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TRPC6 is a Biomarker for Prognosis and Immunotherapy of Stomach Adenocarcinoma Based on Bioinformatic Analysis and Experimental Validation

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Background: Transient receptor potential canonical 6 (TRPC6), a key member of the TRPC family, is involved in diverse physiological and pathological processes. Although previous studies have implicated TRPC6 in the progression of stomach adenocarcinoma (STAD), its precise functions and mechanisms remain unclear. Understanding TRPC6's role in STAD may provide insights into its prognostic and therapeutic potential.

Methods: Using transcriptional and clinical data from The Cancer Genome Atlas (TCGA) database, we assessed the expression and prognostic value of TRPC6 in STAD through Kaplan-Meier survival curve analysis and correlation studies. Immune-related parameters, including immune cell infiltration and immune checkpoint gene expression, were also evaluated. Additionally, drug response analyses explored TRPC6's association with therapeutic agents. In vitro experiments were conducted to investigate TRPC6's role in STAD cell proliferation, migration, and invasion, focusing on its regulation of the PI3K-Akt signaling pathway.

Results: TRPC6 was significantly overexpressed in STAD tissues compared to normal tissues, with high TRPC6 expression associated with poor overall survival. TRPC6 expression correlated strongly with immune cell infiltration, immune checkpoint genes, and sensitivity to therapies such as Lapatinib, anti-CTLA4, and anti-PD1 treatments. Functional assays confirmed that TRPC6 promotes STAD cell proliferation, migration, and invasion by activating the PI3K-Akt signaling pathway.

Conclusion: This study highlights the prognostic significance of TRPC6 in STAD and its potential as a therapeutic target. TRPC6's involvement in immune regulation and cancer cell progression underscores its dual role in STAD pathogenesis and treatment, offering new avenues for targeted therapy development.

Keywords: TRPC6, stomach adenocarcinoma, immunotherapy, biomarkers

Introduction

Stomach adenocarcinoma (STAD) is one of the most common gastrointestinal malignancies. Despite recent decreases in its global incidence and mortality due to intensive research, it remains one of the most prevalent cancers worldwide.[1,2] Currently, the primary challenges in STAD are diagnosis and treatment.[3] Early detection and diagnosis can significantly improve treatment outcomes and even achieve a cure, while surgical resection remains the most effective treatment for STAD.[4,5] Therefore, understanding the mechanisms underlying STAD development and identifying more effective biomarkers is of great importance and urgency.

Transient receptor potential canonical (TRPC) family member TRPC6 is a receptor-activated non-selective cation channel, widely distributed in human tissues and involved in many physiological and pathological processes.[6,7] Aberrant expression of

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TRPC6 is also involved in the progression of various cancers, including breast cancer, lung cancer, liver cancer, and gastric cancer.[8,9] In STAD, studies have suggested that TRPC6 promotes tumor growth and invasion through multiple signaling pathways. For example, Helicobacter pylori infection upregulates TRPC6 expression via the Wnt/β-catenin pathway, promoting STAD cell migration and invasion.[10] Similarly, TRPC6 contributes to the epithelial-mesenchymal transition (EMT) in STAD via the Ras/Raf1/ERK signaling pathway under TGF-β1 stimulation.[11] Pharmacological inhibition of TRPC6 using specific antagonists has also been shown to reduce gastric cancer cell proliferation and migration, further underscoring its role in tumor progression.[12] However, these studies mainly focus on isolated mechanisms or pharmacological interventions, lacking clinically relevant research on TRPC6 and exploration of its relationship with the tumor microenvironment, while its role in gastric cancer mechanisms remains to be further elucidated.

In our study, we found that TRPC6 is highly expressed in STAD. Through bioinformatics analysis, we further explored the relationship between TRPC6 and tumor diagnosis, prognosis, immunotherapy, and its functional mechanisms. Additionally, we investigated the impact of TRPC6 on immune cell infiltration and the efficacy of common chemotherapeutic drugs. Finally, we conducted in vitro experiments to validate the functional mechanisms of TRPC6 in STAD and to explore the potential pathways through which it promotes tumor development.

Materials and Methods

Data Collection and Processing

We obtained the mRNA expression profile of TRPC6 in pan-cancer and corresponding normal tissues from TCGA database (https://portal.gdc.cancer.gov). In addition, we obtained mRNA expression profiles in normal tissues from the GTEx database (https://www.gtexportal.org/). Immunohistochemical data for TRPC6 were obtained from the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/). By retrieving published literature and the GEO database, the GSE27342 dataset was included in this study.[13] Between December 2020 and May 2024, STAD and adjacent non-cancerous tissues were collected from 100 patients undergoing surgery at Zhenjiang First People's Hospital in China. None of the participants had received chemotherapy or other treatments prior to surgery. The adjacent non-cancerous tissues were taken 5 cm away from the tumor margin. The excised tissues were placed in 1 mL of RNAfixer sample preservation solution (Bioteke, Beijing, China) and stored at −80°C until use. All tissue samples were pathologically confirmed. In accordance with the Declaration of Helsinki, all participants provided informed consent voluntarily to ensure their rights and protection. This study was approved by the Ethics Committee of Zhenjiang First People's Hospital (Ethics Approval Number: SH2024062). Data analysis and visualization were performed using R software (v4.0.3) and SangerBox (sangerbox.com).

Immune Infiltration

The interrelationship between the tumor genome and immunity was studied using the TIMER 2.0 (timer.cistrome.org) database.[14] TIMER 2.0 was utilized to explore the infiltration of six types of immune cells in the TRPC6 high expression group and the TRPC6 low expression group, as well as the correlation between TRPC6 expression and these six types of immune cells in STAD.

Functional Enrichment

Gene Ontology (GO) is a multifaceted qualification and description of the function of a gene, including Molecular Function (MF), Biological Process (BP), and Cellular Components (CC).[15] Kyoto Encyclopedia of Genes and Genomes (KEGG) is a comprehensive database integrating genomic, chemical, and phylogenetic functional information.[16] Count value is typically used to indicate the number of genes included in a function or pathway. The -log10(p-adjvalue) value is used to indicate the statistical significance of enrichment analysis results. A larger circle represents greater importance of the function or pathway, while a redder color indicates greater significance of the function or pathway.

Drug Sensitivity Analysis

The IC50 values of Cisplatin, 5-fluorouracil, Paclitaxel, and Lapatinib were obtained from the Genomics of Drug Sensitivity in Cancer (GDSC, https://www.cancerrxgene.org/). We compared the IC50 values of Cisplatin, 5-fluorouracil, Paclitaxel, and Lapatinib between the TRPC6 high expression group and the TRPC6 low expression group and analyzed the correlation between TRPC6 expression and the IC50 values of these drugs in STAD. Immunotherapy scores for samples in the TCGA database were obtained from the Tumor Immune Dysfunction and Exclusion (TIDE, https://tcia.at). We compared the scores for anti-CTLA4 and anti-PD1 inhibitor treatments between high and low TRPC6 expression groups.

qRT-PCR

The mRNA level of TRPC6 was quantified by IQ SYBR Premix Ex Taq Perfect Real-Time (Bio-Rad Laboratories, Hercules, CA) to perform real-time polymerase chain reaction (qRT-PCR) and tracked by β-actin. Primers were synthesized by Sangon Biotech (Sangon Biotech, Shanghai, China). The primers used were designed as follows: β-actin-forward: CAGGGC GTGATGGTGGGCA and β-actin-reverse: CAAACATCATCTGGGTCATCTTCTC; TRPC6-forward: GGGCTGGAGAGT CTCTGTTG and TRPC6-reverse: CGGGGAAAAGTCACCACTTA. Relative mRNA level comparisons were calculated by the 2-ΔΔCT method.

