

# Thrombospondin-2 Holds Prognostic Values and is Associated with the Metastasis and Mismatch Repair Process in Gastric Cancer

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## Research Article

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# Abstract

**Background:** The study aims to investigate the expression level of Thrombospondin 2 (TSP2) in Gastric Cancer (GC) and determine the relationship between TSP2 and clinical characteristics and prognosis.

**Methods:** The online database Gene Expression Profile Interactive Analysis (GEPIA) was used to analyze the mRNA expression level of TSP2 in GC. The Kaplan-Meier plotter prognostic analysis tool was used to evaluate the influence of TSP2 expression on clinical prognosis in GC patients. The expression level of TSP2 was analyzed in paraffin-embedded GC samples and adjacent normal tissues by immunohistochemistry. The relationship between clinicopathological characteristics and prognosis of GC patients. Next, the relationship between clinicopathological characteristics and prognosis of GC patients was assessed. Transwell experiment was used to evaluate the effect of TSP2 on the invasion and migration of HGC27 and AGS cells.

**Results:** Compared with normal tissues, the expression of TSP2 mRNA in GC was significantly up-regulated, and it was closely related to the clinical stage of GC. The high expression of TSP2 significantly affected the OS, FP and PPS of patients with GC. Among them, the expression level of TSP2 did not affect the prognosis of patients with GC in N0 subgroup, but significantly affected the prognosis of patients with GC in N (1+2+3) subgroup. The protein expression level of TSP2 in GC tissue was significantly higher than in normal tissues ( $P < 0.01$ ). The overall survival (OS) rate of patients with high TSP2 expression was lower than the low TSP2 expression group ( $P = 0.013$ ). Knockdown of TSP2 can significantly inhibit the growth of GC cells. Proliferation, migration, invasion ability, and TSP2 expression level significantly correlate with mismatch repair genes such as *PMS2*, *MSH6*, *MSH2*, and *MLH1* ( $P < 0.05$ ).

**Conclusion:** The expression of TSP2 in GC is significantly increased, closely related to the metastasis and mismatch repair process of GC patients and affected GC patients' prognosis. It is a potential marker and treatment target for the prognosis of GC patients.

## Background

Gastric cancer (GC) is one of the most common cancers in the world. With over 1 million estimated new cases annually, GC is the fifth most diagnosed malignancy worldwide[1]. Although the development of surgical techniques and combined chemotherapy have made significant progress in the treatment of GC in recent years, the prognosis of patients with advanced GC is still abysmal[2]. At present, there is still a lack of GC specific treatment targets and precise prognostic markers in clinical practice. GC remains the third leading cause of cancer-related mortality worldwide, with a high mortality rate mostly due to its detection in advanced stages of the disease[3]. Therefore, exploring new prognostic biomarkers and developing therapeutic targets are of great significance for the diagnosis and treatment of GC.

Tumor progression involves a series of complex events, starting with the tumor cells' mutations and ending with invasion and metastasis to distant places. In this process, the normal tissue structure is destroyed, and the surrounding tissues begin to produce a proliferative response similar to wound

healing. This response can be triggered by the highly permeable blood vessels that characterize the tumor vasculature[4]. Tumors are thought to secrete many angiogenic factors. Angiogenesis is regulated by the balance of a variety of pro-angiogenic factors and inhibitors. These blood vessels release plasma protein, which initiates the production of fibrin[5]. The tumor microenvironment also includes activated immune cells, fibroblasts, extracellular matrix, and newly formed capillaries, which constitute connective tissue's proliferation response [6]. Although the basis of tumorigenesis and development has been clarified in many aspects, the molecular genetic basis of tumorigenesis and development is still not completely clear. It is widely accepted that cancer is caused by different mutations attacking specific genes. There is no doubt that the genetic basis of cancer is terrible because it encodes too many molecular changes inherent in basic cellular processes[7].

Studies have shown that Thrombospondins-2 (TSP2) may be closely related to tumor occurrence and development [8]. TSP2 is one of the five members of the human TSP protein family, namely: TSP1 (THBS1) and TSP2 (THBS2), TSP3, TSP4, and TSP5[9]. Thrombospondin (TSP) is a kind of stromal cell protein. Its spatial structure is relatively stable, and it participates in the communication between cells and the intercellular matrix. Its main functions involve early embryonic development, damage repair, and tumorigenesis[10, 11]. The molecular weight of TSP2 is about 145kD; it is a trimeric structure sensitive to  $Ca^{2+}$  and maintained by disulfide bonds. TSP2 has four protein binding domains similar to TSP1, including the N-terminal heparin-binding domain and lysin-like domain, Epidermal growth factor-like area, and  $Ca^{2+}$  binding area[12]. These domains regulate various biological functions by interacting with various cell surface receptors, including proliferation, angiogenesis, cell adhesion, and extracellular matrix remodeling. For example, TSP2 interacts with the cytokines CD47, CD36, and integrin  $\alpha\beta_3$  to promote cell migration[13]. Studies have shown that the TSP2 gene is closely related to the occurrence and development of coronary atherosclerosis, liver disease, and chronic kidney disease[14]. Further studies have found that TSP2mRNA expression is abnormally increased in prostate cancer[15] and oral cancer [16]tissues and affects the prognosis of patients, indicating that TSP2 may be closely related to the occurrence and development of the tumors as mentioned above. However, there are few reports about TSP2 in GC. The relationship between GC is still worthy of further discussion. Therefore, we tried to reveal the clinical significance of TSP2 and its role in GC.

In order to verify the above hypothesis, this study used bioinformatics technology combined with clinical data to preliminarily analyze the expression of TSP2 in GC tissues and explore the possible relationship between TSP2 expression and the clinicopathological characteristics and clinical prognosis of GC patients. In addition, HGC-27 and AGS GC cell lines were used to inhibit the potential target TSP2 and observe the in vitro effects of TSP2 on GC cells. Provide clues and ideas for further study of the mechanism of the TSP2 gene in GC.

## Methods

### Tumor database source

The online database Gene Expression Profile Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/index.html>) is used to analyze and compare the expression levels of TSP2 in GC and normal gastric tissues[17]. The Kaplan-Meier Plotter prognostic analysis tool (<http://kmplot.com/analysis/>) was used to evaluate the effect of TSP2 expression on the prognosis of GC patients[18]; at the same time, the Kaplan-Meier Plotter database was used to analyze the correlation between the TSP2 expression level and clinical characteristics of GC patients Sex.

## Clinical data and follow-up

The random number table method selected 80 GC patients who underwent surgery in the General Surgery Department of the First Affiliated Hospital of Jinan University from January 2016 to December 2017, without prior chemotherapy or radiotherapy. Eighty samples of GC tumor tissue and paired adjacent tissues (3cm from the edge of the cancerous tissue) were collected. Eighty cases of GC tissue and paired adjacent tissues were fixed with formalin and embedded in paraffin. The pathology department of our hospital confirmed these diagnoses. The staging is unified according to the eighth edition TNM staging standard of the International Union Against Cancer (UICC), and the postoperative adjuvant treatment is carried out according to the National Comprehensive Cancer Network (NCCN) GC practice guidelines. The endpoint of this study's follow-up is the follow-up period of four years or the patient's death. Overall survival (OS) was defined as the period from the day of surgery until death from any cause or the end of the follow-up. The Institutional Review Boards approved this study of the First affiliated hospital of Jinan University, and all of the patients provided informed consent.

