Cell Counting Kit-8 assay was used to analyze the cell proliferation rate, while wound healing and Transwell Matrigel assays were performed to determine the cell migratory and invasive abilities, respectively. Cell cycle distribution was determined by flow cytometric analysis, and the expression levels of TC2N, P-glycoprotein (P-gp), cyclin D1, CDK4, cyclin E1, MMP2, MMP9 and N-Myc downstream regulated gene 1 were analyzed using reverse transcription-quantitative PCR or western blotting. Bioinformatics analysis revealed a high expression of TC2N in patients with gastric cancer. The experimental results revealed that TC2N expression levels were significantly unregulated in gastric cancer cell lines. The knockdown of TC2N in AGS cells significantly inhibited the cell proliferation rate and induced cell cycle arrest at the G0/G1 phase, while downregulating cyclin E1, cyclin D1 and CDK4 expression levels. The knockdown of TC2N also inhibited cell migration and invasion. Furthermore, the knockdown of TC2N improved the sensitivity of AGS cells to cisplatin, paclitaxel and 5-fluorouracil, and downregulated the protein expression levels of P-gp. By contrast, TC2N overexpression exerted the opposite effects in AGS cells. In conclusion, the findings of the present study indicated that the genetic knockdown of TC2N may inhibit cell proliferation, migration and invasion, while inducing cell cycle arrest in the G1/S phase and reversing the drug resistance of AGS cells, which may be partly through inhibiting P-gp expression levels. Thus, TC2N may serve as a novel diagnostic marker and therapeutic target for patients with gastric cancer.

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Introduction

There are ~1 million new cases of gastric cancer diagnosed every year worldwide, and the malignancy has become a leading contributor to cancer-induced deaths in East Asia, prompting the World Health Organization to declare gastric cancer a public health concern (1,2). There is a wide geographical variation in the incidence of gastric cancer, with the highest incidence rates observed in China, Japan, Latin America and Eastern Europe, whereas the lowest incidence rates are reported in North America, parts of Africa and Northern Europe (3,4). Despite advancements being made in the clinical treatment, the 5-year survival rate of patients with gastric cancer is <30% in most countries due to a majority of patients being only being diagnosed at an advanced stage (5). Increasing research has focused on determining the molecular mechanisms underlying the development of gastric cancer, which may provide a basis for discovering novel therapeutic targets.

Tac2-N (TC2N), located on human chromosome 14q32.12, belongs to the carboxyl-terminal type tandem C2 protein family, and is a putative C2 domain-containing protein (6). Although the function of the C2 domain was originally related to calcium-dependent phospholipid binding, a previous study has also indicated that the C2 domain may be involved in cellular signal transduction and protein-protein interactions (7). Furthermore, other proteins containing C2 domains were also reported to be involved in the modulation of tumorigenesis. For example, in a previous study, the double C2-like domain β (DOC2B) gene was identified to be closely associated with cervical carcinogenesis, as the downregulated expression levels of DOC2B led to selective cervical tumor growth, which was dependent on the methylation of its DNA promoter (8). In addition, copines were demonstrated to effectively increase cellular migration in breast cancer through binding with c-Jun activation domain binding protein-1 (9). It was also reported that TC2N served as an oncogene and regulated tumor metastasis in lung cancer, while serving as a tumor suppressor in breast cancer (6,10,11). Therefore, due to

various genes serving a dual role in cancer, the role of TC2N in different types of cancer should also be investigated.

The present study aimed to investigate the precise role of TC2N in gastric cancer occurrence and development, and to determine the regulatory mechanism of TC2N in gastric cancer. The results revealed a previously unknown role of TC2N in gastric cancer, and uncovered its potential mechanism underlying the therapeutic targeting of TC2N to inhibit gastric cancer progression.

Materials and methods

Bioinformatics analysis of TC2N expression levels in gastric cancer. Data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov) database, including 375 gastric tumor tissues and 32 normal gastric tissues (adjacent cancer samples), were used to analyze the expression levels of TC2N (12). TC2N expression levels and clinical data, including age, stage and overall survival, were downloaded from the TCGA data portal. Patients with known TC2N expression and complete clinical data were included in the present study, while those with missing clinical data were excluded.

Cell culture. The normal gastric mucosa cell line GES1 was obtained from Beijing Beina Chuanglian Biotechnology Institute and the gastric cancer cell line AGS was obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), and maintained in a humidified atmosphere with 5% CO_2 at 37˚C.

Cell transfection. For the overexpression and knockdown of TC2N, pcDNA3.1-TC2N [overexpression (OE)-TC2N], the specific short hairpin (sh)RNA vector targeting TC2N-1/2 (shRNA-TC2N-1/2), and their corresponding negative control (NC) vectors (OE-NC empty vector and scrambled shRNA-NC, respectively) were purchased from Shanghai GenePharma Co., Ltd. AGS cells ($1x10^4$ cells/well) were seeded into a six-well culture plate and cultured to 60-70% confluence. The vectors (500 ng/μl) were transfected into AGS cells at a dose of 10 nM using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following 48 h of transfection at 37˚C, cells were harvested for reverse transcription-quantitative PCR (RT-qPCR) and western blotting analysis to determine the transfection efficiency.

