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Upregulation of TMEFF2 is involved in the antiproliferative effects of vitamin C and tyrphostin AG490 on GES-1 and AGS cells

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Abstract. Transmembrane protein with epidermal growth factor (EGF)-like and two follistatin motifs 2 (TMEFF2) is downregulated in human gastric cancer, and its levels are associated with tumor aggressiveness. Herein, a positive correlation was identified between serum vitamin C levels (μg/ml) and mRNA levels of TMEFF2 in gastric cancer tissue. TMEFF2 silencing promotes cell proliferation in GES-1 normal human gastric epithelial cells and AGS human gastric adenocarcinoma cells. Notably, vitamin C and AG490 exerted antiproliferative effects on the two cell lines. The present study demonstrated that small interfering (si)-RNA-TMEFF2 exerts pro-proliferative effects on GES-1 and AGS cells. The results revealed that vitamin C significantly inhibited the growth of GES-1 and AGS cells by reducing cell viability, decreasing the expression of proliferating cell nuclear antigen (PCNA), and blocking the STAT3 pathway. Moreover, siRNA-TMEFF2-induced enhanced cell viability and PCNA expression were significantly reversed by additional vitamin C treatment; notably, markedly enhanced TMEFF2 expression was observed. Upregulated TMEFF2 expression was observed in association with the antiproliferative effect of AG490. In conclusion, serum vitamin C content (μg/ml) was positively correlated with the mRNA levels of TMEFF2 in gastric cancer tissue. Exploring novel drugs that target TMEFF2 is a potential therapeutic strategy for blocking human GC.

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Introduction

Gastric carcinoma (GC) is a common and very aggressive malignancy of the digestive system with poor early diagnosis. Adenocarcinoma is a multifactorial disease that usually arises from the epithelium and is categorized into two pathological variants: intestinal and diffuse (1). Currently, GC therapies are mainly focused on surgical removal, radiotherapy and chemotherapy. However, GC therapies are often accompanied by limited efficacy, drug resistance, or even serious side effects (2). Despite numerous improvements in GC therapies, GC patient survival is less than 40% (3). Therefore, identifying highly effective and safe medicines is necessary.

Targeted molecular therapy for GC has attracted interest (2). Transmembrane protein with epidermal growth factor (EGF)-like and two follistatin motifs 2 (TMEFF2) is a potential biomarker for human GC (4). The downregulation of TMEFF2 frequently occurs in human gastric cancer tissue, and its levels correlate with tumor aggressiveness and survival time in patients (5). In contrast, upregulated TMEFF2 suppresses tumor progression and may be a potential effective strategy for preventing human GC. TMEFF2 plays an important role in regulating the cell cycle, apoptosis, and DNA repair and is thus directly associated with tumorigenesis in GC cells (4). Therapeutic strategies for GC that target TMEFF2 include proliferation inhibitors, apoptosis promoters, and genetic stability regulators (6), providing possibilities for exploring more effective medicines to block human GC.

Vitamin C, which is known as a water-soluble hexose derivative, serves as a natural antioxidant (7). Dietary vitamin C prevents the occurrence of human GC by increasing mucosal immune responses, eliminating free radicals, and decreasing the N-nitrosamine content in gastric juice (8). Epidemiological studies revealed that vitamin C deficiency in humans is linked to more severe *H. pylori*-associated gastritis and a higher risk of human GC (9). Serum vitamin C levels may be used to predict GC risk (10). Higher concentrations of circulating vitamin C decrease GC risk. TMEFF2 is a transmembrane protein that participates in various cellular functions by binding to protein tyrosine phosphatase (SPH-1), a key regulator of gastric carcinogenesis, and is thus closely related to tumorigenesis in GC cells (6). Evidence suggests that proinflammatory cytokines, for example, interleukin (IL)-1β and tumor necrosis factor (TNF)-α, induce TMEFF2 ectodomain shedding via the

activation of the nuclear factor (NF)-κB signaling pathway and thus lead to protease inactivity (11). Vitamin C is a highly effective anti-inflammatory agent that reduces the activation of the NF-κB signaling pathway (12). However, data on the interaction between serum vitamin C concentrations and TMEFF2 expression are currently scarce.

Vitamin C prevents the proliferation of normal human gastric epithelial cells (GES-1) (13) and AGS human gastric adenocarcinoma cells (9,14), depending in part on its antioxidant properties. AGS cells are a type of GC cell line. However, the exact molecular mechanisms are not completely elucidated. TMEFF2 silencing promotes the proliferation of human gastric cancer cells or normal gastric epithelial cells, such as AGS, MKN28, SGC7901, MGC803, or GES-1 cells; in contrast, upregulated TMEFF2 inhibits cell proliferation. However, little is known about the roles of TMEFF2 in the antiproliferative effects of vitamin C and tyrphostin AG490 on human gastric cancer cells.