## Immunohistochemical test

Take paraffin sections of GC tissue, make 4 $\mu$ m-thick paraffin sections, bake the slices at 65°C for 30 minutes, after dewaxing, block the endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub>, inactivate for 10 minutes, and rinse twice with PBS; slices are placed 0.01mol /L (pH6.0) citrate buffer 90°C-95°C heating for 15min to perform antigen retrieval. Wash twice with PBS. Block non-specific antigens with 5% BSA, add rabbit anti-human TSP2 monoclonal antibody (BW1441, Santa Cruz) diluted 1:400 with 5% BSA to the slide to cover the tissue completely, incubate overnight in a refrigerator at 4°C, and rinse twice with PBS (5min). Add the secondary antibody (goat anti-rabbit) to the glass slide to completely cover the tissue, incubate at 37°C for 40 min, and rinse with PBS twice (5 min). Use DAB to develop color, observe under microscope, control the reaction time within 2-4min, and stop the display after washing with tap water. It was counterstained with hematoxylin at room temperature, washed with tap water to turn blue, dehydrated with gradient ethanol solution, transparent with xylene, mounted with neutral gum, and observed under a microscope. The immunohistochemical staining area was 0 (0%), 1 (1% - 25%), 2 (26% - 50%), 3 (51% - 75%) and 4 (76% - 100%) according to the percentage of positive cells. TSP2 staining intensity score is 0 (no staining), 1 (weak staining), 2 (medium staining), 3 (strong staining). The final staining score is the product of two parameters, divided into 2 groups: 0-3 groups are low expression groups, and  $\geq 4$  groups are high expression groups.

## Cell culture and transfection

Human HGC-27 and AGS GC cell lines were purchased from the ATCC cell database in 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin mixture (purchased from Guangzhou Genio Biotech Co., Ltd. ). All were cultured in a 37 °C, 5% CO<sub>2</sub> constant temperature incubator, and the medium was changed every 2 days, observed under a microscope. After the cells were 80%-90% fused, 0.25% trypsin was used (purchased from Guangzhou Genio Biotech Co., Ltd.) to digest and continue subculture according to the ratio of 1:2. Cell transfection: Human HGC27 and AGS GC cells were cultured in 1640 medium containing 10% fetal bovine serum to a confluency of about 70%, and then used the target according to the manufacturer's instructions (purchased from Guangzhou Genio Biotechnology Co., Ltd.) The siRNA to TSP2 was transfected into GC cells through Lipo3000 liposome. The specific interference sequence and control sequence were: Si-1: (5'-CCGGCCCTCCTAAGACAAGGAACATCT-3'); Si-2: (5'-CGAGATGTTCTTGTCTTAGGAGGGTTTTTG-3') and control group (Ctrl) (5'-CCCTCCTAAGACAAGGAACAT-3'), the obtained stably transfected interference group cells were named Si-1 and Si-2, respectively.

## Western blotting

After 48 hours of cell transfection, the total protein was extracted with protein lysate, the sample and loading buffer were mixed according to the corresponding ratio, and then the sample was denatured in a boiling water bath. Each lane was loaded with an equal amount of 30µg. After the cell electrophoresis, the protein was transferred to the PVDF membrane. After blocking with 5% skimmed milk powder, incubate the corresponding primary antibody (1:1000, ab112543, Abcam), incubate the secondary antibody the next day, and add ECL developer solution in a dark environment and expose in a gel imager. The final result is expressed as a target strip. The ratio of the belt's optical density to the internal control GAPDH (1:2000, AF1186, Biyuntian) was used as the protein expression level.

## Cell migration and invasion experiments

The HGC-27 and AGS cell lines 48h after transfection were digested with 0.25% trypsin, the digestion was terminated, and the culture medium was discarded by centrifugation. The cells were resuspended in serum-free 1640 medium, and the cell density was adjusted to  $1 \times 10^6$ / ml. After repeated pipetting and mixing of the cell suspension, 0.2 ml of the cell suspension was added to the upper chamber of the Transwell chamber. Add 0.6ml of 1640 medium containing 10% serum to the lower chamber of the 24-well plate, shake it gently, and place it in an incubator for 24 hours. Take out the Transwell chamber, discard the culture medium in the well, gently wipe off the cells in the upper chamber with a cotton swab, rinse 3 times with PBS, fix the cells with 4% paraformaldehyde for 25 minutes, dry the chamber properly, stain with 0.1% crystal violet for 20 minutes, PBS Wash three times and air dry the chamber. Place the chamber under a microscope, randomly select five view fields to observe the cells, take pictures, and count them. In the invasion experiment, the Transwell chamber was pre-added with Matrigel, and the treatment method was the same as above. 0.2ml of cell suspension was added to the upper chamber, and the remaining methods were the same as above.

## Statistical analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA) was used for data analysis and graphing. The analysis of differences between groups used a t-test or one-way analysis of variance. The expression level of related genes and the characteristic clinicopathological parameters were compared using Fisher's exact test or  $\chi^2$  test. Kaplan-Meier survival curve was used to analyze the relationship between TSP2 expression level and OS.  $P < 0.05$  indicates that the difference is statistically significant.

## Results

### Analysis of the expression level of TSP2 in different tumors

GEPIA database analysis of the expression level of TSP2 in tumors showed that the gene expression level of TSP2 in a variety of tumors was significantly increased (Fig. 1A, Fig. 1C), and the expression level of TSP2 in GC (Stomach adenocarcinoma; STAD) samples was significantly higher than normal tissues ( $P < 0.01$ ) (Fig. 1B, 1C); further comparison of the TSP2 expression levels of different GC clinical stages, the results showed that the expression of TSP2 in different stages was statistically significant ( $F = 3.16$ ,  $P = 0.0248$ , Fig. 1D), staging The expression level of TSP2 in GC tissue of  $\text{I-IV}$  stage was significantly increased.

### The relationship between TSP2 expression level and prognosis of GC

Kaplan-Meier Plotter database analysis results show that high TSP2 expression significantly affects the overall survival (OS) of GC patients (HR = 1.55, 95%CI: 1.29–1.85;  $P < 0.01$ ) (Fig. 2A), after recurrence Post progression survival (PPS) (HR = 1.51, 95%CI: 1.19–1.9;  $P < 0.01$ ) (Fig. 2B) and first progression survival (FP) (HR = 1.53, 95%CI) : 1.25–1.88;  $P < 0.01$ ) (Fig. 2C). Besides, Kaplan-Meier analysis was performed on the OS of 80 GC patients, and there was statistical significance between the TSP2 low expression group and the high expression group ( $P = 0.013$ ) (Fig. 3C).

### The effect of TSP2 expression on the prognosis of GC patients in different subgroups

Kaplan-Meier Plotter was used to analyze the effect of TSP2 on different subgroups of GC patients, and the results showed that TSP2 expression level affects the OS of patients with different genders, treatment methods, HER2 expression, M staging, Lauren classification, and differentiation type subgroups ( $P < 0.05$ ). The expression level of TSP2 did not affect the prognosis of GC patients in the N0 subgroup (HR = 1.67, 95%CI: 0.73–3.83,  $P = 0.22$ ), but significantly affected the prognosis of GC patients in the N(1 + 2 + 3) subgroup (HR = 2.36, 95%CI: 1.81–3.09,  $P < 0.01$ ). Furthermore, TSP2 did not affect the prognosis of patients with stage I and II GC ( $P > 0.05$ ) but significantly affected the prognosis of patients with stage III and IV ( $P < 0.01$ ), as shown in Table 1.