Cell treatment. To determine the function of TC2N on the sensitivity of AGS cells to chemotherapy, following transfection for 48 h, AGS cells were collected. Subsequently, the transfected AGS cells were re-seeded into 96-well plates ($1x10^4$ cells/well). Following 24 h, the cells were treated with different concentrations of cisplatin (DPP; 0, 10, 20 or 50 μmol/l), paclitaxel (0, 10, 20 or 50 nmol/l) and 5-fluorouracil (5-FU; 0, 10, 20 or 50 μmol/l; all from Sigma-Aldrich; Merck KGaA) for 24 h in a humidified atmosphere with 5% CO_2 at 37˚C, respectively. The survival rate (viability) of each group was then determined using CCK-8 assay via measuring the absorbance values at 450 nm, as described below.

Cell proliferation assay. To determine the cell proliferation rate of AGS cells, a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay was performed using transfected and non-transfected (control) cells, according to the manufacturer's protocol. Briefly, the cells were seeded into 96-well plates at a density of $1x10^4$ cells/well and incubated in a humidified atmosphere with 5% CO_2 at 37˚C for 24, 48 and 72 h. Subsequently, 10 μl CCK-8 solution was added/well at each time point following incubation for 4 h at 37˚C, and the absorbance (optical density) values were measured using a microplate reader at a wavelength of 450 nm.

Wound healing assay. A wound healing assay was performed to analyze the cell migration rate. Following 48 h of transfection, AGS cells were seeded into 6-well plates ($2x10^5$ cells/well) and cultured in a humidified atmosphere with 5% CO_2 at 37˚C. Subsequently, straight lines were scratched into the 100% confluent cell monolayer using a 20-μl pipette tip. The cell debris was removed by washing with PBS and then the cells were incubated with fresh serum-free RPMI-1640 medium. Following 24 h of incubation at 37˚C, the wound healing process was analyzed under a light microscope (magnification, x100; CKX41, Olympus Corporation). The migratory distance was measured using ImageJ software version 1.46 (National Institutes of Health). Migration rate was determined using representative images obtained at 0 and 24 h using the following formula: Migration rate (%) = (migration distance/original distance) x100. The original distance represented the width of the wound at 0 h, and the migration distance represented the original width of the wound at 0 h, minus the width of the wound at 24 h. The relative migration rate was presented as the fold change relative to the control group.

Cell invasion assay. A Transwell Matrigel assay was performed to determine the cell invasion rate. At 48 h post-transfection, $2x10^4$ cells were suspended in serum-free RPMI-1640 medium and plated into the upper chambers of Transwell plates, which were precoated with 40 μl Matrigel (BD Biosciences) at 37˚C for 30 min. Complete RPMI-1640 medium supplemented with 10% FBS was plated in the lower chambers. Following incubation for 24 h at 37˚C, non-invasive cells remaining in the upper chamber were removed using a cotton swab, while the invasive cells in the lower chamber were fixed with 4% formaldehyde for 10 min at room temperature and stained with 0.05% crystal violet for 10 min at room temperature. Stained cells were visualized and counted using a light microscope (magnification, x100).

Cell cycle assay. Flow cytometric analysis was performed to determine whether TC2N regulated the growth phase of AGS cells. Following 48 h of transfection, cells were harvested by centrifugation (4˚C; 1,000 x g; 5 min) and fixed with 70% ethanol at -20˚C overnight. Cells were washed twice with PBS and stained with a Cell Cycle Detection Kit (Nanjing KeyGen Biotech Co., Ltd.) containing 200 μl PBS, 1 μl propidium iodide (1 mg/ml) and 1 μl RNase (10 mg/ml) for 20 min

at 37˚C in the dark. Cell cycle distribution was analyzed using flow cytometry (CYTOMICS FC 500; Beckman Coulter, Inc.) and BD CellQuest Pro version 5.1 software (BD Biosciences).

RNA extraction and RT-qPCR. Total RNA from the cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration and purity of the RNA was detected using the A_{260}/A_{280} ratio of 1.8-2.0 with NanoDrop 2000 (Thermo Fisher Scientific, Inc.). A total of 1 μg total RNA was reverse transcribed into cDNA using the PrimeScript RT Master mix kit (Takara Bio, Inc.). The following conditions were used for the reverse transcription: 42˚C for 2 min, 37˚C for 15 min and 85˚C for 5 sec. qPCR was subsequently performed using the SYBR Premix Ex Taq kit (Takara Bio, Inc.). The following primer pairs were used for qPCR: TC2N forward, 5'-TGGCTGTACTGAGGATTA TTTGC-3' and reverse, 5'-TGTGAAGGAGTTTCTTGTGTC C-3'; and GAPDH forward, 5'-CCATCTTCCAGGAGCGAG AT-3' and reverse, 5'-TGCTGATGATCTTGAGGCTG-3'. The following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 10 min; 40 cycles at 94˚C for 2 min and 60˚C for 50 sec; and a final extension at 60˚C for 1 min. The relative mRNA expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (13) and normalized to the GAPDH loading control. All experiments were repeated ≥3 times.