In the present study, fifty patients with GC from Jiangsu, China, were enrolled. We tested vitamin C concentrations (μg/ml) in the peripheral blood and mRNA levels of TMEFF2 in gastric cancer tissue. Our study suggested the existence of a positive correlation between serum vitamin C concentrations and TMEFF2 expression in human gastric cancer. In addition, the siRNA-TMEFF2-induced proliferation of GES-1 and AGS cells was successfully established. Vitamin C and tyrphostin AG490, which are known as antiproliferative agents in GC cells, were used for treatment. Our data indicated the involvement of TMEFF2 in the antiproliferative effects of vitamin C and AG490 on these two cell types.

Materials and methods

Plasma vitamin C assay. Fifty patients with GC were recruited. The present study was approved by Yancheng Third People's Hospital (Jiangsu, China), and written informed agreement was obtained from each participant involved in this study. Vitamin C levels (μg/ml) in the peripheral blood of GC patients were determined using commercially available kits (www.nijcbio.com), according to the manufacturer's instructions.

Cell culture and treatment. Cell culture medium was prepared with the following ingredients (9:1:0.01 (v/v/v)): RPMI-1640 (SH30809.01B; HyClone; GE Healthcare Life Sciences, Logan, UT, USA); fetal bovine serum (16000-044, Gibco); and penicillin/streptomycin (100x, P1400-100; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), respectively. The concentrations of penicillin and streptomycin in the medium were 100 U/ml and 100 mg/ml, respectively. After preparation, the mixture was stored at 4˚C. GES-1 and AGS cells were cultured in this medium at 37˚C under 5% CO_2. Cells in log-phase growth were used in the following study.

To establish the knockdown of TMEFF2 within GES-1 and AGS cells, Lipofectamine 2000 (11668-019; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to transfect cells with siRNA-TMEFF2 according to the manufacturer's protocol. After transduction for 48 h, the efficacy of stable TMEFF2 silencing was verified by RT-PCR and western blotting. The siRNA-TMEFF2 sequences targeting TMEFF2 mRNA (GenBank NM_016192.3) were as follows: 5'-GCUGGAAUUGCUCUGGUUATT-3' (sense) and 5'-UAA CCAGAGCAAUUCCAGCTT-3' (antisense) at position 567-585; 5'-GGAGACAUCCACCUGUGAUTT-3' (sense) and 5'-AUCACAGGUGGAUGUCUCCTT-3' (antisense) at position 880-898; and 5'-GCAGGUGUGAUGCUGGUUATT-3' (sense) and 5'-UAACCAGCAUCACACCUGCTT-3' (antisense) at position 1275-1293. In addition, a scrambled siRNA was included as a negative control (siRNA-NC).

To study the effects of vitamin C on the proliferation of GES-1 and AGS cells, cells in log-phase growth were treated with vitamin C (an L form of ascorbic acid, A7506; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at concentrations of 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/l and then cultured as described above.

To study whether the antioxidants vitamin C, vitamin E, N-acetyl cysteine (NAC), resveratrol, and glutathione (GSH) could regulate TMEFF2 expression, GES-1 and AGS cells were treated separately with 20 μmol/l vitamin C, 20 μmol/l vitamin E (CAS no: 14638-18-7; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), 5 mmol/l NAC (CAS no: 616-91-1; Sigma-Aldrich; Merck KGaA), 50 μmol/l resveratrol (CAS no: 501-36-0, Sigma, USA), and 40 μg/l GSH (CAS no: 70-18-8; Shanghai Aladdin Bio-Chem Technology Co., Ltd.) and then cultured as described above.

To study the effects of vitamin C and AG490 on the proliferation of siRNA-TMEFF2-transfected GES-1 and AGS cells, after transduction for 48 h, the cells were treated separately with vitamin C (10^{-7} and 10^{-6} mol/l) and 30 μmol/l AG490 (Sigma-Aldrich; Merck KGaA) and then cultured as described above.

Cell proliferation assay. A Cell Counting Kit-8 (CCK-8) kit (CP002, SAB) was used to assess cell viability. Cells with or without siRNA-TMEFF2 transfection were fixed in a 96-well plate ($3x10^3$ cells/well) in 100 μl of culture medium. After treatment, 10 μl of CCK-8 solution was added to each well at 0, 24, 48 or 72 h and incubated for an additional 1 h. The optical density (OD) was detected via a microplate reader (Thermo Fisher Scientific, Inc.) at 450 nm.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). To assess the mRNA expression of TMEFF2 in gastric cancer tissue, as well as in GES-1 and AGS cells, RT-qPCR was conducted following the previously reported procedure (6). Primers targeting the mRNA sequence of TMEFF2 (GenBank NM_001305134.1) at position 851-1089 were designed as follows: 5'-CATGAAGGCTCTGGAGAA AC-3' (forward) and 5'-CATCGACCCAAAGACATGAC-3' (reverse). Primers targeting the mRNA sequence of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank NM_0,012,56799.1) at position 1065-1174 were designed as follows: 5'-CACCCACTCCTCCACCTTTG-3' (forward) and 5'-CCACCACCCTGTTGCTGTAG-3' (reverse). The $2^{-\Delta\Delta Cq}$ method was used for relative quantification (15).