Cell Culture

STAD cell lines BGC-823 and AGS, and normal gastric epithelial cells GES-1 were obtained by purchase from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). 10% FBS (Gibco, Carlsbad, CA) and DMEM (HyClone, Beijing, China) were mixed proportionally for cell culture, and cells were stored in a humid environment at 37°C and 5% CO_2 for culture.

Transfection

Short hairpin (sh)-TRPC6 plasmids were obtained from GenePharma (Suzhou, China). When cells reached approximately 60% confluence, they were transfected with sh-TRPC6, with sh-NC used as a control. Transfection was carried out using Lipofectamine 2000 reagent (Invitrogen) after 6 hours of serum-free incubation, following the manufacturer's instructions. Cells were cultured for an additional 48 hours post-transfection before subsequent experiments were conducted.

Cell Proliferation Assay

Cells were seeded into a 96-well plate with 1000 cells per well. Each experimental group included 6 wells, while wells without cells served as the blank control group. Cell Counting Kit-8 (CCK-8) was added on days 1, 2, 3, 4, and 5, respectively. After a 1-hour incubation in the dark at 37°C, absorbance was measured at 490 nm using a microplate reader.

Xenograft Tumor Assay

Four-week-old female BALB/c nude mice, purchased from Jiangsu Hua Chuang Sino Pharmaceutical Technology Co., Ltd., were randomly divided into two groups. Lentivirus-transduced cells were injected into the dorsal region of each mouse at a density of 2×10^6 cells per mouse. Tumor dimensions were measured using calipers, and the volume was calculated using the formula $a2 \times b \times 0.5$, where a represents the width and b represents the length. Four weeks after injection, all mice were euthanized, and the xenograft tumors were excised and weighed. All animal studies were conducted with the approval of the Animal Ethics Committee of Jiangsu University and in accordance with the guidelines for the use of laboratory animals.

Wound Healing Assay

Cells were seeded at a density of 30,000 cells per well in a 6-well plate. A linear scratch was gently made using the tip of a 200 μL pipette. Samples were washed with PBS to remove any loose cells. Photographs were taken after 36 hours, and images were captured and analyzed for results.

Transwell Migration and Invasion Assay

Cells were seeded into the upper chambers of a Transwell plate (24-well, 8-mm, Corning, Life Sciences) for the migration assay without Matrigel coating. Approximately 30,000 cells were added to each chamber and allowed to incubate overnight. The lower chambers were filled with culture medium containing 10% FBS, while the upper chambers contained medium without FBS. After 48 hours of incubation at 37°C, non-migrating cells were gently removed. The migrated cells were fixed in 90% ethanol and stained with 1% crystal violet (Sigma-Aldrich). Similarly, for the invasion assay, the upper chambers were coated with Matrigel (BD Biosciences, USA) prior to seeding cells, following the same incubation and staining procedures after 48 hours to assess invaded cells. Using an inverted microscope, capture images from at least 5 random fields and analyze the collected images.

Western Blotting

Antibodies used include anti-TRPC6 (ab62461, Abcam), GAPDH (no.5174, Cell Signaling), anti-phosphor-PI3K (no.17366, Cell Signaling), anti-PI3K (no.4257, Cell Signaling), anti-phospho-Akt (no.4060, Cell Signaling), and anti-Akt (no.4691, Cell Signaling). The grayscale values of the strips were measured and counted by Image J (NIH, Bethesda) software.

Statistical Analysis

This study utilized the Wilcoxon rank-sum test or Student's *t*-test to compare differences between two groups, depending on the data distribution. For comparisons among three or more groups, the Kruskal–Wallis test was applied. Spearman correlation analysis was conducted to evaluate relationships between variables. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic efficacy of TRPC6, and time-dependent ROC analysis was employed to evaluate its time-related diagnostic performance. Survival analyses were carried out using Kaplan-Meier curves with the Log rank test and univariate Cox regression analysis. All statistical analyses were conducted using GraphPad Prism 7.00 and IBM SPSS 23.0 software, with statistical significance set at $p < 0.05$.

Results

TRPC6 is Significantly Overexpressed in STAD

To understand the basic information of TRPC6 in cancer, we used TCGA databases to assess the expression levels of TRPC6 in cancer versus normal tissues. In the TCGA database, we observed significant upregulation in 8 tumors, including ESCA, STES, STAD, HNSC, KIRC, LIHC, PCPG, and CHOL, and significant downregulation in 15 tumors, including LGG, CESC, LUAD, COAD, COADREAD, BRCA, KIRP, and KIPAN (Figure 1A). However, the significant overexpression of TRPC6 in STAD has piqued our interest. We further incorporated normal tissue data from the GTEx database and found that TRPC6 remained significantly expressed in gastric cancer. The area under the curve (AUC) value between the two groups, STAD and normal tissues, was 0.64 (Figure 1B and C). Subsequently, we found that the GSE27342 dataset from the GEO database also indicates a marked increase in TRPC6 expression in STAD tissues compared to adjacent normal tissues, with an area under the curve (AUC) value of 0.78 (Figure 1D and E). Interestingly, qRT-PCR experiments conducted on a subset of STAD and adjacent tissues revealed a significant up-regulation of TRPC6 expression, with an AUC value of 0.85 (Figure 1F and G). This suggested that TRPC6 might not only be an important oncogene but also hold potential as a diagnostic biomarker for STAD.

Assessment of the Prognostic Value of TRPC6 in STAD

Figure 2A displayed STAD samples arranged by TRPC6 expression levels, divided into high and low expression groups based on the median cut-off value. The scatter plot illustrated patient survival time and status, with red dots indicating survival and blue dots indicating death. The TRPC6 high-expression group exhibited lower overall survival rates compared to the TRPC6 low-expression group (Figure 2B). The AUC values for OS at 1, 3, and 5 years in the TRPC6 expression groups were 0.585, 0.568, and 0.687, respectively (Figure 2C). This also indicated that TRPC6 held potential as a prognostic indicator.