Western blotting. Total protein was extracted from cells in six-well plates on ice using RIPA lysis buffer (Invitrogen; Thermo Fisher Scientific, Inc.). The lysate was centrifuged at 12,000 x g at 4˚C for 10 min and the supernatant was collected. Total protein was quantified using a Bradford assay and equal amounts of protein (25 μg per lane) were separated via 12% SDS-PAGE at 120 V. The separated proteins were subsequently transferred onto PVDF membranes (EMD Millipore) and blocked at room temperature with 5% skimmed milk for 1 h. The membranes were then incubated overnight at 4˚C with the following primary antibodies (all diluted 1:1,000): Anti-TC2N (cat. no. PA5-32086; Invitrogen; Thermo Fisher Scientific, Inc.), anti-cyclin D1 (cat. no. ab134175; Abcam), anti-CDK4 (cat. no. ab108357; Abcam), anti-cyclin E1 (cat. no. ab33911; Abcam), anti-matrix metalloproteinase (MMP)2 (cat. no. ab92536; Abcam), anti-MMP9 (cat. no. ab38898; Abcam), anti-N-Myc downstream regulated gene 1 (NDRG1; cat. no. ab124689; Abcam), anti-P-glycoprotein (P-gp; cat. no. ab170904; Abcam) and anti-GAPDH (cat. no. ab9485; Abcam). Following the primary antibody incubation, the membranes were washed three times with TBS-0.05% Tween-20 (Sigma-Aldrich; Merck KGaA) and incubated with a mouse anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3,000; cat. no. sc-2357; Santa Cruz Biotechnology, Inc.). Protein bands were visualized using an ECL western blotting substrate (GE Healthcare) and semi-quantitatively analyzed using Quantity One version 4.4.0 software (Bio-Rad Laboratories, Inc.).

Statistical analysis. All data are presented as the mean ± SD from ≥3 independent experiments. Statistical analysis was performed using SPSS version 18.0 software (SPSS, Inc.). Statistical significance was analyzed using a one-way ANOVA, followed by a Tukey's post hoc test for multiple comparisons (Figs. 2-5), whereas the expression levels of TC2N in normal tissues and tumors tissues were analyzed using a U-Mann Whitney test (Fig. 1A). Low and high expression levels of TC2N in gastric cancer samples were defined according to the median, and those above the median were defined as high expression and those under the median were defined as low expression. The prognosis was analyzed using a Kaplan-Meier test, followed by a log-rank test. The association between TC2N expression levels and clinical parameters were analyzed using a U-Mann Whitney test between 2 groups, or a Kruskal Wallis test for >2 groups (Fig. 1B and D). $P<0.05$ was considered to indicate a statistically significant difference.

Results

TC2N expression levels are upregulated in gastric cancer tissues and cell lines. The data from TCGA database revealed that the expression levels of TC2N were significantly upregulated in gastric cancer tissues compared with the adjacent tumor tissues (Fig. 1A). Notably, TC2N was discovered to be differentially expressed in patients with gastric cancer depending on the age; the expression levels of TC2N were significantly upregulated in patients of ≥65 years old compared with patients that were <65 years old, excluding those without clinical information on age (Fig. 1B). In addition, the survival rate in patients with high expression levels of TC2N was observed to be increased compared with patients with low expression levels of TC2N, excluding the patients without clinical information on survival (Fig. 1C); however, there was no significant difference identified between the two groups in the overall survival rate. Furthermore, the expression levels of TC2N were indicated to be independent of the tumor stage of patients, as no significant differences were observed between patients with different stages of gastric cancer, excluding the patients without information on the clinical stage (Fig. 1D). These findings indicated that the expression levels of TC2N may be upregulated in gastric cancer; however, this was likely to be independent of the degree of malignancy.

To further investigate the role of TC2N, TC2N expression levels in GES1 and AGS cells were analyzed using RT-qPCR and western blotting. The present data demonstrated that compared with the GES1 cells, the mRNA and protein expression levels of TC2N were significantly upregulated in AGS cells (Fig. 2A-C).

TC2N regulates the cell proliferation of AGS cells. To determine the function of TC2N in gastric cancer, the effects of TC2N knockdown or overexpression on AGS cell proliferation were analyzed. Both the mRNA and protein expression levels of TC2N were significantly downregulated following the transfection with shRNA-TC2N-1/2 compared with the shRNA-NC group (Fig. 3A and B), and shRNA-TC2N-1 was selected for experiments due to its higher knockdown efficacy. The expression levels of TC2N were significantly upregulated following the transfection with OE-TC2N compared with the OE-NC group (Fig. 3C and D). Flow cytometric analysis revealed a significant increase in the percentage of cells in the G0/G1 phase and a decrease in the percentage of