Western blot analysis. Western blotting methods were conducted as previously reported (6). The following antibodies were adopted: A TMEFF2 antibody (1 μg/ml, Ab50002; Abcam), a PCNA antibody (1:1,000 dilution, cat. no: 13110, CST), a STAT3 antibody (1:100 dilution, Ab50761; Abcam),

a p-STAT3 antibody (1:200,000 dilution, cat. no: Ab76315, Abcam), and a GAPDH antibody (1:2,000 dilution, cat. no: 5174; Cell Signaling Technology, Inc., Danvers, MA, USA).

Statistical analysis. Triplicate experiments were conducted. The data are presented as the mean (n=3) ± standard error of the mean. Pearson's correlation coefficients (r) and linear regression were carried out to analyze the correlation between serum vitamin C levels (μg/ml) and mRNA levels of TMEFF2 in gastric cancer tissue. One-way analysis of variance with Tukey's post hoc test was employed to assess differences sing GraphPad Prism v7.00 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Positive correlation between vitamin C and TMEFF2 in GC patients. To study the association between vitamin C and TMEFF2 in the development of human GC, fifty GC patients from Jiangsu, China, were recruited. Vitamin C concentrations in the peripheral blood and the mRNA levels of TMEFF2 in gastric tissue from GC patients were measured. The serum vitamin C levels of healthy people living in Wuwei City, a GC endemic region in China, ranged from 2.9-8.5 μg/ml (16). In Europe, the mean concentrations of plasma vitamin C in patients of different ages with GC ranged from 31.4-36.6 μmol/ml (5.5-6.4 μg/ml) but were 30.5-35.0 μmol/ml (5.4-6.2 μg/ml) in the corresponding group of healthy people (17). In our present study, the vitamin C levels (μg/ml) in peripheral blood from fifty GC patients ranged from 1.94-7.67 μg/ml. In addition, RT-PCR was performed to measure the mRNA levels of TMEFF2 in gastric tissue from GC patients. Pearson's r and linear regression were carried out to analyze correlations. Fig. 1 reveals a positive correlation between vitamin C content and TMEFF2 mRNA levels in GC patients (r^2=0.9289, P<0.0001).

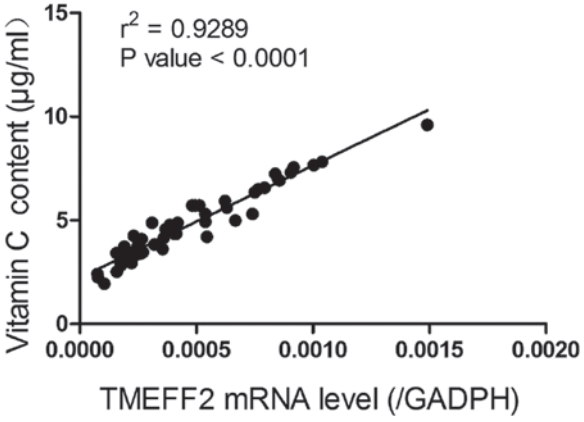

Figure 1. Positive association between vitamin C and TMEFF2 in gastric carcinoma patients. Vitamin C concentrations in the peripheral blood were assessed using a commercially available vitamin C detection kit, and reverse transcription-quantitative polymerase chain reaction was performed to measure the mRNA levels of TMEFF2 in human gastric cancer tissue (n=50). TMEFF2, two follistatin motifs 2.

Effect of vitamin C on cell proliferation in the GES-1 and AGS cell lines. GES-1 and AGS cells were treated with different concentrations of vitamin C (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/l) (Fig. 2). At 0, 24, 48 and 72 h, CCK-8 was performed to detect cell viability. In Fig. 2A and C, with vitamin C treatment, significantly decreased cell viability was observed in a dose-dependent manner, with minimum values observed at 10^{-6} mol/l, confirming the inhibitory effects of vitamin C on these two cell lines. We selected two optimal concentrations (10^{-7} and 10^{-6} mol/l) of vitamin C to treat GES-1 and AGS cells for 48 h, and protein levels of proliferating cell nuclear antigen (PCNA), which is known as a proliferation marker, and the signal transducer and activator of transcription 3 (STAT3) pathway were then evaluated. As shown in Fig. 2B and D, vitamin C had almost no effect on total STAT3 levels but decreased the levels of phosphorylated-STAT3 (p-STAT3) and PCNA in a dose-dependent manner, further confirming the antiproliferative effect of vitamin C at the molecular level.

Vitamin C promotes TMEFF2 expression in the GES-1 and AGS cell lines. In the present study, we assessed the effects of vitamin C and several other antioxidants on the protein and mRNA levels of TMEFF2 in GES-1 and AGS cells. Cells were treated separately with vitamin C (20 μmol/l), vitamin E (20 μmol/l), NAC (5 mmol/l), GSH (40 μg/l), and resveratrol (50 μmol/l). After treatment for 48 h, the protein and mRNA levels of TMEFF2 in the two cell lines were determined. As shown in Fig. 3, vitamin C and NAC significantly increased the protein and mRNA levels of TMEFF2, with the strongest effect observed in the vitamin C-treated group, suggesting a positive correlation between vitamin C and TMEFF2 at the molecular level. Unfortunately, vitamin E, resveratrol, and GSH enhanced the expression of TMEFF2 slightly or moderately.