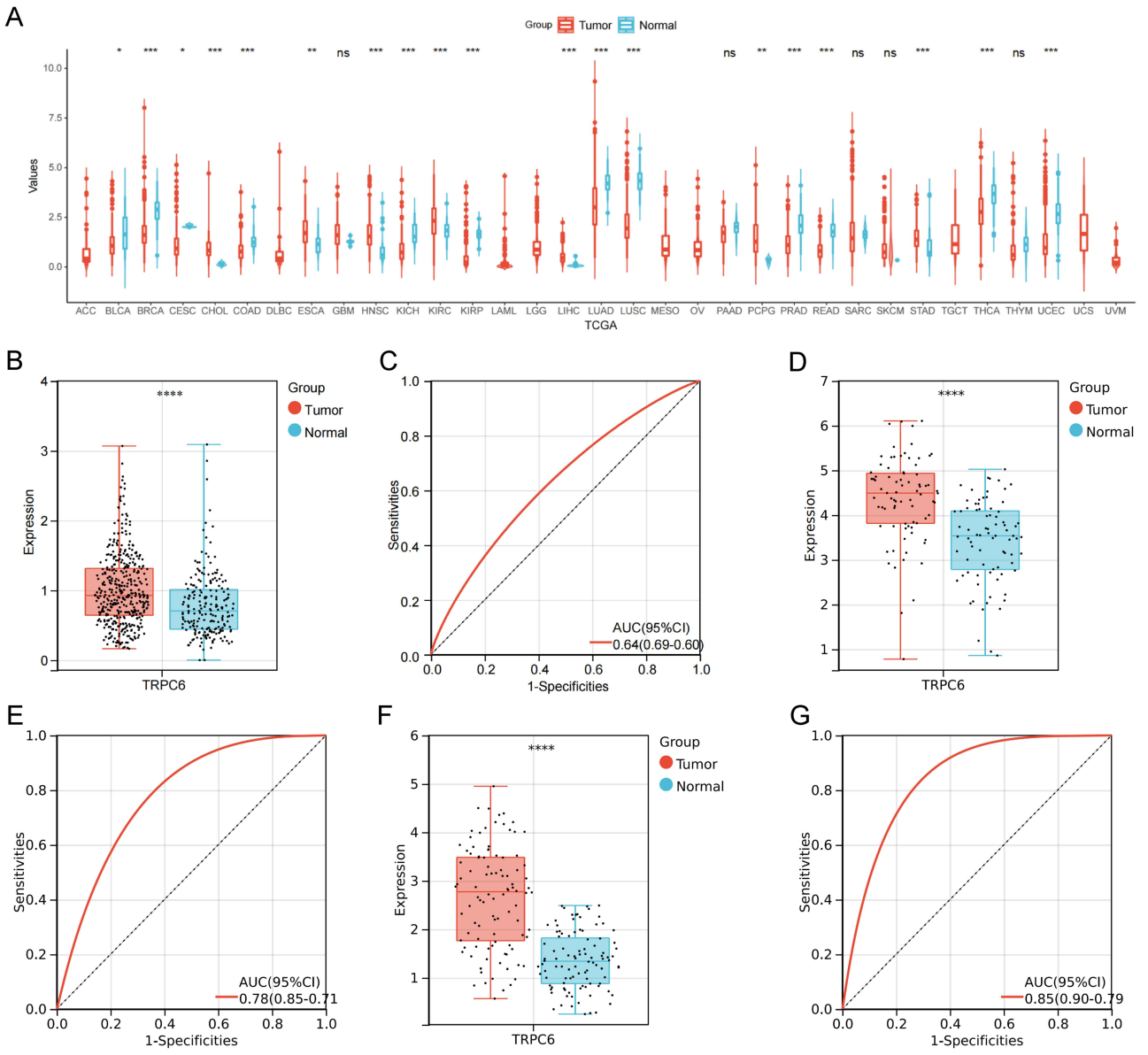

Figure 1 TRPC6 is significantly overexpressed in STAD. (**A**) Expression data of TRPC6 gene in 33 cancer species based on TCGA database. (**B**) TRPC6 expression in tumor samples and normal samples from TCGA and GTEx dataset. (**C**) ROC curve of TRPC6 was based on TCGA and GTEx dataset. (**D**) TRPC6 expression in tumor samples and normal samples from the GSE27342 dataset. (**E**) ROC curve of TRPC6 was based on the GSE27342 dataset. (**F**) TRPC6 expression in clinically collected tumor samples as well as in normal samples. (**G**) ROC curve of TRPC6 was based on data from clinically collected samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Immune Landscape Analysis

The difference in immune cell infiltration between high and low TRPC6 expression groups was compared using the TIMER database. Compared to the TRPC6 low expression group, the TRPC6 high expression group had higher infiltration of T cell CD4+, T cell CD8+, Neutrophil, Macrophage, and Myeloid dendritic cells (Figure 3A). Additionally, TRPC6 expression was positively correlated with the infiltration levels of T cell CD4+, T cell CD8+, Neutrophil, Macrophage, and Myeloid dendritic cells (Figure 3B). Furthermore, the difference in immune checkpoint gene expression between high and low TRPC6 expression groups was analyzed. Compared to the TRPC6 low expression group, the TRPC6 high expression group showed significantly higher expression levels of CD274, CTLA4, HAVCR2, PDCD1LG2, and TIGIT (Figure 3C). Additionally, TRPC6 expression was positively correlated with the expression of HAVCR2, PDCD1LG2, and TIGIT (Figure 3D).

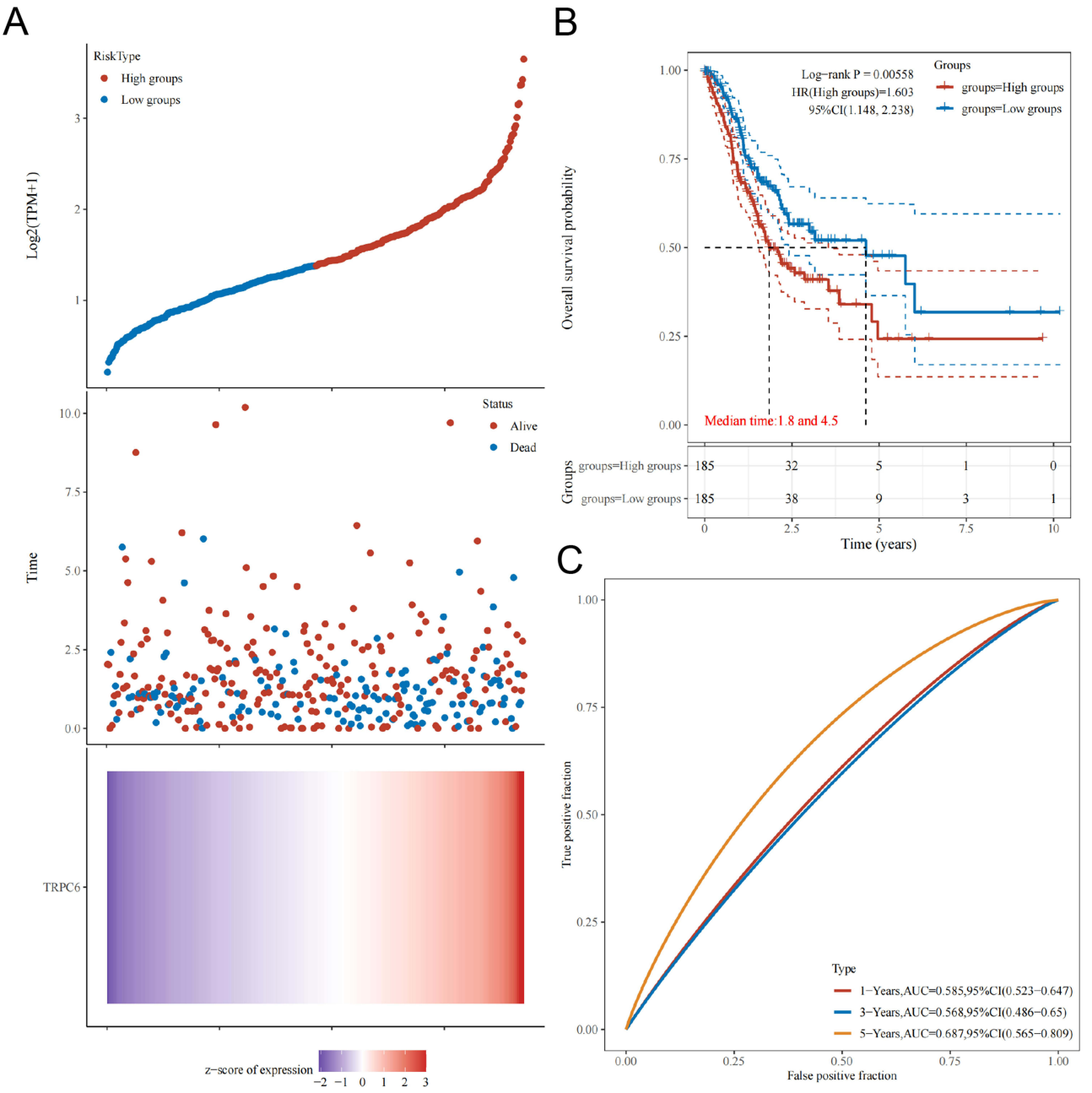

Figure 2 Assessment of the prognostic value of TRPC6 in STAD. (**A**) Relationship between TRPC6 expression and survival time and survival status. (**B**) KM survival curve analysis according to TRPC6 expression. (**C**) ROC curves and AUC values of TRPC6 at 1 year, 3 years, and 5 years.