Table 1  
The effect of TSP2 in the Kaplan-Meier Plotter database on the prognosis of patients with different subgroups of GC

<b>Clinical characteristics</b>	<b>Items</b>	<b>Cases</b>	<b>HR (95% CI)</b>	<b>P value</b>
Gender	Female	236	2.05 (1.44 – 2.92)	4.9e – 05
	Male	545	1.52 (1.22 – 1.89)	0.00015
Treatment	Surgery alone	380	1.71 (1.28 – 2.29)	0.00023
	5 FU based	153	0.65 (0.46–0.92)	0.014
	Other adjuvant	76	2.82(1.17–6.79)	0.015
HER2 status	Negative	532	1.58(1.25–1.99)	9.4e – 05
	Positive	344	1.58 (1.22 – 2.05)	0.00049
Stage	StageⅠ	67	0.45 (0.17–1.24)	0.11
	StageⅡ	140	0.62(1.34–1.14)	0.12
	StageⅢ	305	1.89(1.41–2.53)	1.4e – 05
	StageⅣ	148	1.87(1.27 – 2.77)	0.0014
Stage T	T2	241	1.84(1.18 – 2.86)	0.0061
	T3	204	1.87(1.32 – 2.63)	3.0e – 04
	T4	38	1.91(0.82 – 4.47)	0.13
	N0	74	1.67(0.73 – 3.83)	0.22
Stage N	N (1 + 2 + 3)	422	2.36(1.81 – 3.09)	8.2e – 11
	N1	225	2.31(1.53 – 3.48)	3.8e – 05
	N2	121	2.92(1.84 – 4.63)	1.9e-6
Stage M	N3	76	2.27(1.31 – 3.91)	0.0026
	M0	444	2.14(1.62 – 2.83)	3.9e-8
	M1	56	1.84(1.01 – 3.33)	0.042
Lauren classification	Instestinal	320	2.49 (1.8 – 3.46)	1.6e-8
	Diffuse	241	1.89 (1.34–2.66)	0.00023

Clinical characteristics	Items	Cases	HR (95% CI)	P value
Differentiation	Mixed	33	4.55 (1.56–13.32)	0.0025
	Poorly	165	1.79 (1.16–2.75)	0.0074
	Moderately	67	2.71 (1.35–5.44)	0.0036
	Well	32	4.36 (1.8–10.54)	4.0e-04

## The relationship between TSP2 expression and clinicopathological characteristics of GC patients

In order to explore whether the expression of TSP2 is related to clinicopathological characteristics, immunohistochemistry was used to detect the expression of TSP2 in GC and paracancerous tissues. The representative diagram is shown in Fig. 3A. Among them, 76.3% (58/80) of high TSP2 expression in GC tissues. The high expression of TSP2 in normal gastric tissue accounted for 23.7% (19/80), and the expression level of TSP2 in GC tissue was significantly higher than that in normal tissue adjacent to cancer (Table 2, Fig. 3B). In addition, the expression level of TSP2 in GC was significantly positively correlated with TNM staging ( $P < 0.01$ ), lymph node metastasis N staging ( $P = 0.038$ ), and distant organ metastasis pM staging ( $P = 0.025$ ), and also correlated with pMMR/MSI-L /MSS ratio is positively correlated (Table 3).

Table 2  
TSP2 expression in 80 cases of gastric cancer tissues (n%)

Tissue	Cases	TSP2 expression		$\chi^2$	P-value
		Low	High		
Normal	80	61(76.3)	19(23.7)	38.079	< 0.01
Tumor	80	22(27.5)	58(72.5)		

Table 3  
Relationship between TSP2 expression and clinicopathological characteristics in 80 cases of gastric cancer (n%)

Clinical characteristics	Cases	TSP2 expression		$\chi^2$	P-value
		Low	High		
Age (years)				1.802	0.180
< 65	34	12(35.3)	22(64.7)		
≥ 65	46	10(21.7)	36(78.3)		
Gender				1.863	0.172
Male	41	14(34.1)	27(65.9)		
Female	39	8 (20.5)	31(79.5)		
TNM stage				12.406	< 0.01
I/II	33	16(48.5)	17(51.5)		
III/IV	47	6 (12.8)	41(87.2)		
T stage				2.190	0.139
T1/T2	23	9 (39.1)	14(60.9)		
T3/T4	57	13(22.8)	44(77.2)		
N stage				4.296	0.038
N0	33	5 (15.2)	28(84.8)		
N(1 + 2 + 3)	47	17(36.2)	30(63.8)		
M stage				5.035	0.025
M0	68	15(22.1)	53(77.9)		
M1	12	7 (58.3)	5 (41.7)		
Differentiation				0.348	0.555
Poor/undifferentiated	43	13(30.2)	30(69.8)		
Well/moderate	37	9 (24.3)	28(75.7)		
MSI/MMR				16.425	< 0.01
pMMR/ MSI-L/MSS	66	12(18.2)	54(81.8)		
dMMR/MSI-H	14	10(71.4)	4 (28.6)		
Abbreviations: pMMR, proficient mismatch repair; MSI-L, microsatellite instability low; MSS, microsatellite stable; dMMR, deficient mismatch repair; MSI-H, microsatellite instability high.					

# Effect of TSP2 on the invasion and metastasis of GC cells in vitro

Western-blot results showed that the TSP2 protein levels of HGC27 and AGS cells decreased significantly (Fig. 4A,4B,S1). The results of Transwell migration experiments showed that after knocking down the TSP2 expression levels of HGC27 and AGS cells, the invasion and migration ability of cells decreased significantly (Fig. 4C-4F). The above results indicate that targeted knockdown of TSP2 can inhibit the migration and invasion ability of HGC27 and AGS cells.

## Correlation between TSP2 gene expression and mismatch repair gene expression in GC

GEPIA database analysis results showed that the expression level of TSP2 in gastric adenocarcinoma (Stomach adenocarcinoma; STAD) was significantly positively correlated with the expression of a series of mismatch repair genes, PMS2, MSH6, MSH2, and MLH1 ( $P < 0.05$ , Fig. 5).

## Discussion

GC is one of the common types of gastrointestinal tumors. Its occurrence is related to various factors, including genetic factors and non-genetic factors, and genetic factors have been found to play an essential role in the pathogenesis of GC [2]. The specific pathogenesis of GC is still worthy of further investigation. Although the comprehensive treatment of GC has made meaningful progress, there is still a lack of more accurate markers for its prognosis. This study uses bioinformatics technology combined with clinical experiments to determine that the expression of TSP2 in GC is significantly increased. Simultaneously, the high expression of TSP2 significantly affects the prognosis of GC patients and significantly affects the prognosis of GC patients with lymph node metastasis. This study also determined that the expression level of TSP2 is related to a variety of genes that affect the biological behavior of GC, which provides theoretical direction and data support for the further study of TSP2 in GC.