Successful establishment of a pro-proliferative effect in GES-1 and AGS cells after siRNA-TMEFF2 treatment. Fig. 4 shows significantly decreased protein and mRNA levels of TMEFF2 in GES-1 and AGS cells, demonstrating the successful establishment of TMEFF2 silencing in the two cell lines. In parallel, cell viability and protein levels of PCNA were also assessed in siRNA-TMEFF2-transfected cells. Our data suggested that the knockdown of TMEFF2 significantly inhibited cell viability and downregulated PCNA levels (Fig. 5), demonstrating the antiproliferative effect of TMEFF2 silencing on GES-1 and AGS cells.

Upregulated TMEFF2 expression is directly involved in the antiproliferative effects of vitamin C and AG490 on GES-1 and AGS cells. To explore whether upregulated TMEFF2 was involved in the antiproliferative effect of vitamin C or AG490 on GES-1 and AGS cells, siRNA-TMEFF2-transfected cells were treated with vitamin C (10^{-6} mol/l) or AG490 (30 μmol/l) for 48 h. Cell viability, mRNA levels of TMEFF2, and protein levels of TMEFF2 and PCNA were assessed. As shown in Fig. 6, the knockdown of TMEFF2 significantly promoted cell viability, decreased the expression of TMEFF2 and increased the expression of PCNA, and these changes could be obviously reversed with additional vitamin C or AG490 treatment. Vitamin C exerted effects similar to those of AG490; cell viability and PCNA expression were significantly reduced and basal TMEFF2 expression was remarkably increased compared with the values of the siRNA-TMEFF2 group,

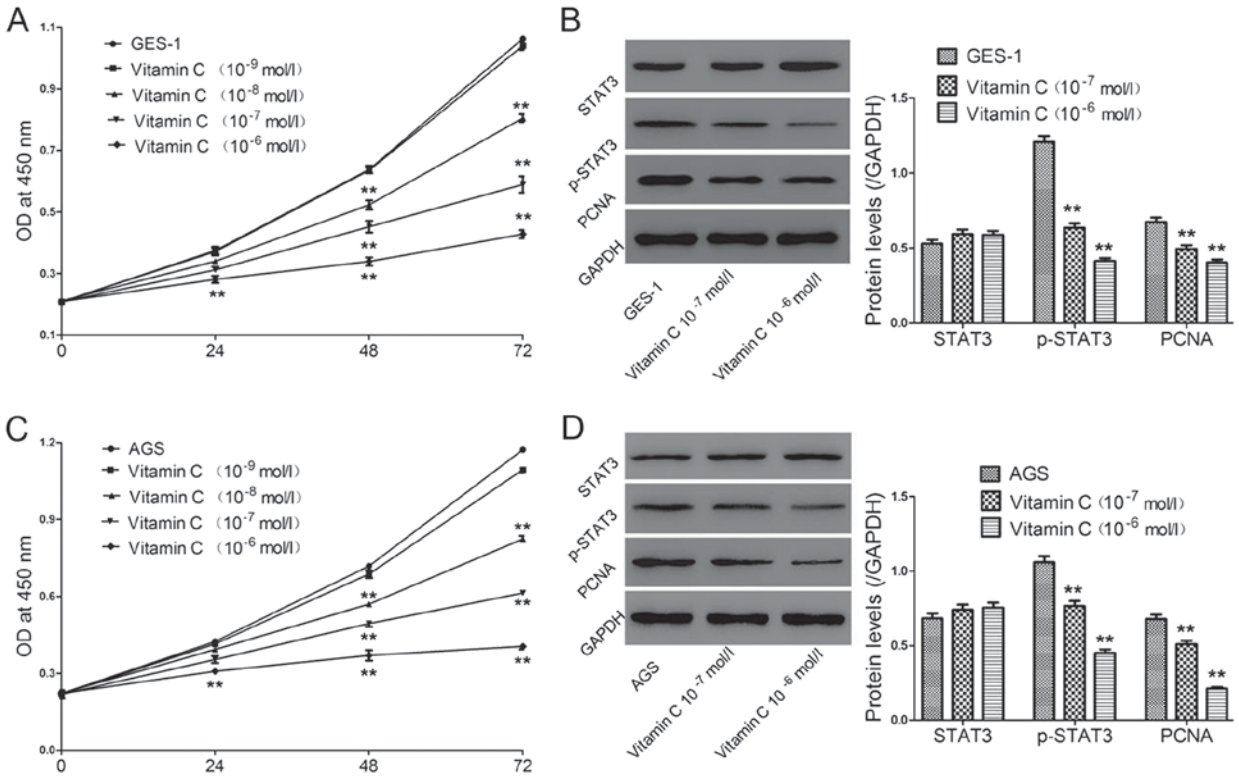

Figure 2. Effect of vitamin C on cell proliferation in the GES-1 and AGS cell lines. Cells were treated with vitamin C at concentrations of 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/l and (A) GES-1 and (C) AGS cell viability was assessed by the Cell Counting Kit-8 method at 0, 24, 48 and 72 h. In addition, (B) GES-1 and (D) AGS cells were treated with vitamin C at concentrations of 10^{-7} and 10^{-6} mol/l for 48 h, and the expression levels of p-STAT3 and PCNA were quantified via western blot analysis. **P<0.01 vs. GES-1 or AGS cells only. STAT3, signal transducer and activator of transcription 3; p-, phosphorylated; PCNA, proliferating cell nuclear antigen; OD, optical density.