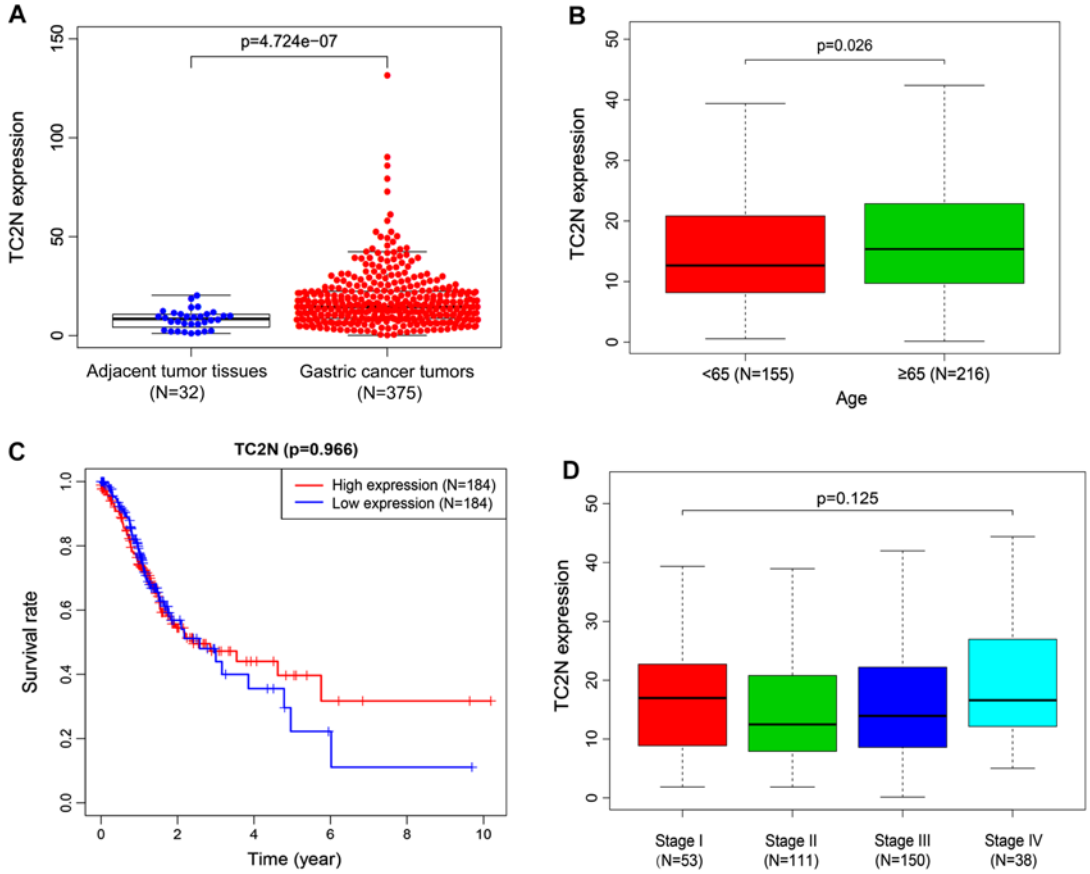

Figure 1. TC2N expression levels in gastric cancer. Data from TCGA were analyzed to determine the expression levels of TC2N in (A) Adjacent tumor tissues and tumor tissue from patients with gastric cancer and (B) patients with gastric cancer of ≥65 or <65 years old. (C) Survival analysis based on TC2N expression levels in patients with gastric cancer from TCGA. (D) Analysis of TC2N expression levels in different stages of gastric cancer using data from the TCGA. TC2N, Tac2-N; TCGA, The Cancer Genome Atlas.

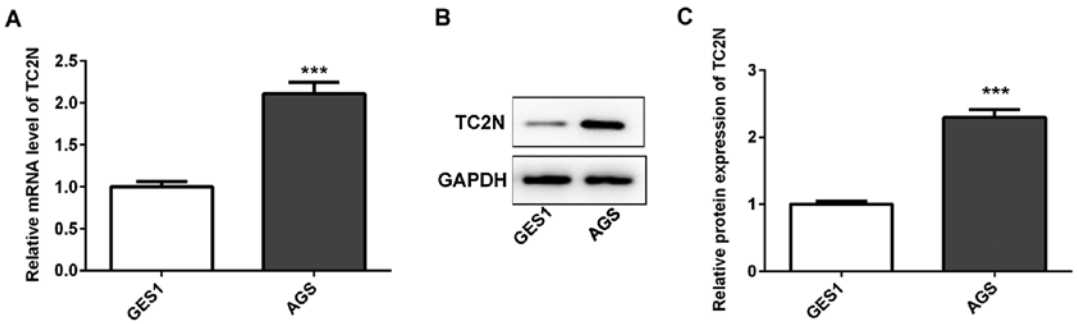

Figure 2. TC2N expression levels are upregulated in gastric cancer cell lines. The normal gastric mucosa cell line GES1 and gastric cancer cell line AGS were used to analyze the (A) mRNA and (B) protein expression levels of TC2N using reverse transcription-quantitative PCR and western blotting, respectively. (C) Semi-quantification of the expression levels presented in part (B). ***P<0.001 vs. GES1. TC2N, Tac2-N.

cells in the S phase following TC2N knockdown compared with the shRNA-NC group (Fig. 3E and F). By contrast, the percentage of cells in the G0/G1 phase was significantly decreased, while the percentage in the S phase was significantly increased following TC2N overexpression compared with the OE-NC group. The effect of TC2N knockdown or TC2N overexpression on G2/M was not significantly different (Fig. 3E and F). These results indicated that TC2N knockdown may induce G1/S phase arrest, whereas TC2N overexpression may inhibit G1/S phase arrest. CCK-8 analysis also revealed that the cell proliferative ability was significantly decreased in the shRNA-TC2N group compared with the shRNA-NC group at 72 h, whereas the proliferative ability was significantly increased following TC2N overexpression compared with the OE-NC group at 72 h (Fig. 3G). Moreover, the protein expression levels of cyclin D1, CDK4 and cyclin E1 were significantly downregulated following TC2N knockdown and upregulated following TC2N overexpression, compared with their respective NCs (Fig. 3H), which is consistent with the previous results from CCK-8 and flow cytometry assays. These findings indicated that TC2N may have a regulatory role over cell proliferation, as the knockdown of TC2N inhibited cell