The occurrence of tumor is the result of multiple factors. Its development is a multi-stage process. It requires tumor cells to have unlimited self-proliferation ability and the ability to escape apoptosis. It also requires continuous nutritional support, including blood vessel growth and infiltration [19, 20]. New blood vessels are vital for tumor cell growth, infiltration, and metastasis [5]. When the normal balance between inhibiting tumor angiogenesis and promoting tumor angiogenesis is broken, tumor blood vessels are rapidly formed in large numbers, and tumor cells gain the ability of infinite proliferation, infiltration, and metastasis. However, the blood vessel of the tumor is a very abnormal new blood vessel [21]. Among them, TSP2 is a member of the TSP series. It is a new, naturally occurring inhibitor of angiogenesis [22]. It can significantly inhibit the expression of vascular endothelial growth factor (VEGF) [23]. It is currently the most studied in the TSP family. One of the members with the most complex functions and closely related to various cell behaviors. Related studies have shown that TSP2 is synthesized, secreted, and transported

to the corresponding extracellular matrix by fibroblasts, smooth muscle cells, endothelial cells and keratinocytes, and other types of cells[24]. Since each subunit of TSP2 is composed of many discrete domains, these different domains can mediate TSP2 binding to various ligands, such as heparin, collagen, fibronectin, plasminogen, and plasminogen activation, etc. [25]Related studies have confirmed that at least two different receptors interact with these ligands. The role of receptors and ligands is precisely mediated by the above domains so that TSP2 participates in a variety of cell behaviors, including cell adhesion, migration, and proliferation[26]. Therefore, TSP2 is considered to play an essential role in cell growth and embryonic development and angiogenesis.

In this study, GEPIA and Kaplan-Meier Plotter database systems were used to analyze the expression level of TSP2 in GC to understand the relationship between their expression and clinical prognosis. The results showed that the expression level of TSP2 in GC tissues was significantly higher than that in normal tissues. At the same time, TSP2 mainly affects the clinical prognosis of GC patients with lymphatic stages N1-3. This indicates that high expression of TSP2 is closely related to lymph node metastasis and tumor invasion in GC. At the same time, it has been shown that high expression of TSP2 affects the clinical prognosis of GC patients in various GC subgroups, indicating that TSP2 has the potential to become an essential biomarker for predicting tumor prognosis in GC. To further clarify the expression of TSP2 in GC tissues, this study performed immunohistochemical analysis on 80 pairs of fresh GC tissues and adjacent tissues. The results showed that TSP2 expression level was significantly related to TNM staging ( $P < 0.01$ ), pM staging of distant metastasis ( $P = 0.025$ ), and other clinicopathological characteristics, but not to gender and age ( $P > 0.05$ ). Kaplan-Meier survival analysis showed that those with high TSP2 expression had a shorter OS and lower OS expression had a worse prognosis. Since the cohort of 80 pairs of GC tissues includes only twelve cases of M1 disease, such a low-metastasis disease group may lead to narrow statistical results. Considering that our research was conducted in small-scale patients, it is necessary to conduct larger-scale prospective clinical studies to fully understand and develop the prognostic and therapeutic value of TSP2.

In order to explore the role of TSP2 in the metastatic activity of GC cells, this study selected GC cell lines HGC27 and AGS for transfection. The results showed that the expression of Si-1 and Si-2 histones was significantly lower than that of the control group, verifying the success of the transfection model set up. Through Transwell cell migration and invasion experiments, it was found that after interfering with the TSP2 gene of GC cells, the expression of TSP2 gene was reduced, and the cell's invasion and metastasis ability was reduced. In conclusion, this experiment shows that the expression of TSP2 gene can promote the migration and invasion of GC cells.

On the other hand, chromosomal instability and microsatellite instability are two key mechanisms in the occurrence of gastrointestinal tumors[27, 28]. Mismatch repair system (MMR) is responsible for maintaining genome stability. The MMR is responsible for maintaining the stability of the genome. When the MMR function is abnormal, the weakened or even missing function of the MMR will cause the instability of the microsatellite[29]. Microsatellites change, and the total mutation rate of specific cells increases[30]. Therefore, MMR plays a vital role in the cause of cancer and its biological behavior[31].

The current immune checkpoint inhibitor therapy for tumors is in the "MSI era" because microsatellite instability (MSI) or mismatch repair gene status (MMR) is currently the best predictor of efficacy[32, 33]. Based on the MSI status, GC patients can be divided into two groups according to the efficacy of immunotherapy: "dominant population" MSI-H/dMMR GC cancer (MSI-H); "ineffective population" MSS/pMMR GC cancer (MSS)[34]. Mismatch repair gene family members include *MLH1*, *MSH2*, *MSH6*, and *PMS2*[35], all of which play an essential role in GC's occurrence and progression [36]. Through gene correlation analysis, this study also found that TSP2 gene has a significant correlation with the expression of four crucial genes in the mismatch repair family, further confirming that TSP2 gene has potential guiding significance for clinical molecular typing and treatment options in GC.

## Conclusion

In summary, this study analyzed the correlation between TSP2 and GC through bioinformatics technology combined with clinical samples and in vitro experiments. It was found that TSP2 was highly expressed in GC and played a cancer-promoting effect. Down-regulating its expression by exogenous means can inhibit the proliferation, migration, and invasion of GC cells. High expression of TSP2 affects the prognosis of GC patients, and the expression of TSP2 is closely related to the poor prognosis of patients with GC lymph node metastasis. It is a potential marker for GC lymph node metastasis and patient prognosis.

## Abbreviations

TSP2, THBS2, Thrombospondin 2;

TSP, Thrombospondin;

GC, Gastric Cancer ;

STAD, Stomach adenocarcinoma;

GEPIA, Gene Expression Profile Interactive Analysis;

UICC, The International Union Against Cancer;

NCCN, The National Comprehensive Cancer Network;

OS, Overall survival;

PPS, Post progression survival;

FP, First progression survival;

VEGF, Vascular endothelial growth factor;

pMMR , proficient mismatch repair;

MSI-L, microsatellite instability low;

MSS, microsatellite stable;

dMMR, deficient mismatch repair;

MSI-H, microsatellite instability high.

## Declarations

### Ethics approval and consent to participate

This study was conducted under the approval of the Ethics Committee of the First Affiliated Hospital of Jinan University in strict accordance with the *Declaration of Helsinki*. All patients and/or legal guardians were informed prior to experiments and signed written informed consents.

### Consent for publication

Not applicable.

### Availability of data and materials

All analyzed data are included in this published article and its supplementary information file. The data underlying this study are freely available from GEPIA data portal (<http://gepia.cancer-pku.cn/index.html>) and the Kaplan-Meier Plotter prognostic analysis tool (<http://kmplot.com/analysis/>). The authors did not have special access privileges. The original data are available upon reasonable request to the corresponding author.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contribution

Research idea: YLP and JHP.

Data extraction and integrated analysis: XDC, YRZ, ZZ, SCHF and ZBL.

Quality assessment and result interpretation: TH, HD, XLC, YGG and SHQ.

Modification and polishing: YLP and JHP.

Manuscript writing: All authors.

Final approval of manuscript: All authors.