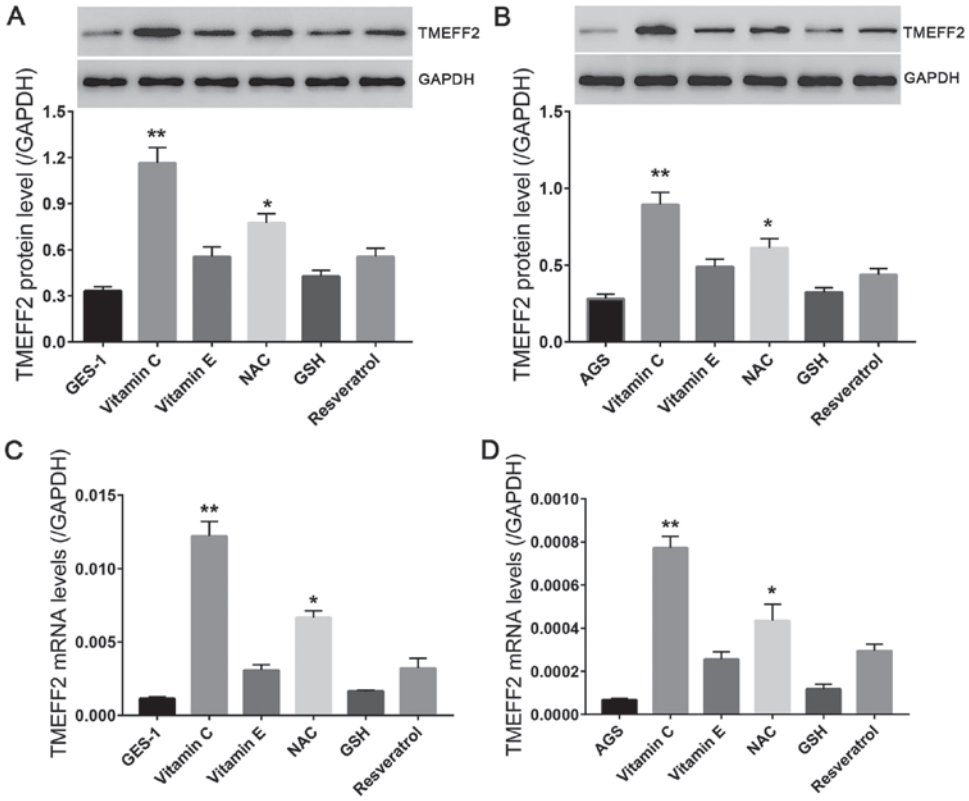

Figure 3. Effects of different antioxidants on TMEFF2 levels in GES-1 and AGS cells. Cells were treated with vitamin C (20 μmol/l), vitamin E (20 μmol/l), NAC (5 mmol/l), GSH (40 μg/l) and resveratrol (50 μmol/l) for 48 h, and western blotting and reverse transcription-quantitative polymerase chain reaction analysis were conducted to assess TMEFF2 protein expression in (A) GES-1 and (B) AGS cells, and mRNA levels in (C) GES-1 and (D) AGS cells. *P<0.05 and **P<0.01 vs. GES-1 or AGS cells only. TMEFF2, two follistatin motifs 2.

