Original Article

Validation of the interaction between PRDX4 and TXNDC5 in gastric cancer and the significance of the *PRDX4* gene in gastric cancer based on a data mining analysis

Lin Zhang[#], Kai Wu[#], Yanhong Hou, Xianghui Li

Department of Gastroenterology, The Eighth Medical Center, PLA General Hospital, Beijing, China

Contributions: (I) Conception and design: L Zhang, Y Hou, K Wu; (II) Administrative support: L Zhang, K Wu; (III) Provision of study materials or patients: K Wu; (IV) Collection and assembly of data: L Zhang, X Li; (V) Data analysis and interpretation: L Zhang, X Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Yanhong Hou, PhD. Department of Gastroenterology, The Eighth Medical Center, PLA General Hospital, 17 Heishanhu Road, Haidian District, Beijing 100091, China. Email: houyan211@sina.com.

Background: We successfully screened the important interacting protein peroxiredoxin 4 (PRDX4) of thioredoxin domain-containing protein 5 (TXNDC5) in gastric cancer. However, its specific molecular mechanism in gastric cancer remains unclear. This study aimed to verify the interaction between PRDX4 and TXNDC5 protein molecules in gastric cancer and analyze the expression and functional significance of PRDX4 in gastric cancer using bioinformatics methods.

Methods: The interaction between TXNDC5 and PRDX4 was verified by the coimmunoprecipitation (co-IP) of the total protein of gastric cancer cells, and tissues with high expressions of TXNDC5. The Human Protein Atlas (HPA) database, UCSC Xena (University of California Santa Cruz xenabrowser) platform, the Kaplan-Meier Plotter platform, and the TIMER (Tumor IMmune Estimation Resource) platform were used to analyze the expression and subcellular localization of the PRDX4 molecule in normal human gastric tissue, the difference in expression between gastric cancer tissue and normal gastric tissue, the relationship between the expression of PRDX4 and survival, its functional significance in gastric cancer cells, and its effect on the tumor immune microenvironment (TIME).

Results: The data analysis results showed that the expression of PRDX4 messenger RNA (mRNA) in the gastric cancer tissues was significantly higher than that in the normal tissues ($P<0.05$). PRDX4 could affect the occurrence and development of tumors by participating in the neutrophil degranulation signaling pathway to regulate tumor immunity. The expression level of PRDX4 has a certain relationship with the TIME; that is, it is mainly negatively correlated with the infiltration of B lymphocytes and $CD4^+$ T lymphocytes ($P<0.05$). The expression level of PRDX4 was positively correlated with the expression of LILRB2 (leukocyte immunoglobulin-like receptor subfamily B member 2), and negatively correlated with BLTA (B and T lymphocyte attenuation factor) and VISTA (V-type immunoglobulin domain-containing suppressor of T cell activation) ($P<0.05$).

Conclusions: There is an interaction between PRDX4 and TXNDC5 protein molecules in gastric cancer. *PRDX4* gene expression is significantly up-regulated in gastric cancer. It may reduce the infiltration of B lymphocytes and $CD4^+$ T lymphocytes and affect the expression of LILRB2, BLTA, and VISTA immune checkpoints, leading to anti-tumor immunosuppression.

Keywords: *PRDX4* gene; survival curve; gastric cancer; molecular signaling pathways; tumor immune microenvironment (TIME)

Submitted May 26, 2023. Accepted for publication Oct 20, 2023. Published online Jan 15, 2024.
doi: 10.21037/tcr-23-904
View this article at: https://dx.doi.org/10.21037/tcr-23-904

Introduction

Verrucous gastritis is a precancerous disease with significant cancerous potential. However, the specific molecular mechanism of its carcinogenesis remains unclear. In a previous study (1), we conducted a differential proteomic analysis of verrucous gastritis and normal gastric mucosa to explore the molecular mechanism of carcinogenesis of an important precancerous disease (i.e., verrucous gastritis). The results showed that thioredoxin domain-containing protein 5 (TXNDC5) is an important and significant differential protein.

TXNDC5, also known as ERP46 (endoplasmic reticulum protein 46) or endo-protein disulfide isomerase (PDI), is a newly discovered protein molecule that is a member of the epithelial PDI family and consists of 324 amino acid residues (2). In previous studies, our research team confirmed that the TXNDC5 molecule has a cancer promoting effect in gastric cancer, promoting tumor cell proliferation, inhibiting cell apoptosis, and enhancing cell invasiveness (3). A preliminary analysis suggested that this gene may be a potential cancer promoting gene in gastric cancer (3,4). In a further study (5), we successfully 'identified the interacting proteins of the TXNDC5 protein in gastric cancer cells using tandem affinity coupled proteomics [fluorescence activating and absorbing tag (FLAG) pull-down coupled mass spectrometry]. Through these studies, we identified some TXNDC5 interacting proteins in gastric cancer, among which peroxiredoxin 4 (PRDX4) is an important interacting molecule.

Combined with other research studies, our previous research confirmed that TXNDC5 has a significant cancer promoting effect in gastric cancer. However, until now, the molecular mechanism of TXNDC5 promoting cancer in gastric cancer has not been elucidated. Thus, we used FLAG pull-down coupled proteomics technology to screen the interacting protein molecules of TXNDC5 in gastric cancer and identified a group of protein molecules, which included PRDX4.