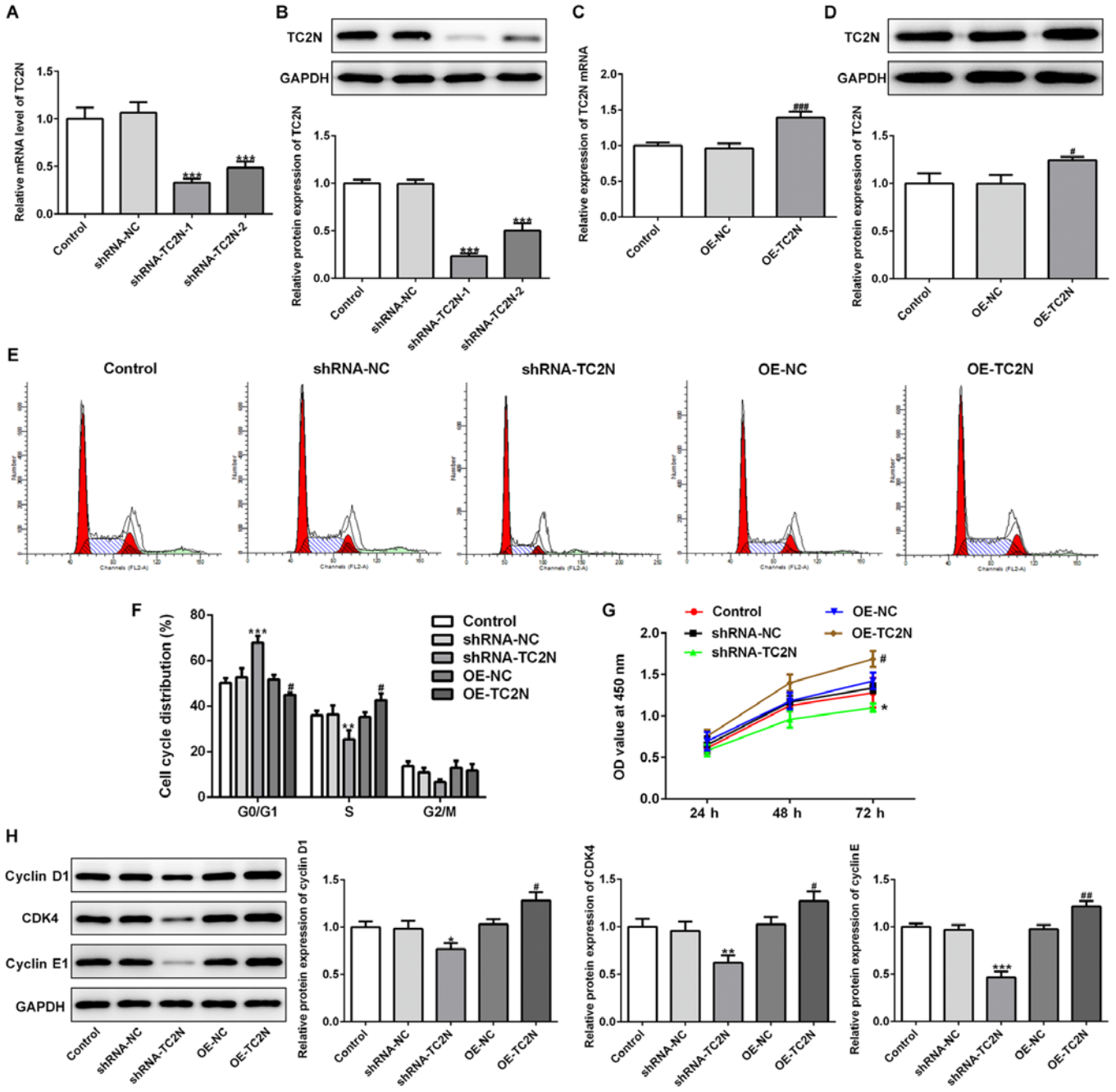

Figure 3. TC2N regulates the cell proliferation of AGS cells. To determine the function of TC2N in gastric cancer, AGS cells were transfected with shRNA-TC2N and the (A) mRNA and (B) protein expression levels of TC2N were analyzed using RT-qPCR and western blotting, respectively. AGS cells were transfected with OE-TC2N and the (C) mRNA and (D) protein expression levels of TC2N were determined using RT-qPCR and western blotting, respectively. (E) Cell cycle distribution was analyzed using flow cytometric analysis. (F) Quantification of cell cycle distribution presented in part (E). (G) Cell Counting Kit-8 assay was performed to determine the cell proliferation rate. (H) Protein expression levels of cyclin D1, CDK4 and cyclin E1 were analyzed using western blotting. *P<0.05, **P<0.01, ***P<0.001 vs. shRNA-NC; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. OE-NC. TC2N, Tac2-N; shRNA, short hairpin RNA; RT-qPCR, reverse transcription-quantitative PCR; OE, overexpression; NC, negative control; OD, optical density.

proliferation, whereas the overexpression of TC2N promoted cell proliferation.

TC2N regulates the cell migration and invasion of AGS cells. The effects of TC2N knockdown or overexpression on the migratory and invasive ability of AGS cells were further investigated. The wound healing and Transwell Matrigel assays demonstrated that TC2N knockdown significantly inhibited both cell migration and invasion compared with the shRNA-NC group, while TC2N overexpression significantly promoted cell migration and invasion compared with the OE-NC group (Fig. 4A-C). MMP2 and MMP9 are known to be involved in cell invasion and tumor metastasis (14). NDRG1 has been demonstrated to repress tumor metastasis, and downregulate MMP2 and MMP9 expression levels (15,16). Therefore, MMP2, MMP9 and NDRG1 were investigated in the present study to determine whether they were involved in the effects of TC2N on cell migration and invasion. The