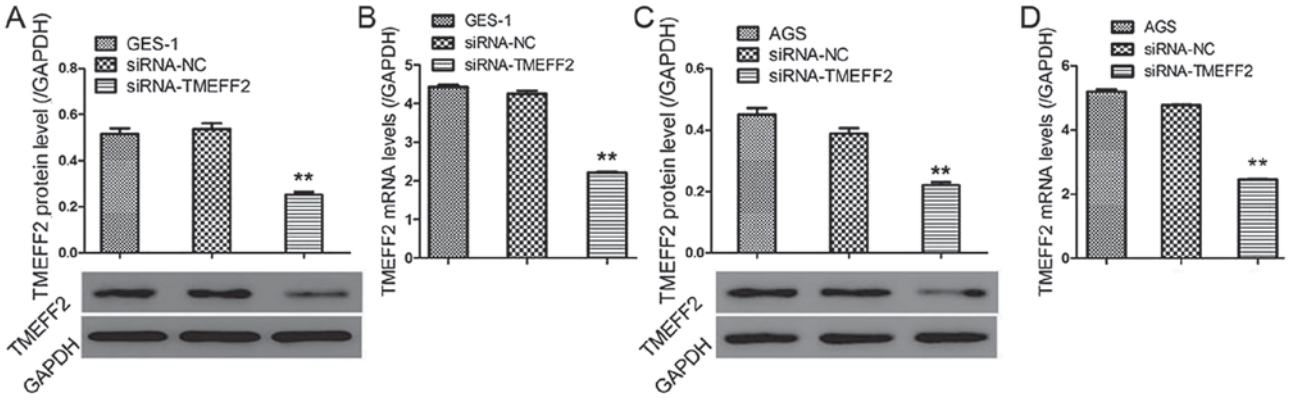

Figure 4. Successful establishment of TMEFF2 silencing in GES-1 and AGS cells. Western blotting results from (A) GES-1 and (C) AGS cells indicating the protein levels of TMEFF2. Reverse transcription-quantitative polymerase chain reaction analysis of (B) GES-1 and (D) AGS cells demonstrating the mRNA levels of TMEFF2. **P<0.01 vs. siRNA-NC. TMEFF2, two follistatin motifs 2; siRNA, small interfering RNA; NC, negative control.

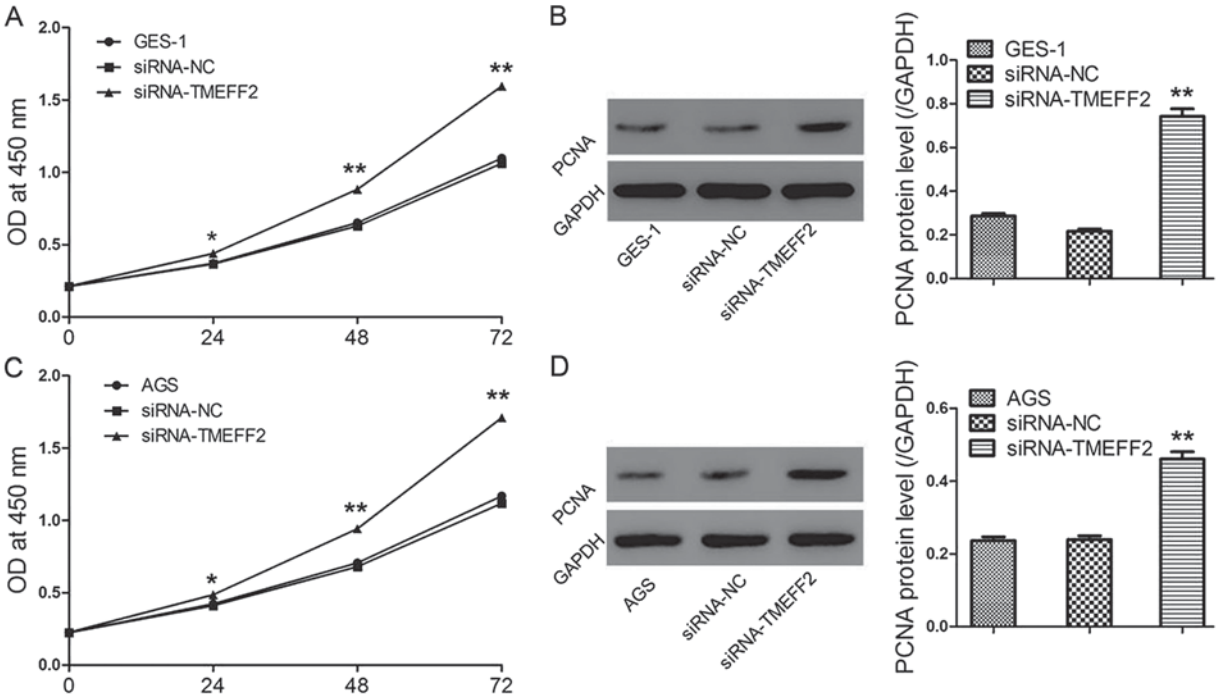

Figure 5. siRNA-TMEFF2 promotes cell proliferation in GES-1 and AGS cells. (A) CCK-8 and (B) Western blot analyses were performed to assess cell viability and PCNA expression, respectively in GES-1. (C) CCK-8 and (D) Western blot analyses were performed to assess cell viability and PCNA expression, respectively in AGS cells. *P<0.05 and **P<0.01 vs. siRNA-NC. CCK-8, Cell Counting Kit-8; TMEFF2, two follistatin motifs 2; siRNA, small interfering RNA; NC, negative control; PCNA, proliferating cell nuclear antigen; OD, optical density.

demonstrating that vitamin C and AG490 inhibited cell proliferation by increasing TMEFF2 levels.