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## References

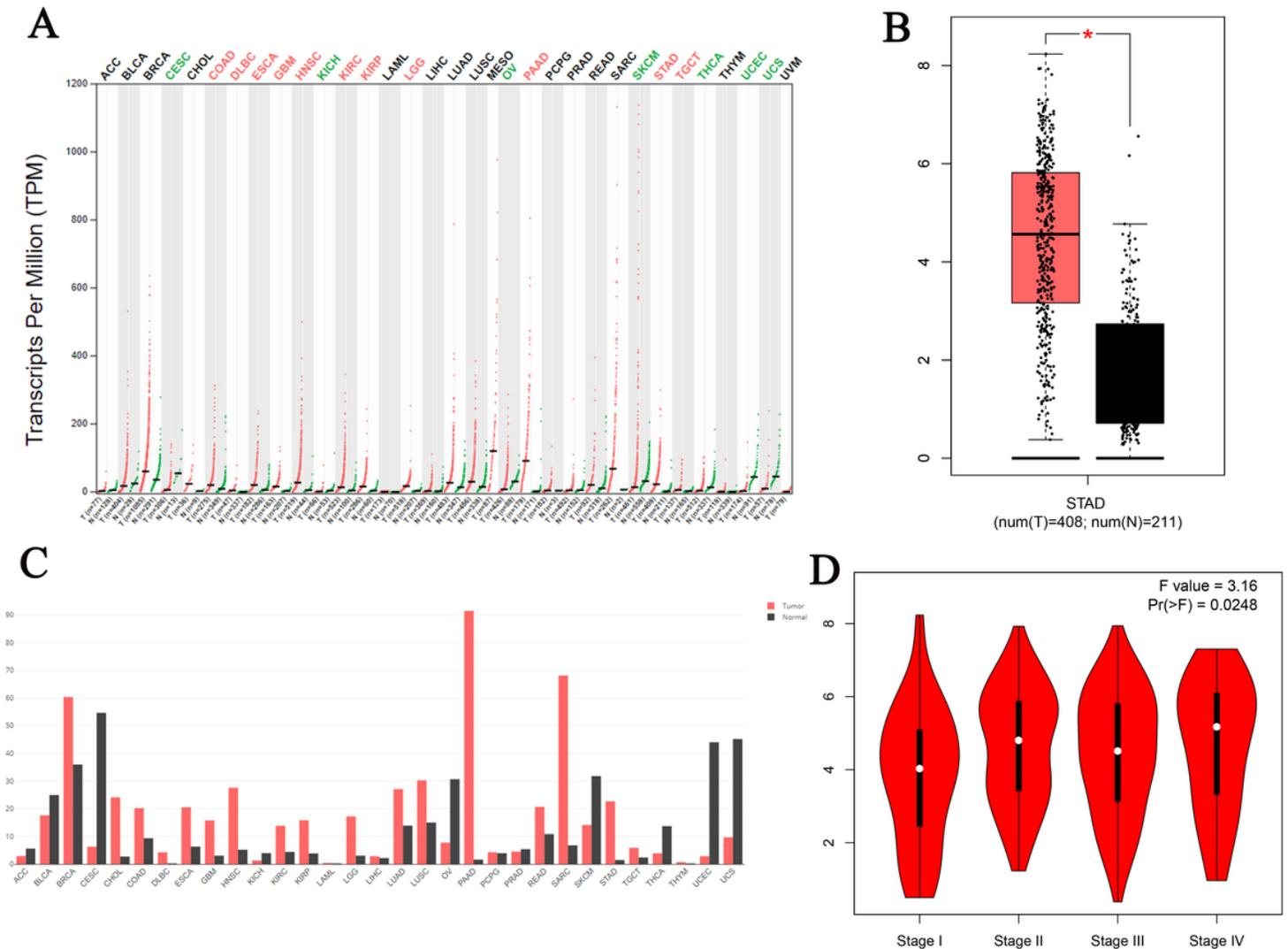
1. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F: **Gastric cancer**. *Lancet (London, England)* 2020, **396**(10251):635-648.
2. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2019**. *CA: a cancer journal for clinicians* 2019, **69**(1):7-34.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries**. *CA: a cancer journal for clinicians* 2018, **68**(6):394-424.
4. Dvorak HF: **Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing**. *The New England journal of medicine* 1986, **315**(26):1650-1659.
5. Folkman J: **Tumor angiogenesis: therapeutic implications**. *The New England journal of medicine* 1971, **285**(21):1182-1186.
6. DeBerardinis RJ: **Tumor Microenvironment, Metabolism, and Immunotherapy**. *The New England journal of medicine* 2020, **382**(9):869-871.
7. Somarelli JA, Gardner H, Cannataro VL, Gunady EF, Boddy AM, Johnson NA, Fisk JN, Gaffney SG, Chuang JH, Li S *et al*: **Molecular Biology and Evolution of Cancer: From Discovery to Action**. *Molecular biology and evolution* 2020, **37**(2):320-326.
8. Kazerounian S, Yee KO, Lawler J: **Thrombospondins in cancer**. *Cellular and molecular life sciences : CMLS* 2008, **65**(5):700-712.
9. Chistiakov DA, Melnichenko AA, Myasoedova VA, Grechko AV, Orekhov AN: **Thrombospondins: A Role in Cardiovascular Disease**. *International journal of molecular sciences* 2017, **18**(7).

10. Kassem MM, Helkin A, Maier KG, Gahtan V: **Thrombospondins Differentially Regulate Proteins Involved in Arterial Remodeling.** *Physiological research* 2019, **68**(6):893-900.
11. Kirk JA, Cingolani OH: **Thrombospondins in the transition from myocardial infarction to heart failure.** *Journal of molecular and cellular cardiology* 2016, **90**:102-110.
12. Rusnati M, Borsotti P, Moroni E, Foglieni C, Chiodelli P, Carminati L, Pinessi D, Annis DS, Paiardi G, Bugatti A *et al.*: **The calcium-binding type III repeats domain of thrombospondin-2 binds to fibroblast growth factor 2 (FGF2).** *Angiogenesis* 2019, **22**(1):133-144.
13. Liu JF, Lee CW, Tsai MH, Tang CH, Chen PC, Lin LW, Lin CY, Lu CH, Lin YF, Yang SH *et al.*: **Thrombospondin 2 promotes tumor metastasis by inducing matrix metalloproteinase-13 production in lung cancer cells.** *Biochemical pharmacology* 2018, **155**:537-546.
14. Mustonen E, Ruskoaho H, Rysä J: **Thrombospondins, potential drug targets for cardiovascular diseases.** *Basic & clinical pharmacology & toxicology* 2013, **112**(1):4-12.
15. Chen PC, Tang CH, Lin LW, Tsai CH, Chu CY, Lin TH, Huang YL: **Thrombospondin-2 promotes prostate cancer bone metastasis by the up-regulation of matrix metalloproteinase-2 through down-regulating miR-376c expression.** *Journal of hematology & oncology* 2017, **10**(1):33.
16. Hsu CW, Yu JS, Peng PH, Liu SC, Chang YS, Chang KP, Wu CC: **Secretome profiling of primary cells reveals that THBS2 is a salivary biomarker of oral cavity squamous cell carcinoma.** *Journal of proteome research* 2014, **13**(11):4796-4807.
17. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses.** *Nucleic acids research* 2017, **45**(W1):W98-w102.
18. Nagy Á, Lánckzy A, Menyhárt O, Gyórfy B: **Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets.** *Scientific reports* 2018, **8**(1):9227.
19. Wang Y, Wang L, Chen C, Chu X: **New insights into the regulatory role of microRNA in tumor angiogenesis and clinical implications.** *Molecular cancer* 2018, **17**(1):22.
20. Pan F, Li W, Yang W, Yang XY, Liu S, Li X, Zhao X, Ding H, Qin L, Pan Y: **Anterior gradient 2 as a supervisory marker for tumor vessel normalization induced by anti-angiogenic treatment.** *Oncology letters* 2018, **16**(3):3083-3091.
21. Zhao X, Pan J, Li W, Yang W, Qin L, Pan Y: **Gold nanoparticles enhance cisplatin delivery and potentiate chemotherapy by decompressing colorectal cancer vessels.** *International journal of nanomedicine* 2018, **13**:6207-6221.
22. Roudnicky F, Yoon SY, Poghosyan S, Schwager S, Poyet C, Vella G, Bachmann SB, Karaman S, Shin JW, Otto VI *et al.*: **Alternative transcription of a shorter, non-anti-angiogenic thrombospondin-2 variant in cancer-associated blood vessels.** *Oncogene* 2018, **37**(19):2573-2585.
23. Bausch D, Fritz S, Bolm L, Wellner UF, Fernandez-Del-Castillo C, Warshaw AL, Thayer SP, Liss AS: **Hedgehog signaling promotes angiogenesis directly and indirectly in pancreatic cancer.** *Angiogenesis* 2020, **23**(3):479-492.
24. Sun XY, Han XM, Zhao XL, Cheng XM, Zhang Y: **MiR-93-5p promotes cervical cancer progression by targeting THBS2/MMPS signal pathway.** *European review for medical and pharmacological*