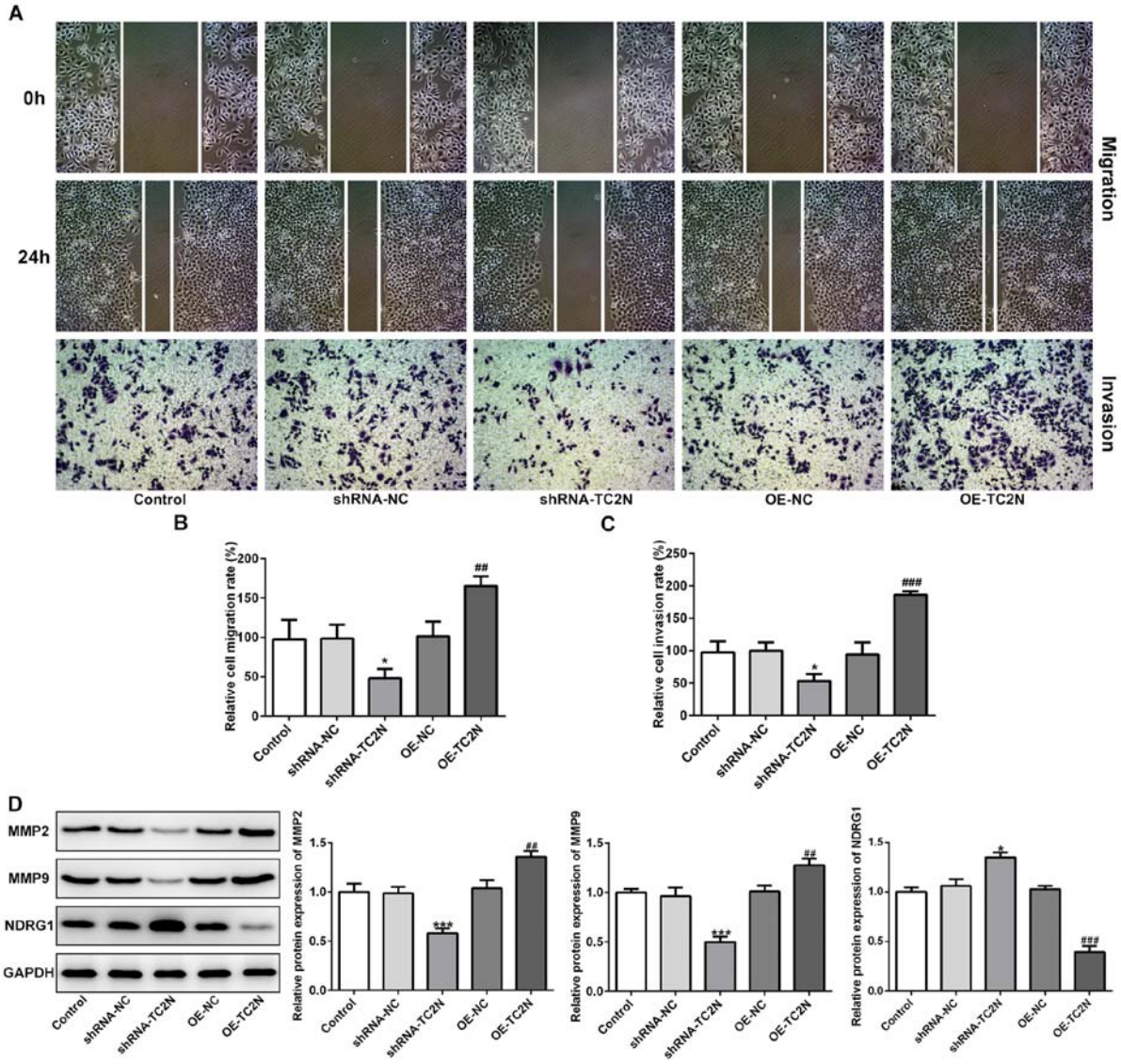

Figure 4. TC2N regulates the cell migration and invasion of AGS cells. (A) Wound healing and Transwell Matrigel assays were performed to analyze the cell migratory and invasive abilities, respectively (magnification, x 100). (B) Semi-quantification of the cell migration rate from part (A). (C) Semi-quantification of the cell invasion rate from part (A). (D) Protein expression levels of MMP2, MMP9 and NDRG1 were analyzed using western blotting. *P<0.05, ***P<0.001 vs. shRNA-NC; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. OE-NC. TC2N, Tac2-N; shRNA, short hairpin RNA; OE, overexpression; NC, negative control; MMP, matrix metalloproteinase; NDRG1, N-Myc downstream regulated gene 1.

results revealed that TC2N knockdown significantly downregulated the protein expression levels of MMP2 and MMP9, whereas the protein expression levels of NDRG1 were significantly upregulated, compared with the shRNA-NC group (Fig. 4D). Conversely, the overexpression of TC2N exhibited the opposite effects in AGS cells (Fig. 4D), indicating that TC2N may affect the gastric cancer migrative and invasive abilities partly via regulating MMP2 and MMP9 expression levels.