Discussion

Multiple steps and multiple factors contribute to the occurrence of GC. TMEFF2 dysregulation is involved in human GC and is an auxiliary indicator for the early diagnosis of GC (6). Vitamin C exerts protective effects on human gastric tissue and is a potential chemopreventive drug for human GC (18). In 2005, Luo *et al* (19) reported that the average levels of serum vitamin C in 293 healthy people were 5.74±2.79 mg/l (μg/ml); however, in this study, the peripheral blood levels of vitamin C in fifty GC patients were 2-10 μg/ml, suggesting a statistically nonsignificant difference in serum vitamin C content between healthy people and GC patients; thus, there may be multiple factors that contribute to the GC process. TMEFF2 is inactivated by proinflammatory cytokines (11), whereas vitamin C is an effective anti-inflammatory agent (12). However, the interaction between serum vitamin C concentrations and TMEFF2 expression remained poorly understood. In our present study, we tested the interaction between vitamin C content in the peripheral blood and TMEFF2 mRNA levels in gastric cancer tissue, and the result was statistically significant. In addition, we found that vitamin C increased the protein and mRNA levels of TMEFF2 in a dose-dependent manner in GES-1 and AGS cells, revealing a positive correlation between vitamin C and TMEFF2 at the molecular level and suggesting that

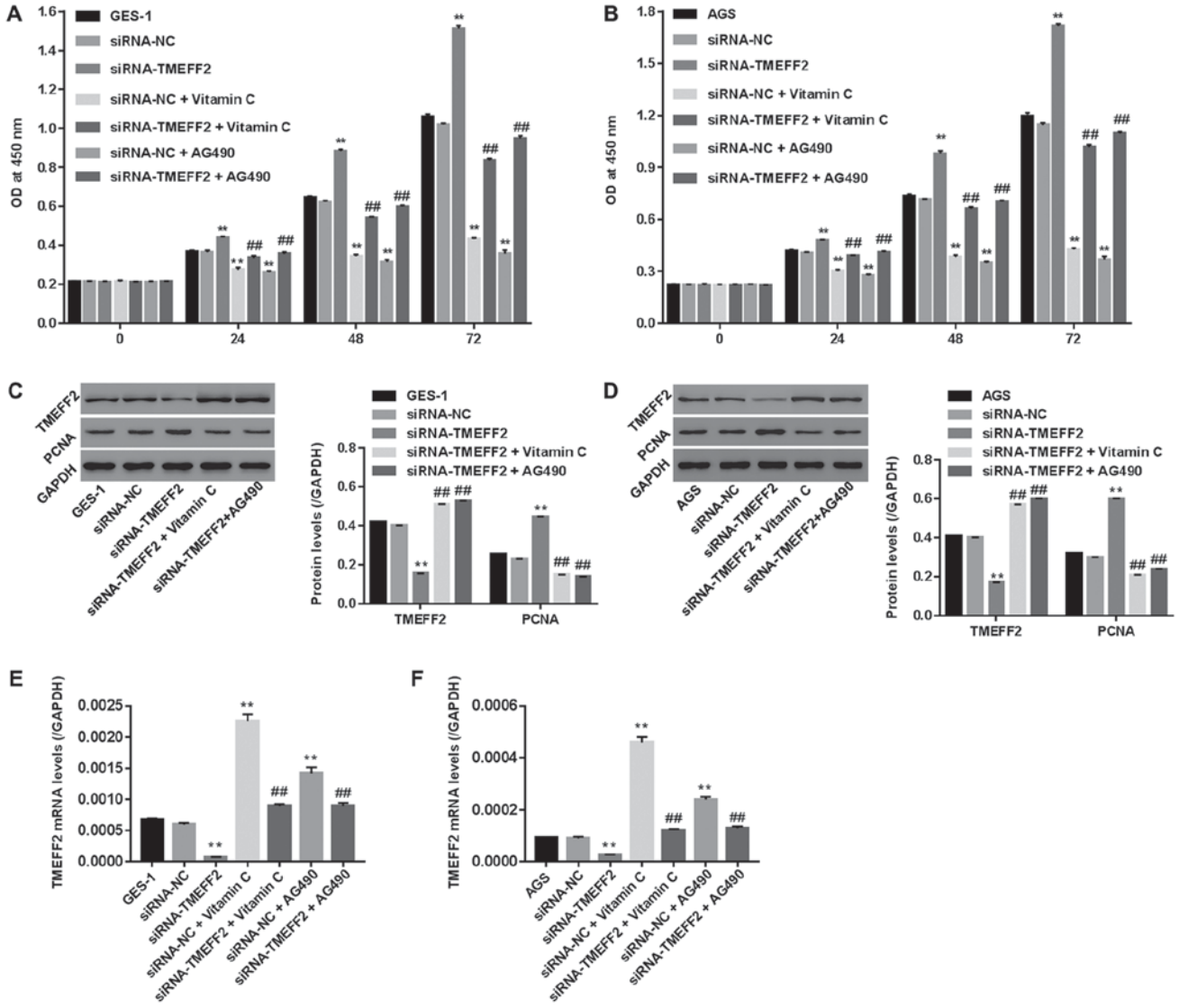

Figure 6. Vitamin C and AG490 inhibits cell proliferation in the GES-1 and AGS cell lines via upregulated TMEFF2 expression. (A) GES-1 and (B) AGS cell viability levels were assessed by Cell Counting Kit-8. Western blotting indicated the protein levels of TMEFF2 and PCNA in (C) GES-1 and (D) AGS cells. Reverse transcription-quantitative polymerase chain reaction revealed the mRNA levels of TMEFF2 in (E) GES-1 and (F) AGS cells. **P<0.01 vs. siRNA-NC; $^{\#\#}$P<0.01 vs. siRNA-TMEFF2. TMEFF2, two follistatin motifs 2; siRNA, small interfering RNA; NC, negative control; PCNA, proliferating cell nuclear antigen; OD, optical density.

enhanced transcription and translation of TMEFF2 mRNA was the underlying biological mechanism. Our data indicated that serum vitamin C content may be a predictor for TMEFF2 levels in gastric tissue from GC patients.