*sciences* 2019, **23**(12):5113-5121.

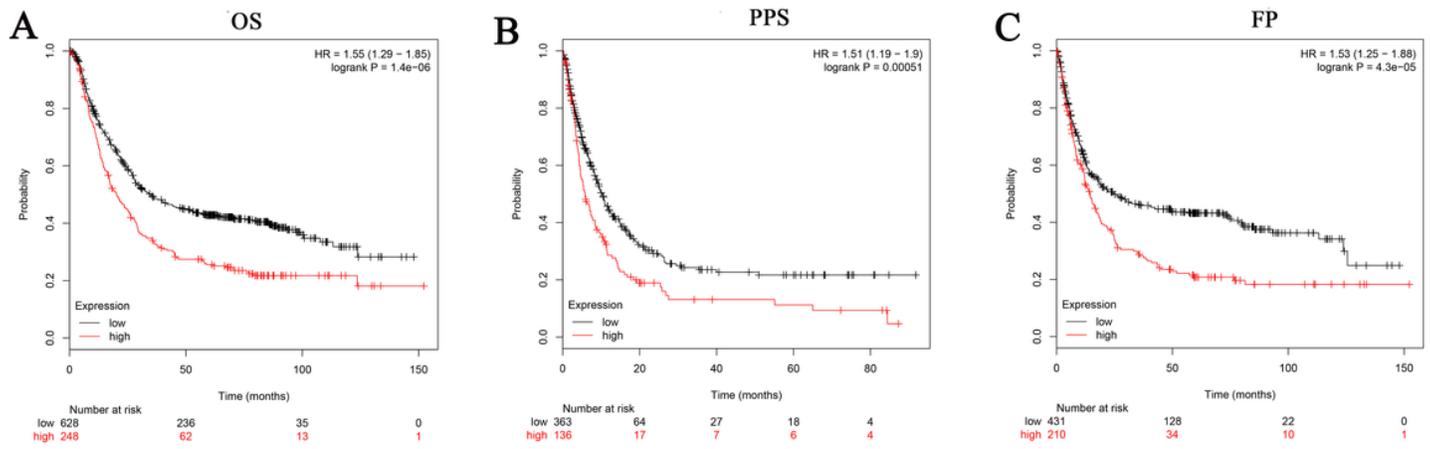
25. MacLauchlan SC, Calabro NE, Huang Y, Krishna M, Bancroft T, Sharma T, Yu J, Sessa WC, Giordano F, Kyriakides TR: **HIF-1 $\alpha$  represses the expression of the angiogenesis inhibitor thrombospondin-2.** *Matrix biology : journal of the International Society for Matrix Biology* 2018, **65**:45-58.
26. Morris AH, Stamer DK, Kunkemoeller B, Chang J, Xing H, Kyriakides TR: **Decellularized materials derived from TSP2-KO mice promote enhanced neovascularization and integration in diabetic wounds.** *Biomaterials* 2018, **169**:61-71.
27. Pećina-Šlaus N, Kafka A, Salamon I, Bukovac A: **Mismatch Repair Pathway, Genome Stability and Cancer.** *Frontiers in molecular biosciences* 2020, **7**:122.
28. Bach DH, Zhang W, Sood AK: **Chromosomal Instability in Tumor Initiation and Development.** *Cancer research* 2019, **79**(16):3995-4002.
29. McGrail DJ, Garnett J, Yin J, Dai H, Shih DJH, Lam TNA, Li Y, Sun C, Li Y, Schmandt R *et al*: **Proteome Instability Is a Therapeutic Vulnerability in Mismatch Repair-Deficient Cancer.** *Cancer cell* 2020, **37**(3):371-386.e312.
30. Jun JK, Choi KS, Lee HY, Suh M, Park B, Song SH, Jung KW, Lee CW, Choi IJ, Park EC *et al*: **Effectiveness of the Korean National Cancer Screening Program in Reducing Gastric Cancer Mortality.** *Gastroenterology* 2017, **152**(6):1319-1328.e1317.
31. Baretta M, Le DT: **DNA mismatch repair in cancer.** *Pharmacology & therapeutics* 2018, **189**:45-62.
32. Kim H, Hwang Y, Sung H, Jang J, Ahn C, Kim SG, Yoo KY, Park SK: **Effectiveness of Gastric Cancer Screening on Gastric Cancer Incidence and Mortality in a Community-Based Prospective Cohort.** *Cancer research and treatment : official journal of Korean Cancer Association* 2018, **50**(2):582-589.
33. Lemery S, Keegan P, Pazdur R: **First FDA Approval Agnostic of Cancer Site - When a Biomarker Defines the Indication.** *The New England journal of medicine* 2017, **377**(15):1409-1412.
34. Lee V, Murphy A, Le DT, Diaz LA, Jr.: **Mismatch Repair Deficiency and Response to Immune Checkpoint Blockade.** *The oncologist* 2016, **21**(10):1200-1211.
35. Fishel R: **Mismatch repair.** *The Journal of biological chemistry* 2015, **290**(44):26395-26403.
36. Mas-Ponte D, Supek F: **DNA mismatch repair promotes APOBEC3-mediated diffuse hypermutation in human cancers.** *Nature genetics* 2020, **52**(9):958-968.