TC2N mediates drug resistance of AGS cells. To determine the function of TC2N on the sensitivity of AGS cells to chemotherapy, cells were treated with different concentrations of DPP, paclitaxel and 5-FU for 24 h and the survival rate of each group was detected using a CCK-8 assay. The cell survival rate was discovered to be closely associated with the drug concentration, as the survival rate of the cells reduced upon increasing the drug concentration (Fig. 5A-C). Furthermore, TC2N knockdown significantly decreased the survival rate of the cells following 50 μmol/l DPP or 5-FU or 50 nmol/l paclitaxel treatment compared with the shRNA-NC group, whereas TC2N overexpression exerted the opposite effects, indicating that TC2N knockdown may improve the sensitivity of AGS cells to DDP, paclitaxel and 5-FU. The inhibition of P-gp has been considered to be an effective approach for reversing cancer-related drug resistance (17). In the present study, TC2N knockdown significantly downregulated the expression levels of P-gp compared with the shRNA-NC group, whereas TC2N overexpression significantly upregulated the expression levels of P-gp compared with the OE-TC2N group (Fig. 5D and E), indicating that TC2N knockdown may improve the sensitivity of AGS cells to chemotherapeutics via the inhibition of P-gp expression.

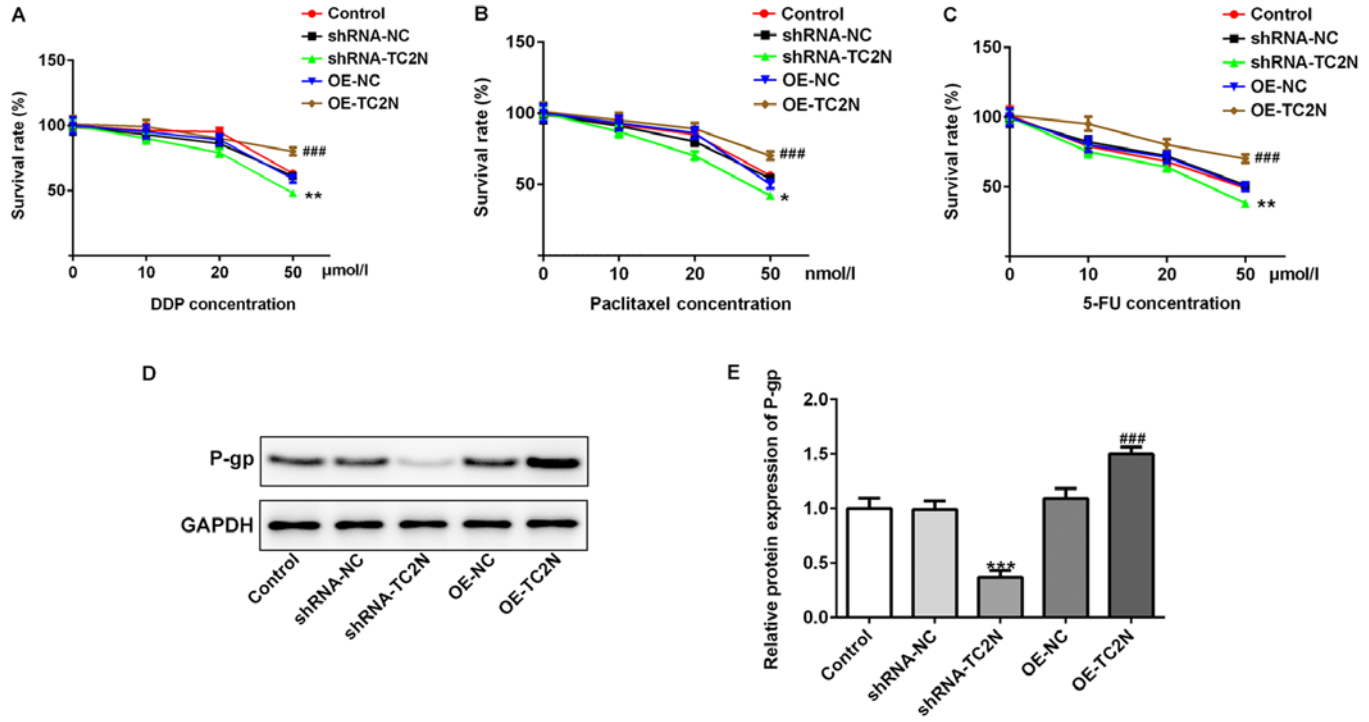

Figure 5. TC2N mediates the drug resistance of AGS cells. AGS cells were treated with different concentrations of DPP (0, 10, 20 or 50 μmol/l), paclitaxel (0, 10, 20 or 50 nmol/l) and 5-FU (0, 10, 20 or 50 μmol/l) for 24 h. The survival rate of each group following (A) DDP, (B) paclitaxel and (C) 5-FU treatment was analyzed using Cell Counting Kit-8 assays. (D) Protein expression levels of P-gp were determined using western blotting. (E) Semi-quantification of the P-gp expression levels from part (D). *P<0.05, **P<0.01, ***P<0.001 vs. shRNA-NC; $^{\#\#\#}$P<0.001 vs. OE-NC. TC2N, Tac2-N; shRNA, short hairpin RNA; OE, overexpression; NC, negative control; DPP, cisplatin; 5-FU, 5-fluorouracil; P-gp, P-glycoprotein.

Discussion

Gastric cancer is one of the most common types of malignant tumor worldwide (18). To date, surgical resection remains the only curative modality for localized gastric cancer, accompanied with perioperative chemotherapy or postoperative chemoradiotherapy to improve the outcomes (18). For advanced and metastatic disease, the combination of a fluoropyrimidine and a platinum compound-containing chemotherapy, with the incorporation of trastuzumab for the HER2-enriched population, remains the standard care for first-line therapy (3). However, due to the high degree of biological malignancy of gastric cancer itself, especially at the advanced stage, the 5-year survival rate of gastric cancer remains poor and does not exceed 30% in the USA and Europe (18). Therefore, determining the molecular mechanism underlying the development of gastric cancer is required. TC2N was recently identified as a novel oncogene in lung cancer (10,11); however, the role of TC2N in gastric cancer remains unclear. In the present study, upregulated expression levels of TC2N were identified in gastric cancer cells and tumors of patients with gastric cancer via TCGA analysis. To determine the role of TC2N in gastric cancer, further studies revealed that TC2N regulated the cell proliferation, migration and invasion of gastric cancer cells. Furthermore, the knockdown of TC2N strengthened the sensitivity of AGS cells to DPP, paclitaxel and 5-FU, and downregulated P-gp expression levels, which indicated that TC2N may be an important regulator in gastric cancer.