Evidence suggests that vitamin C significantly prevents the proliferation of human SGC-7901 gastric adenocarcinoma cells at concentrations of 10^{-4} to 10^{-8} mol/l (20). Other antioxidants, such as N-acetyl cysteine (NAC) and resveratrol, have been demonstrated to exert antiproliferative effects on GES-1 or AGS cells at mM concentrations (21,22), demonstrating less sensitivity than vitamin C. In our present study, human GES-1 and AGS cells were treated with vitamin C at doses of 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/l. We confirmed obvious inhibitory effects of vitamin C on the proliferation of GES-1 and AGS cells at doses of 10^{-8}, 10^{-7} and 10^{-6} mol/l after 24, 48 and 72 h. PCNA, which serves as a proliferation marker, is widely used to measure cell proliferation. Vitamin C increased the protein expression of PCNA in nitrofen-stimulated human pneumocytes (23). However, whether PCNA could be regulated by vitamin C in GES-1 and AGS cells remained largely unknown. In our present study, we found that vitamin C significantly decreased PCNA expression, suggesting the involvement of PCNA in the antiproliferative effects of vitamin C. Moreover, the activation of the STAT3 pathway is widely implicated in the growth and survival of human gastric cancer cells. The generation of reactive oxygen species (ROS) is required for STAT3 activation. Vitamin C, as a powerful antioxidant reagent, abrogates STAT3 activation in COS-7 cells (24). However, little is known about whether vitamin C acts on the STAT3 pathway in GES-1 and AGS cells. Our data suggested that vitamin C decreased p-STAT3 expression but had no effect on the expression of total STAT3, indicating that blocking the STAT3 signaling pathway was the underlying mechanism in this process.

TMEFF2 is downregulated in human gastric cancer cells (6). The basal mRNA level of TMEFF2 was much lower

in AGS cells than that in GES-1 cells (6), which was further confirmed in our study. TMEFF2 overexpression in gastric cancer cells inhibits cell proliferation (4). In our present study, the siRNA-mediated knockdown of TMEFF2 was established. We confirmed that TMEFF2 silencing induced proliferation in GES-1 and AGS cells by obviously promoting cell viability and increasing PCNA expression. More importantly, with additional vitamin C treatment (10^{-6} mol/l), TMEFF2 expression was enhanced above basal levels, and cell proliferation was significantly reduced, demonstrating the upregulation of TMEFF2 in response to the antiproliferative effect of vitamin C in GES-1 and AGS cells. Vitamin C exerts its antiproliferative effect on human gastric cancer cells as an antioxidant. In this study, we further assessed the effects of other antioxidants (vitamin E, NAC, resveratrol, and GSH) on the protein and mRNA levels of TMEFF2 in GES-1 and AGS cells. We found that NAC remarkably upregulated TMEFF2 expression ($P<0.05$), whereas vitamin E, GSH, and resveratrol increased TMEFF2 expression slightly or moderately.

Tyrphostin AG490, as an inhibitor of STAT3, is widely used to produce an antiproliferative response in human normal gastric epithelial or GC cells. High doses of AG490 (greater than 20 μmol/l) significantly inhibit cell growth in GES-1 and AGS cells (25). TMEFF2 and STAT3 are mutually regulated and negatively correlated (5). However, whether TMEFF2 was involved in the antiproliferative effect of AG490 remained unknown. To further substantiate the hypothesis that the upregulation of TMEFF2 could represent an effective strategy to prevent the proliferation of GES-1 and AGS cells, siRNA-TMEFF2-transfected cells were treated with AG490 (30 μmol/l). AG490 exerted an effect similar to that of vitamin C, and cell viability and PCNA protein levels were dramatically reduced, while basal TMEFF2 expression was obviously increased, suggesting that AG490 inhibited cell proliferation by increasing TMEFF2 levels and verifying that the upregulation of TMEFF2 could represent an effective strategy for preventing tumorigenesis in GES-1 and AGS cells.

In brief, our data revealed a positive correlation between serum vitamin C levels (μg/ml) and mRNA levels of TMEFF2 in gastric cancer tissue. In addition, vitamin C inhibited the proliferation of GES-1 and AGS cells by downregulating the expression of PCNA and blocking the STAT3 signaling pathway. Our data also revealed that the upregulation of TMEFF2 expression was involved in the antiproliferative effects of vitamin C and AG490 on GES-1 and AGS cells, further substantiating the role of TMEFF2 as a target for exploring novel drugs that block human GC.

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Availability of data and materials

The datasets used in the present study are available from the corresponding author on reasonable request.

Authors' contributions

AW and LW designed the study. HH and JX performed the experiments. WJ analyzed the data and wrote the paper. AW reviewed and edited the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by Yancheng Third People's Hospital (Jiangsu, China), and written informed consent was obtained from each participant involved in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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