Immunotherapy Analysis

Exploring the therapeutic value of TRPC6 in STAD. Based on the GDSC database, there were no significant differences in the IC50 values of Cisplatin, 5-fluorouracil, and Paclitaxel between high and low TRPC6 expression groups (Figure 4A–C). However, the IC50 value of Lapatinib was significantly higher in the TRPC6 high expression group compared to the TRPC6 low expression group (Figure 4D). Further correlation analysis revealed that the expression of TRPC6 was not correlated with the IC50 values of Cisplatin, 5-fluorouracil, and Paclitaxel, but was positively correlated with the IC50 value of Lapatinib (Figure 4E–H). A higher therapeutic score for anti-CTLA4 and anti-PD1 inhibitors indicates a better therapeutic effect. Compared to the TRPC6 low expression group, the TRPC6 high expression group showed significantly decreased IPS for CTLA4-negative-PD1-negative, CTLA4-negative-PD1-positive, CTLA4-positive

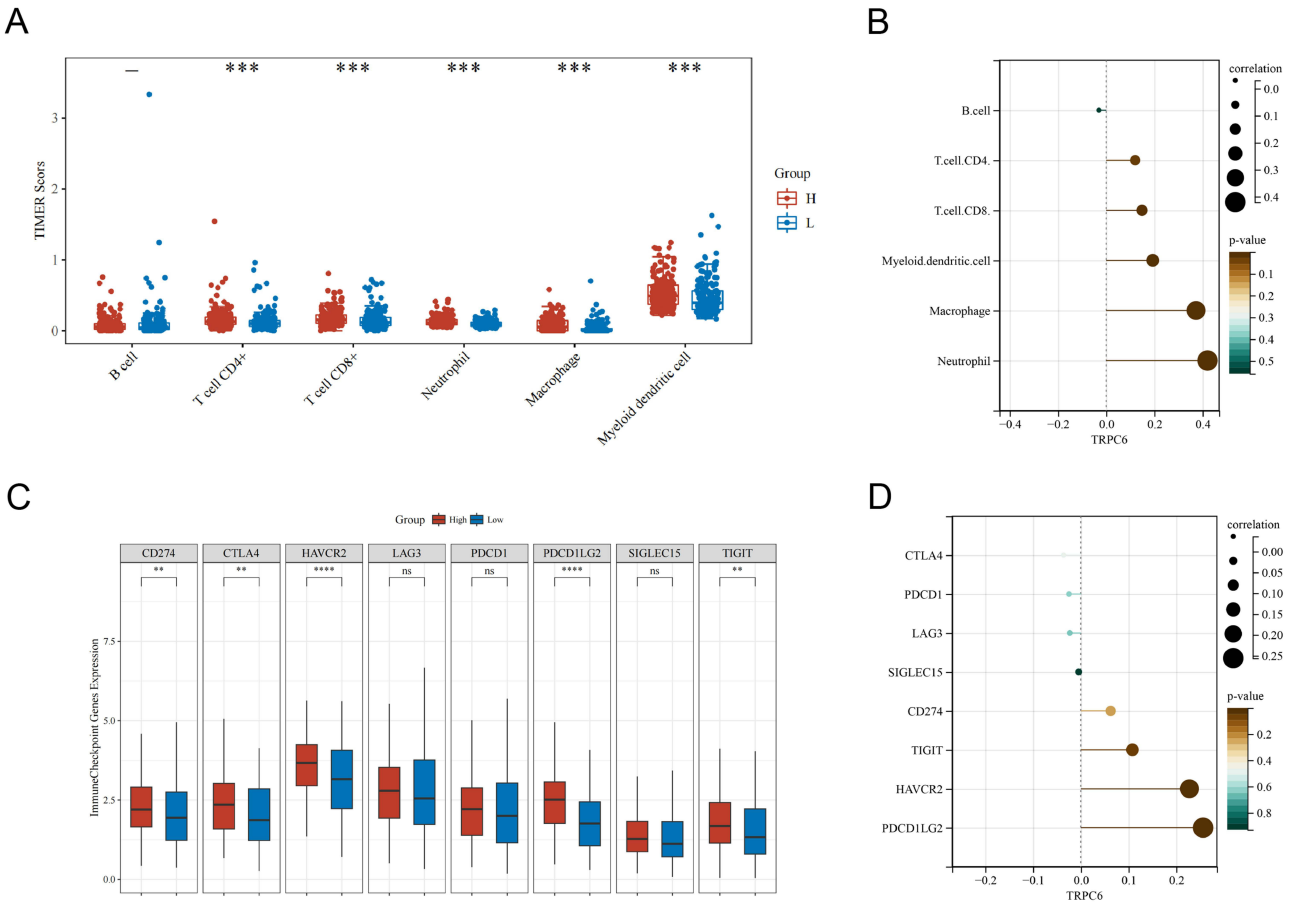

Figure 3 Immune infiltration analysis. (**A**) The differences in immune infiltration scores between high and low TRPC6 expression groups were compared based on the TIMER algorithm. (**B**) The correlation between TRPC6 expression and the infiltration levels of B cell, T cell CD4+, T cell CD8+, neutrophils, macrophages, and myeloid dendritic cells. (**C**) Comparison of immune checkpoint gene expression differences between high and low TRPC6 expression groups. (**D**) Correlation between TRPC6 expression and immune checkpoint genes expression. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

-PD1-negative, and CTLA4-positive-PD1-positive (Figure 4I–L). This suggested that anti-CTLA4 therapy or anti-PD1 therapy might be more effective in patients with low TRPC6 expression.

TRPC6 Knockdown Inhibited the Proliferation of STAD Cells

The immunohistochemical results from the HPA database showed that the staining range and intensity of TRPC6 protein expression in STAD were higher than those in normal gastric tissues (Figure 5A). We analyzed TRPC6 mRNA levels in GES-1, BGC-823, and AGS cells by qRT-PCR. Compared with TRPC6 mRNA levels in GES-1 cells, TRPC6 mRNA levels were significantly higher in both BGC-823 and AGS cells (Figure 5B). In BGC-823 and AGS cells, effective interference with TRPC6 expression was followed by inhibition of cell proliferation ability detected by CCK8 assay (Figure 5C and D). To investigate whether TRPC6 also promotes tumorigenesis in vivo, subcutaneous tumor-bearing experiments were performed. The tumor volume and weight of sh-NC group were significantly larger than those of sh-TRPC6 group (Figure 5E–G). This suggest that TRPC6 could promote the growth of STAD.