The overexpression of TC2N in AGS cells significantly increased the cell proliferation, and promoted cell migration and invasion, whereas the knockdown of TC2N exerted the opposite effects, which is consistent with previous studies, which reported an oncogenic function of TC2N (10,11). Cell proliferation is predominantly regulated by the cell cycle, which is a complex network of regulatory process involving multiple signaling pathways (19). For example, T-Box transcription factor TBX3 has been indicated to function as an immortalizing gene promoting proliferation via actively repressing negative cell cycle regulators, which has been associated with the c-Myc-mediated pathway (20). Moreover, xanthoxyletin has been revealed to inhibit proliferation and induce cell cycle arrest via modulating the MEK/ERK signaling pathway (20). Thus, the effect of TC2N on cell cycle progression and cell cycle-related proteins was further determined The experimental results revealed that TC2N overexpression increased the percentage of cells in the S phase, and upregulated the expression levels of cyclin D1, cyclin E1 and CDK4, while TC2N knockdown achieved the opposite effects. Cyclin D1, cyclin E1 and CDK4 all serve important roles during G1-S phase cell cycle progression, and cyclins and CDKs can directly participate in cell cycle regulation (21). In particular, cyclin D1 and cyclin E1 are important members of the cyclin family, which are responsible for cell transformation from the G1 to S phase (22). Therefore, these results implied that TC2N knockdown may result in the downregulation of cell cycle-related proteins to prolong the cell cycle, regulating the progression of gastric cancer.

To investigate the role of TC2N in drug resistance and determine the potential mechanism of action, three different types of chemotherapeutic drugs (DPP, paclitaxel and 5-FU)

were used to treat AGS cells following TC2N overexpression or knockdown. The survival rate of AGS cells varied depending on the expression levels of TC2N; TC2N knockdown significantly decreased the cell survival rate, reflecting a higher sensitivity of AGS cells to DPP, paclitaxel and 5-FU when TC2N expression was inhibited. Acquired drug resistance is one of the predominant reasons for the low cure rate and high recurrence rate in patients with gastric cancer, especially at an advanced stage (23). Thus, improving the sensitivity of cancer cells to chemotherapeutics may be an important strategy for cancer treatment. Reducing the resistance of cancer cells to chemotherapeutics has been indicated as a potential strategy to improve the therapeutic effects in numerous types of cancer (24-26). In the present study, the findings suggested that TC2N may serve as an oncogene, which not only promoted gastric cancer cell proliferation, migration and invasion, but also regulated the sensitivity of gastric cancer cells to DPP, paclitaxel and 5-FU. The predominant mechanism underlying cancer cell resistance involves the drug efflux pump, P-gp (27). P-gp pumps intracellular drugs out of cells as an energy-dependent drug efflux pump, resulting in drug resistance caused by a reduced intracellular concentration of drugs (28). In a previous study, P-gp expression levels were discovered to be upregulated in gastric cancer cells presenting with drug resistance (29). In the present study, the expression levels of P-gp were significantly upregulated following TC2N overexpression and AGS cells had a higher sensitivity to DPP, paclitaxel and 5-FU, whereas the opposite effects were observed when TC2N expression was knocked down. These results indicated that TC2N may influence the sensitivity of AGS cells to drugs via regulating P-gp expression levels.

To the best of our knowledge, this study was the first to determine a previously unknown role of TC2N in gastric cancer and uncover its potential mechanism; however, further experiments and clinical data are required to validate and make the conclusions reliable. However, there are some limitations to the present study. Firstly, the knockdown of TC2N significantly improved the sensitivity of AGS cells to chemotherapeutics; however, whether the improvement of drug sensitivity is also reflected in the processes of cell migration, invasion and cell cycle distribution remains to be elucidated. Secondly, the data in this study were predominantly based on cell lines, and data from an animal model were lacking; thus, *in vivo* experiments are required to verify the therapeutic effect of TC2N in gastric cancer. Finally, further clinical data are also required to determine the diagnostic and prognostic value of TC2N in gastric cancer. These limitations are worthy of being studied and our future work aims to investigate these points further.

In conclusion, the present study provided evidence that suggested that TC2N expression levels may be upregulated in AGS cells. The knockdown of TC2N expression inhibited the proliferation, migration and invasion of cells, in addition to inducing cell cycle G1/S phase arrest. Furthermore, the knockdown of TC2N partially reversed the cell resistance to DPP, paclitaxel and 5-FU, which was indicated to be partly through inhibiting P-gp expression levels, whereas TC2N overexpression exerted the opposite effects on AGS cells. Thus, TC2N may serve as an important regulator in gastric cancer.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

PJ conceptualized and designed the study, wrote and proof-read the manuscript. PZ created the figures and provided important comments during the revision of the manuscript. LS, PZ and JW participated in the literature collection, experiment conduction and analysis of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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