## Figures



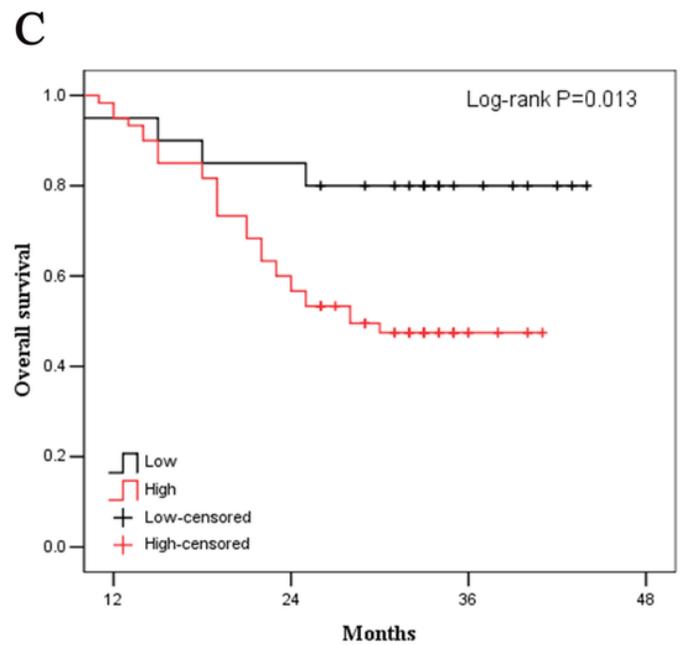
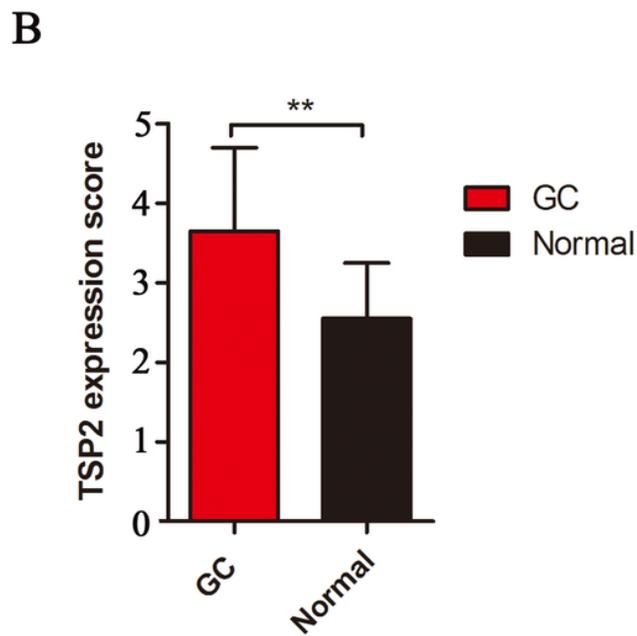
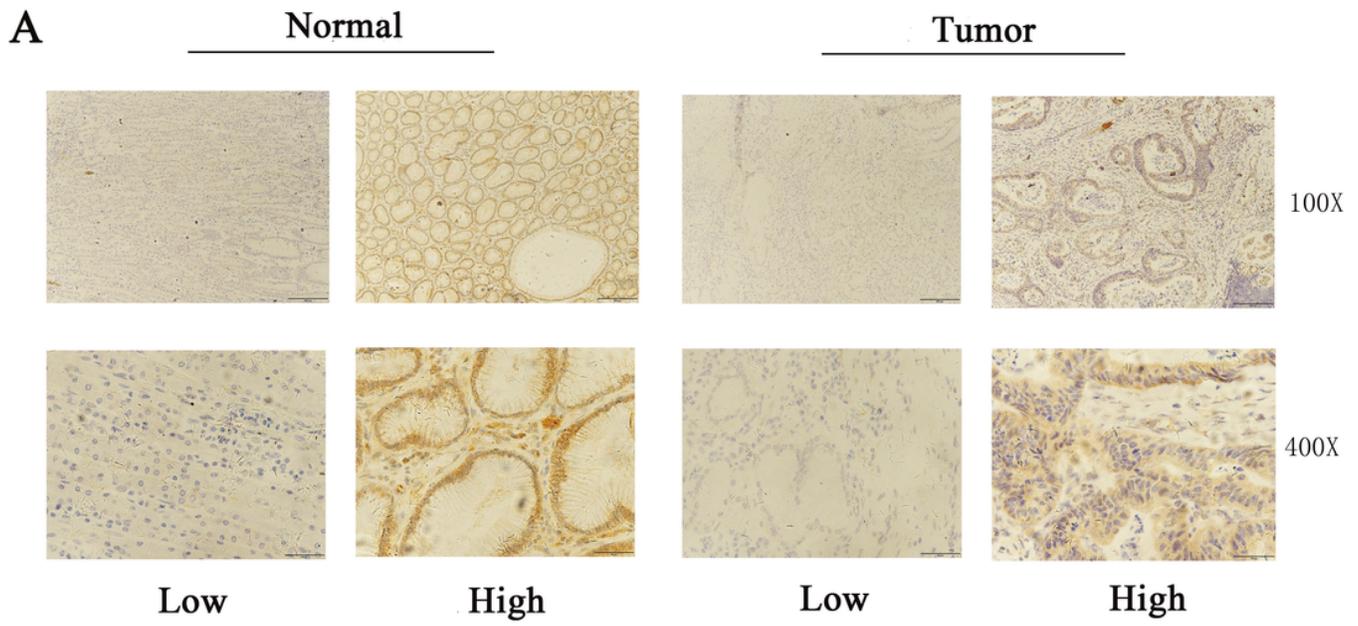
**Figure 1**

Analysis expression level of TSP-2 in different tumors and GC. Analyze the results with the GEPIA database: (A) Dot plots of gene expression profiles in all tumor samples and matched normal tissues; (B) Comparison of expression levels of TSP2 gene in GC and normal stomach tissues ( $P < 0.01$ ); (C) The gene expression profile bar graphs of all tumor samples and paired normal tissues. The height of the bar represents the median expression of certain tumor types or normal tissues, where Stomach adenocarcinoma (STAD,  $T = 22.67$ ;  $N = 1.45$ ); (D) The expression of TSP2 gene in GC clinical stage ( $P < 0.01$ ).