TRPC6 Knockdown Inhibited Migration and Invasion of STAD Cells

To investigate the impact of TRPC6 on the migration and invasion abilities of STAD cells, we performed wound healing assays and Transwell migration and invasion assays. In BGC-823 and AGS cells, TRPC6 knockdown inhibited their wound healing ability (Figure 6A). In the Transwell migration assay, TRPC6 knockdown significantly reduced the migration ability of BGC-823 and AGS cells (Figure 6B). Additionally, in the Transwell invasion assay, TRPC6 knockdown significantly diminished the invasion capacity of BGC-823 and AGS cells (Figure 6C). EMT is the process

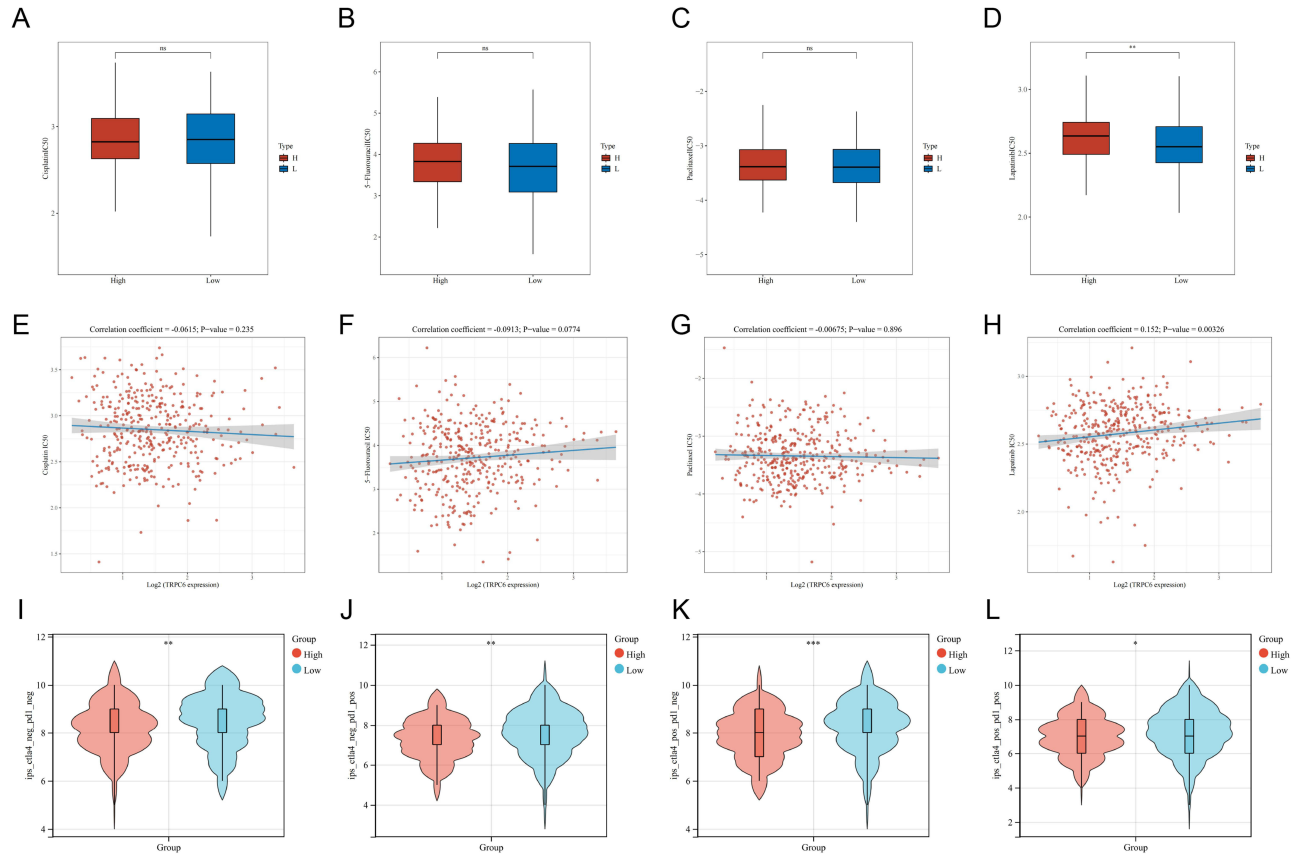

Figure 4 Prediction of therapeutic target for TRPC6. The IC50 values of (**A**) Cisplatin, (**B**) 5-fluorouracil, (**C**) Paclitaxel, and (**D**) Lapatinib were compared between high and low TRPC6 expression groups. TRPC6 expression was associated with IC50 values of (**E**) Cisplatin, (**F**) 5-fluorouracil, (**G**) Paclitaxel, and (**H**) Lapatinib. Immune therapy scores of anti-CTLA4 and anti-PD1 inhibitors indicating response of (**I**) no immunotherapy, (**J**) anti-PD1, (**K**) anti-CTLA4, (**L**) anti-PD1 and anti-CTLA4 therapies between high and low TRPC6 expression groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

by which epithelial cells acquire mesenchymal cell characteristics, which is closely related to extracellular matrix (ECM). This process also enables tumor cells to gain enhanced metastatic and invasive capabilities. In BGC-823 and AGS cells, TRPC6 knockdown increased the protein expression levels of E-cadherin, but decreased the protein expression levels of N-cadherin, vimentin, and MMP9 (Figure 6D). These results suggested that TRPC6 might promote the migration and invasion of STAD cells.

TRPC6 Knockdown Inhibited the PI3K-Akt Signaling Pathway in STAD Cells

Differentially expressed genes between the TRPC6 high expression group and the TRPC6 low expression group were subjected to KEGG and GO analyses. KEGG analysis revealed that these genes were mainly enriched in the PI3K-Akt signaling pathway (Figure 7A). GO analysis showed that they were primarily enriched in extracellular structure organization and extracellular matrix organization (Figure 7B). This was also consistent with our previous findings. We further examined the effect of TRPC6 on the PI3K-Akt signaling pathway using Western blotting. In BGC-823 and AGS cells, TRPC6 knockdown inhibited the protein expression levels of p-PI3K and p-Akt (Figure 7C–E). This suggested that TRPC6 might play a role in promoting cancer through the PI3K-Akt signaling pathway.

TRPC6 Knockdown Inhibited the Proliferation, Migration, and Invasion of Gastric Cancer Cells Through the PI3K-Akt Signaling Pathway

To further explore whether TRPC6 exerts its effects through the PI3K-Akt signaling pathway, we treated AGS cells with a combination of the PI3K activator 740Y-P and sh-TRPC6. The treatment with 740Y-P partially rescued the inhibitory effects of TRPC6 suppression on AGS cell proliferation (Figure 8A). Additionally, 740Y-P partially reversed the

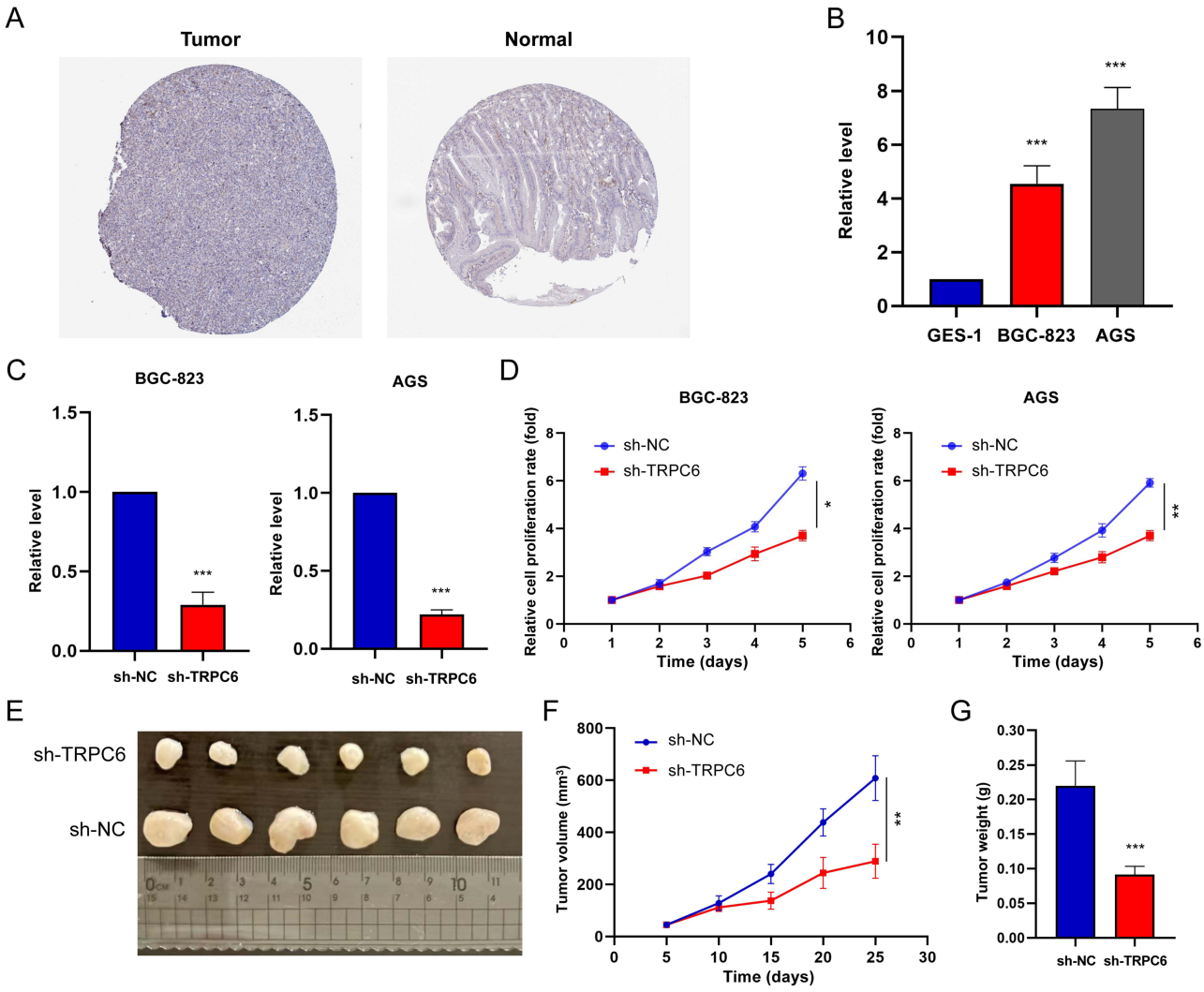

Figure 5 The impact of TRPC6 on the proliferative capacity of STAD cells. (**A**) Representative images of immunohistochemical staining for TRPC6 in STAD tissues and normal tissues were based on the HPA database. (**B**) Expression of TRPC6 in GES-1, BGC-823, and AGS cells by qRT-PCR. (**C**) In BGC-823 and AGS cells transfected with sh-TRPC6, the expression of TRPC6 was detected by qRT-PCR. (**D**) The effect of TRPC6 knockdown on the proliferation of BGC-823 and AGS cells was determined using the CCK-8 assay. (**E**) AGS cells stably transfected with sh-NC and sh-TRPC6 were subcutaneously injected to establish subcutaneous xenograft models in nude mice. (**F**) The growth trend of tumor volume was analyzed and evaluated. (**G**) Tumor weight was analyzed and evaluated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

suppression of migratory and invasive abilities caused by TRPC6 inhibition in AGS cells (Figure 8B–D). Furthermore, 740Y-P partially reversed the upregulation of E-cadherin expression and the downregulation of N-cadherin, vimentin, and MMP9 expression induced by TRPC6 inhibition (Figure 8E and F). This suggested that TRPC6 inhibition suppressed STAD progression through the PI3K-Akt signaling pathway.

Discussion

The transient receptor potential (TRP) channels were first identified in 1969 and, as research progressed, were found to be overexpressed in some cancers, thereby triggering a massive activation of the Ca2+ signaling cascade, ultimately leading to enhanced cancer growth and resistance to apoptosis as well as promoting cancer cell migration and invasiveness.[17] Interestingly, most TRP channels are present on the cell surface, which makes them more easily exposed and thus easier to be targeted for inhibition and become more suitable targets.[18] As a key member of the TRP channel family, TRPC6 was found to be highly expressed in STAD based on the TCGA database. Patients with high TRPC6 expression had worse overall survival, suggesting that TRPC6 might be an important factor influencing the prognosis of