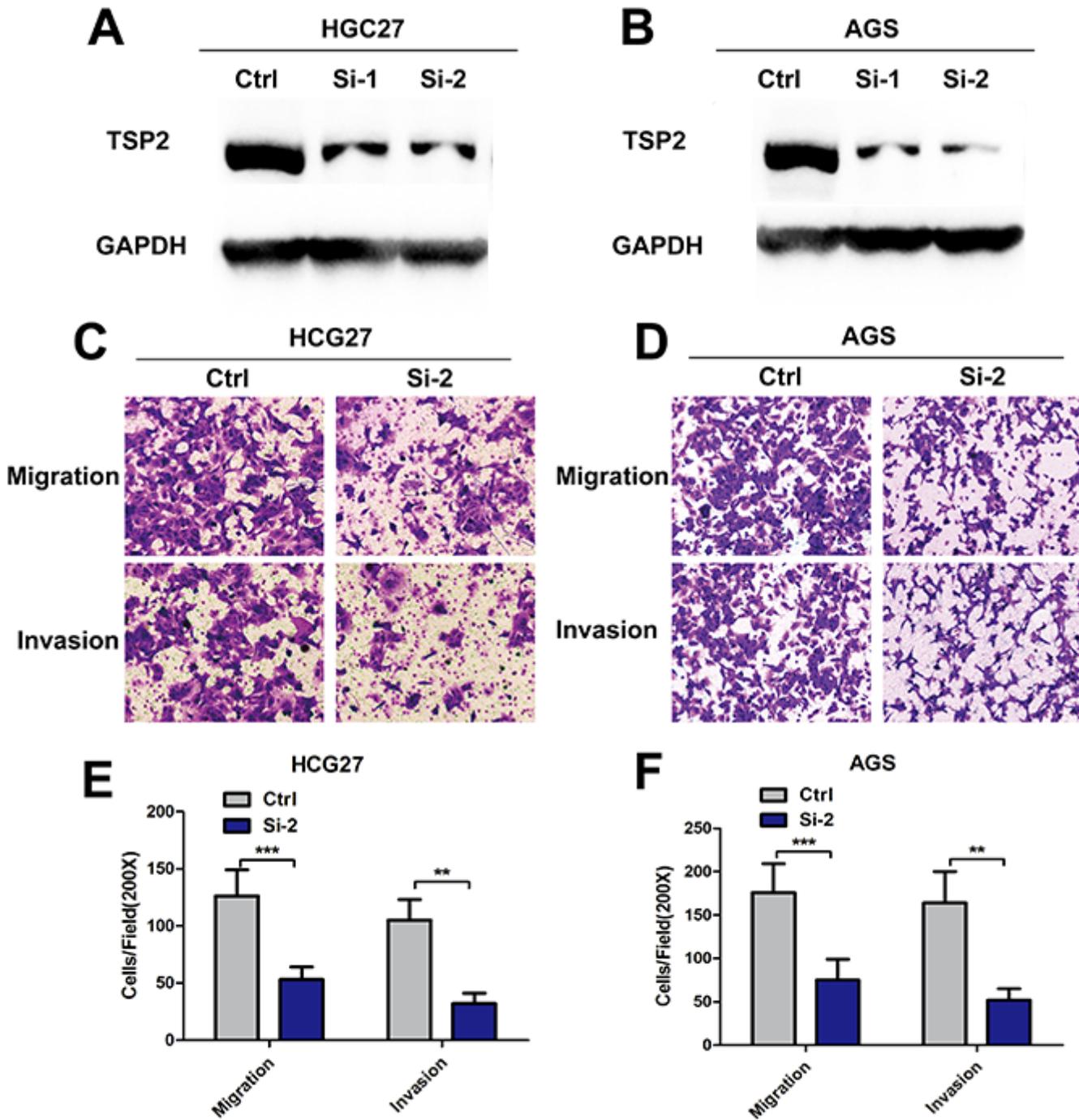
**Figure 2**

Kaplan-Meier Plotter database analysis of the relationship between TSP2 mRNA expression level and prognosis in GC patients: (A) TSP2 expression affects OS of GC patients, (B) Progressive survival after recurrence (PPS) and (C) First progression survival (FP).



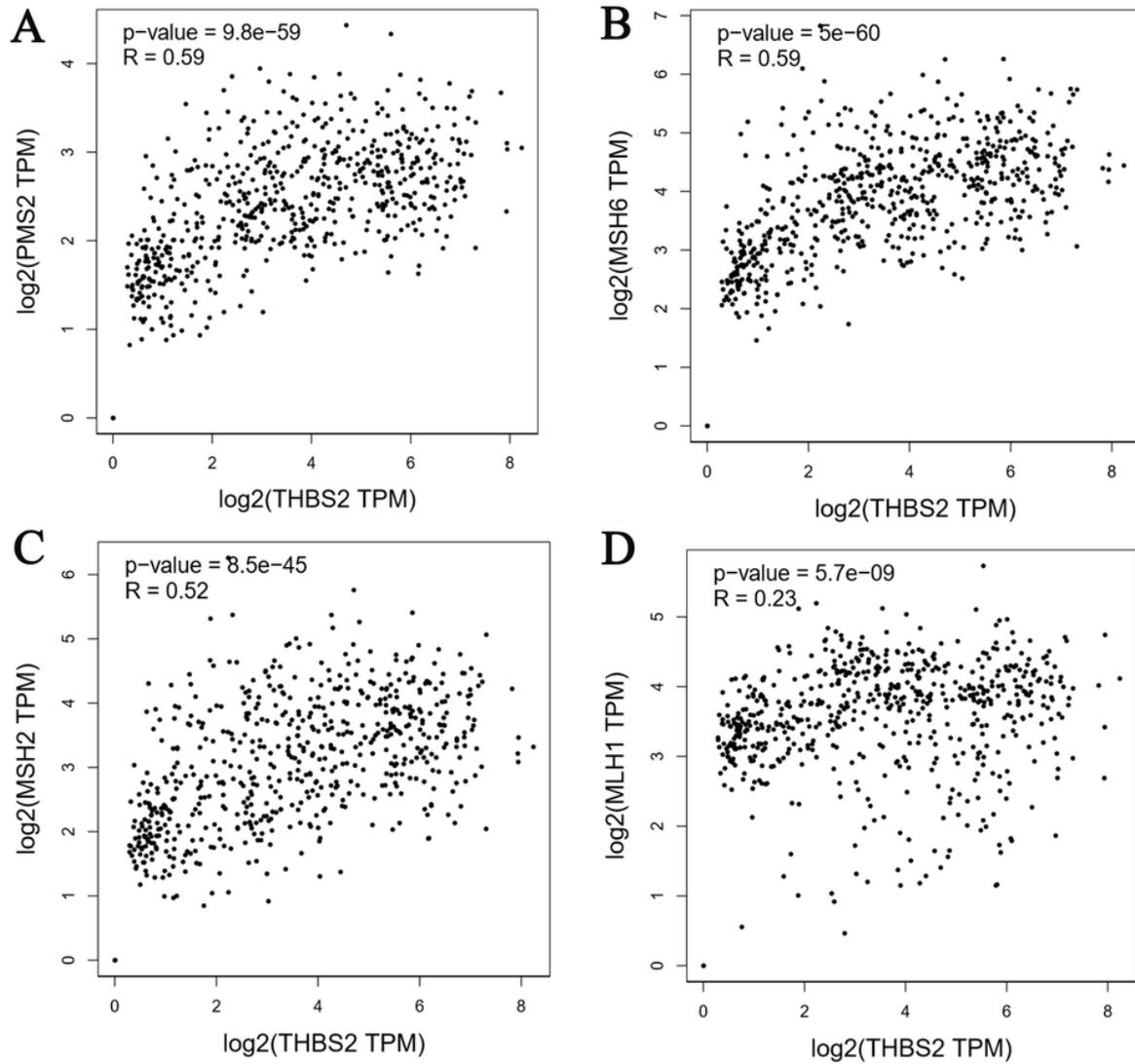
**Figure 3**

The relationship between TSP2 expression and clinicopathological characteristics in 80 cases of GC tissues: (A) TSP2 immunohistochemical staining of 80 cases of GC tissues showed low expression and high expression. (B) TSP2 expression level in 80 cases of GC tissues (\*\*P<0.01); (C) The effect of TSP2 expression on OS in 80 cases of GC (P=0.013).



**Figure 4**

Western blot detection of TSP2 protein expression level: (A) HGC27 GC cell line, (B) AGS GC cell line, Ctrl: control group; Si-1, Si-2: cell line after transfection. Transwell cell test detects the migration and invasion ability of GC cells: Migration: migration test; Invasion: invasion test; comparison between Ctrl control group and Si-2 experimental group (\*\*P<0.05; \*\*\*P<0.01).



**Figure 5**

The correlation between TSP2 gene and mismatch repair gene expression in the GEPIA database. (A-D) respectively indicate a significant positive correlation with the mismatch repair genes PMS2, MSH6, MSH2, MLH1, and TSP2 (THBS2) genes.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.pdf](#)