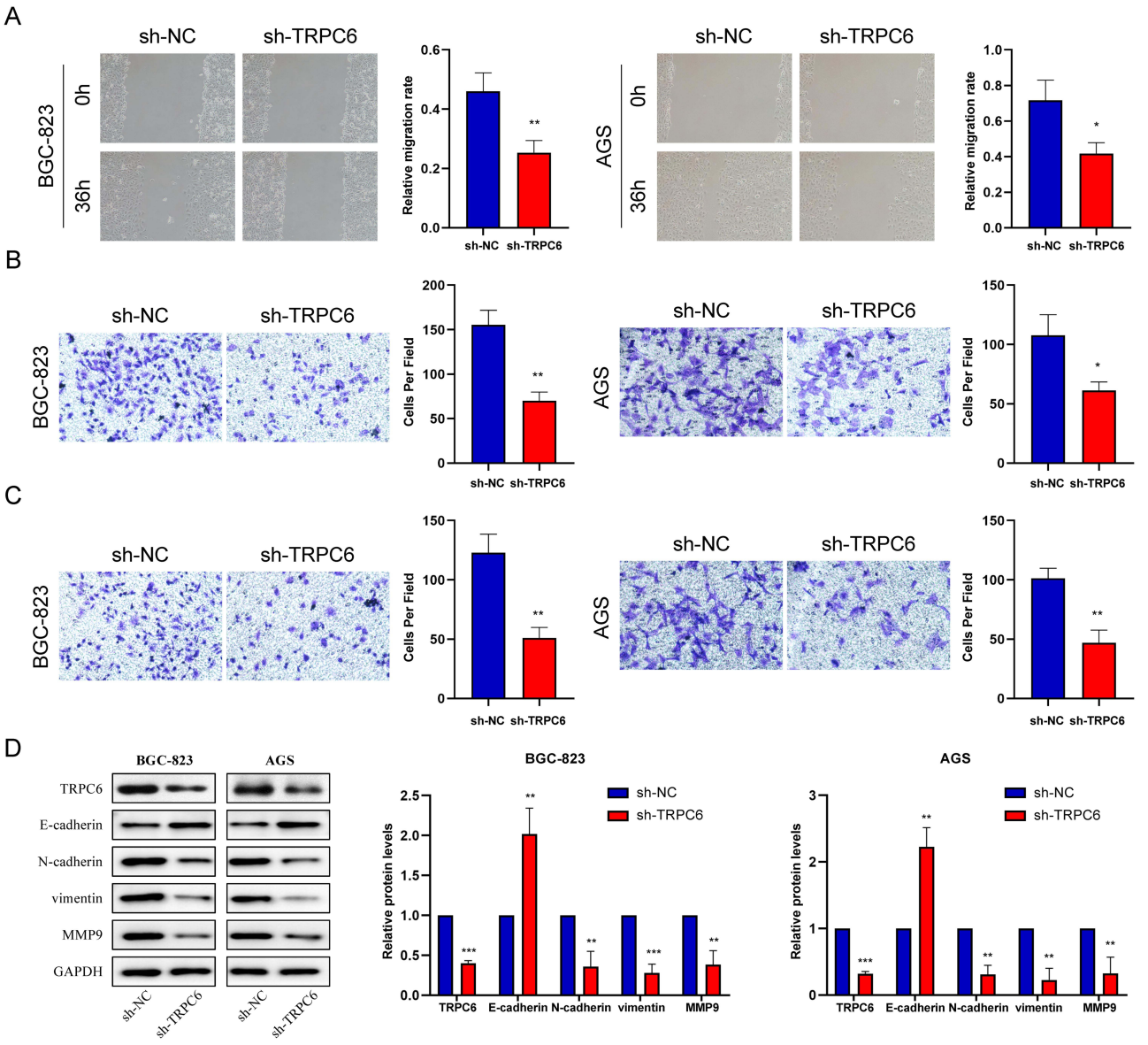

Figure 6 The effect of TRPC6 on the migration and invasion abilities of STAD cells. (**A**) The effect of TRPC6 on the migratory capacity of BGC-823 and AGS cells was assessed using the wound healing assay. (**B**) The effect of TRPC6 on the migration ability of BGC-823 and AGS cells was assessed using the Transwell migration assay. (**C**) The effect of TRPC6 on the invasion ability of BGC-823 and AGS cells was assessed using the Transwell invasion assay. (**D**) The expression of proteins related to EMT was detected by Western blotting. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

STAD. More importantly, we validated the high expression of TRPC6 in STAD through the GSE27342 dataset and in tumor and normal tissues from 100 STAD patients, demonstrating its potential as a reliable diagnostic marker.

In the results of KEGG and GO analysis of differential genes with high TRPC6 expression and low TRPC6 expression, it was found that they were mainly enriched in PI3K-Akt signaling pathway, Focal adhesion, ECM-receptor interaction, extracellular structure organization, and extracellular matrix organization. The PI3K-Akt signaling pathway is one of the major cellular signaling pathways in the human body, but it is over-activated in cancer, thus promoting cancer progression, and inhibition of pathway activation can inhibit cancer progression.[19,20] We found that knockdown of TRPC6 inhibited the activation of PI3K-Akt signaling pathway in vitro. In addition, the complex division and role of extracellular matrix as an important part of the tumor microenvironment (TME) provide great convenience to tumor growth and metastasis.[21] Focal adhesion coordinates the relationship between the cell and the extracellular matrix, yet in cancer, it can instead contribute to the proliferation and metastasis of cancer cells and the formation of TME.[22] We

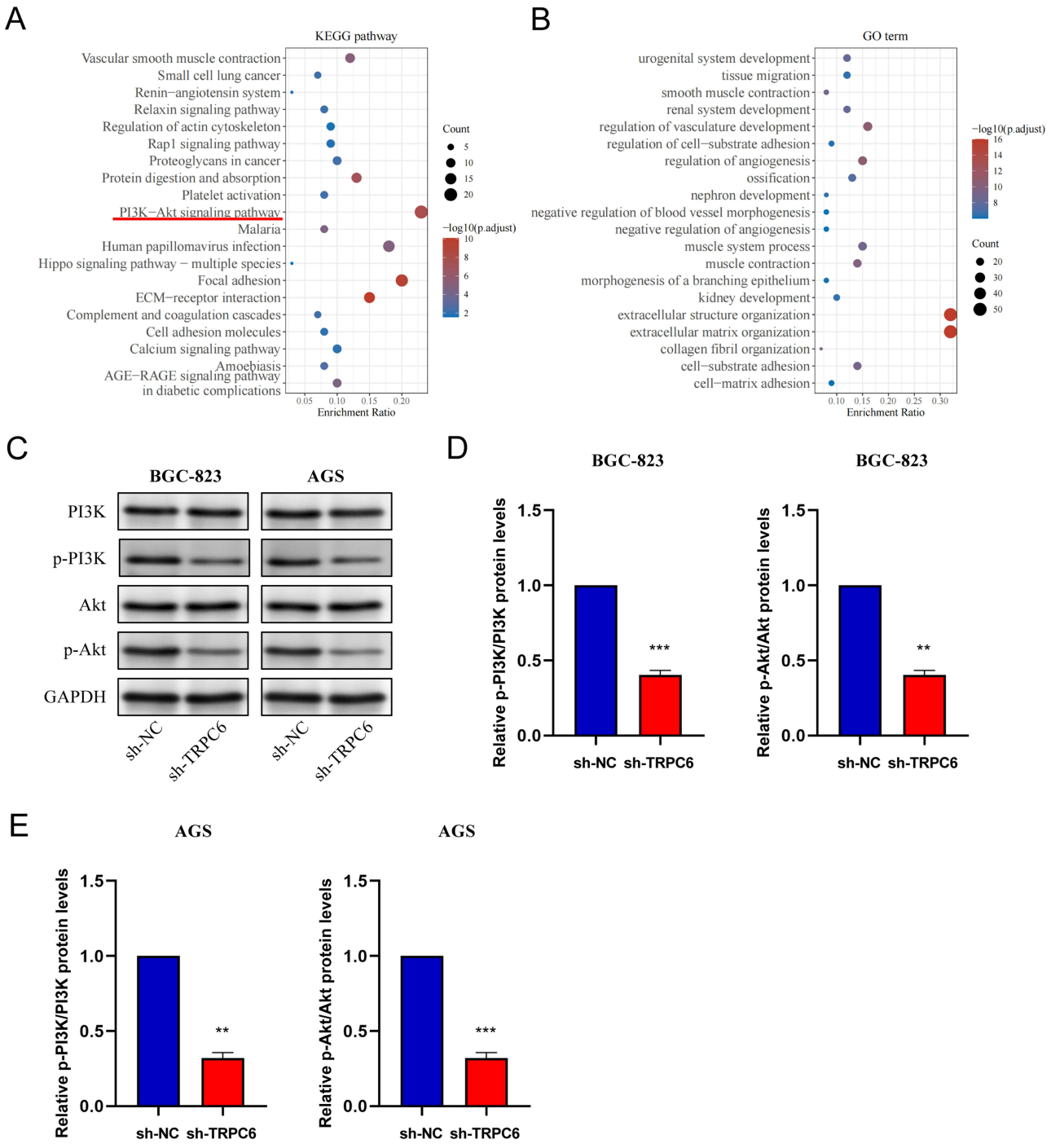

Figure 7 The effect of TRPC6 on the PI3K-Akt signaling pathway in STAD cells. (**A**) KEGG analysis of DEGs. (**B**) GO analysis of DEGs. (**C-E**) The expression of proteins related to the PI3K-Akt signaling pathway was detected by Western blotting. $^{**}p < 0.01$, $^{***}p < 0.001$.

found that knockdown of TRPC6 expression in vitro inhibited the proliferation, migration, and invasion of STAD cells. This also suggested that TRPC6 could be an important regulator in the occurrence and development of STAD.

TME consists of all non-cancerous host cells and non-cellular components of the tumor, of which tumor-associated immune cells and the extracellular matrix are important components.[23] We found that the expression of TRPC6 and T cell, CD4 + T cell CD8 +, Neutrophil, Macrophage, and Myeloid dendritic cells infiltration was a positive correlation. Tumor-associated macrophages (TAMs) are abundant in TME of most cancer types, promote tumor inflammation thereby

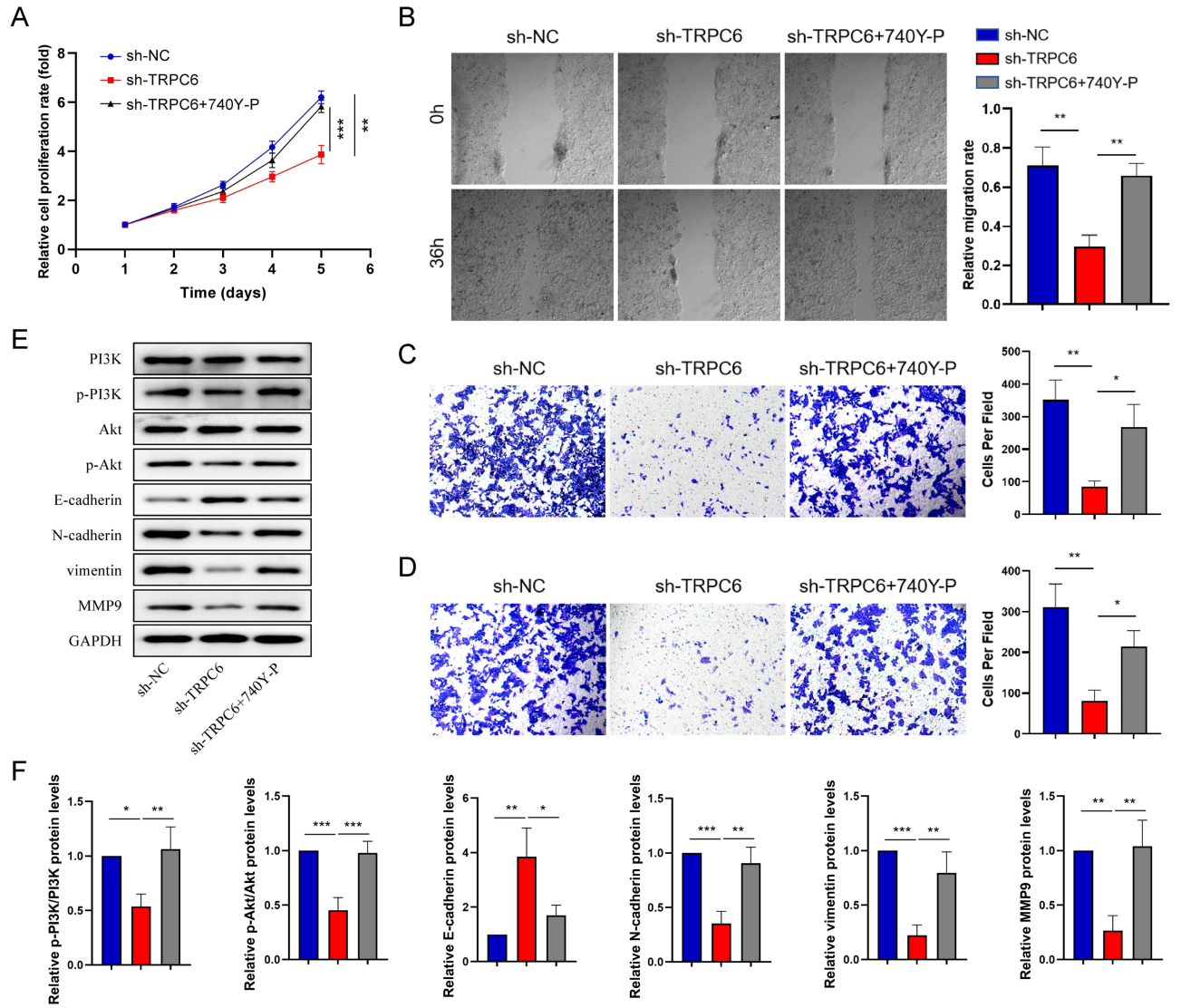

Figure 8 Effects of combined administration of sh-TRPC6 and 740Y-P on proliferation, migration, and invasion of AGS cells. (**A**) The CCK-8 assay was used to assess the effect of the combined use of sh-TRPC6 and 740Y-P on the proliferation of AGS cells. (**B**) The wound healing assay was performed to evaluate the impact of the combined treatment of sh-TRPC6 and 740Y-P on the migratory capacity of AGS cells. (**C**) The Transwell migration assay was employed to evaluate the impact of sh-TRPC6 combined with 740Y-P on the migration of AGS cells. (**D**) The Transwell invasion assay was utilized to assess the effect of the combined use of sh-TRPC6 and 740Y-P on the invasion of AGS cells. (**E-F**) Western blotting was used to assess the effect of the combined use of sh-TRPC6 and 740Y-P on EMT-related proteins in AGS cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

aiding cancer cell immune escape, and promote angiogenesis to accelerate tumor growth.[24] The large number of TAMs and their reciprocal achievement relationship with cancer cells are making TAMs a key direction for immunotherapy research.[25] Neutrophils, the first responders of innate immunity, are the most abundant population of leukocytes in peripheral blood.[26] Tumor-associated neutrophils (TANs) are an important component of TME, releasing various proteases, inflammatory factors, growth factors, and immunosuppressive factors, thereby modifying TME more conducive to cancer cell survival and ultimately promoting tumor progression and metastasis.[27,28] One study validated the high sensitivity of TME evaluation to predict the effect of immunotherapy in advanced gastric cancer.[29] This also suggested that TRPC6 might influence the prognosis of STAD by affecting immune cell infiltration. TRPC6 expression was positively correlated not only with the IC50 of lapatinib, but also with the immune checkpoints HAVCR2, PDCD1LG2, and TIGIT. Immune checkpoints act as regulators of the body's immune system, preventing excessive T-cell activation.[30] Abnormal expression of immune checkpoint molecules in tumor cells inhibits immune cell function, allowing the tumor to complete its immune escape and continue to grow.[31] At present, immunotherapy with immune

checkpoint blockade (ICB) targeting CTLA4 and PD1 is the main choice for cancer treatment.[32] By blocking immunomodulatory receptors such as PD1 and CTLA4, T cells restore function and proliferation, thereby restoring antitumor immune responses.[33] We found that patients with low TRPC6 expression benefited more from CTLA4 or anti-PD1 immunotherapy. This suggests that TRPC6 not only influences cancer progression but may also mediate immune escape in gastric cancer. These findings expand the understanding of TRPC6 beyond its previously identified roles, emphasizing its potential as both a therapeutic target and an immunotherapy biomarker.

However, there are some limitations of this study. More data from clinical samples and more in-depth molecular experiments are needed to prove the clinical significance of TRPC6 in STAD and the mechanism of action.

Conclusion

In summary, this study provides a comprehensive analysis of TRPC6 in STAD, highlighting its overexpression, functional role in cancer progression, and potential impact on TME. Our work uniquely integrates bioinformatics, clinical sample validation, and functional assays to offer new insights into TRPC6's role in cancer biology. These findings not only deepen our understanding of STAD pathogenesis but also propose TRPC6 as a promising target for therapeutic and immunotherapeutic strategies.

Data Sharing Statement

The data analyzed in this study were obtained from online public databases, and the databases are given names and accession number(s) in the article.

Ethical Approval

The study was conducted in accordance to with the Declaration of Helsinki (as revised in 2013). Approval was granted by the Ethics Committee of The Affiliated People's Hospital of Jiangsu University.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

The authors affirm that human research participants provided informed consent for publication.

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Disclosure

The authors declare that there are no conflicts of interest.

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