However, these studies had a number of limitations. First, the interaction between TXNDC5 and these protein molecules still requires experimental verification. Second, the functional significance, prognostic relationship, relationship with the tumor immune microenvironment (TIME), and clinical significance of these molecules in gastric cancer still requires further clarification. If the molecular significance of the TXNDC5 interacting proteins, such as PRDX4, are elucidated, we may be able to clarify the molecular pathway by which TXNDC5 promotes cancer and discover target molecules with clinical therapeutic potential.

To address these limitations, in this study, we first validated the interaction between one of the interacting proteins (i.e., PRDX4) and TXNDC5 in gastric cancer using coimmunoprecipitation (co-IP) technology. Subsequently, the significance of this molecule in gastric cancer was explored using bioinformatics methods to provide a model for further research on other molecules.

The association between the protein molecule PRDX4 and gastric cancer, including its possible mechanism of action, is not fully understood. Presently, this gene is considered a tumor-related gene, but its specific role is not clear. Its protein molecule is a member of the peroxidase reductase family, and it is an endogenous antioxidant enzyme in cells (6,7). It can anchor in the endoplasmic reticulum to regulate protein oxidative folding and can also secrete in the extracellular matrix for an antioxidant action (8,9); however, its functional significance in various tumor cells is quite complex.

This study first verified the interaction of TXNDC5 and PRDX4 in gastric cancer tissues and cells using co-IP technology. Bioinformatics methods were then used to conduct data mining to analyze its expression differences in gastric cancer, prognostic relevance, the possible molecular signaling pathways involved, and its effect on the TIME.

Highlight box

Key findings

- This study confirms that the peroxiredoxin 4 (PRDX4) protein molecule is an interacting protein of the gastric cancer promoting gene TXNDC5 protein, and this molecule may promote tumor progression by regulating the tumor immune microenvironment.

What is known and what is new?

- TXNDC5 has a promoting effect on cancer progression in gastric cancer, and this protein molecule plays a pro cancer role by interacting with other protein molecules to affect downstream molecular signaling pathways.
- PRDX4 protein molecule is an important interacting protein of TXNDC5 protein in gastric cancer, and TXNDC5 protein may regulate downstream pro cancer molecular signaling pathways through PRDX4 protein.

What is the implication, and what should change now?

- These findings suggest that PRDX4 may be an important hub for the pro cancerous effect of TXNDC5 molecule in gastric cancer. Intervention of PRDX4 gene expression may affect the pro cancer effect of TXNDC5 molecule in gastric cancer.

Our findings lay the foundation for further in-depth research.

Bioinformatics is a discipline that studies the collection, processing, storage, dissemination, analysis, and interpretation of biological information. This new discipline was established following the rapid development of life science and computer science, and the combination of life science and computer science. It reveals the biological mysteries inherent in a large and complex amount of biological data through the comprehensive use of biology, computer science, and information technology.

Shao *et al.* (10). successfully conducted research on gastric cancer–related genes using bioinformatics methods, and their research results suggest that FN1 (fibronectin-1), COL1A1 (collagen type I alpha 1), TIMP1 (tissue inhibitor of metalloproteinase 1), COL1A2 (collagen type I alpha 2), SPARC (secreted protein, acidic, cysteine-rich), COL4A1 (collagen type IV alpha 1 chain), and SERPINE1 (serpin peptidase inhibitor 1) could contribute to the development of novel molecular targets and biomarker-driven treatments for gastric cancer. Due to different sources of research samples, different methods of use, and different research focuses, the experimental results may vary, which reflects the complexity of tumor diseases themselves and provides richer clues for further research. Based on the current research status on *PRDX4* gene, we used bioinformatics methods for research and exploration in this study. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-904/rc).

Methods

Materials

Human gastric cancer SGC7901 cells were purchased from Shanghai Enzymes Research Biotechnology Co. Ltd. (catalog number: CC-Y1456; Shanghai, China). The cells were maintained at the Department of Gastroenterology of the Eighth Medical Center of the PLA General Hospital and sub-cultured with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum. DMEM, calf serum, and TRIzo were purchased from the GIBCO company (Grand Island Biological Company, New York, USA). TXNDC5 primary antibody was purchased from the Abcam company (catalog number: ab237697; Cambridge, UK), PRDX4 primary antibody was purchased from the Abcam company (catalog number: ab184167), and secondary antibody was purchased from the Abcam company (catalog number: ab150080).

The gastric cancer tissue sample was obtained from a 49-year-old male patient (medical record number H007895, Institutional Review Board: 00896) who underwent radical surgery for gastric cancer at the Eighth Medical Center of the PLA General Hospital in April 2022. The tumor was located in the upper part of the gastric body and the pathological result was poorly differentiated adenocarcinoma. The study was approved by the Medical Ethics Committee of The Eighth Medical Center of PLA General Hospital (No. 309202309121507*)*. Informed consent was obtained from the patient. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Verification of the TXNDC5-PRDX4 interaction using the Co-IP method

Following the experimental protocol of Zhang *et al.* (11), the total protein samples extracted from the human gastric cancer SGC7901 cells were stably transfected with the pcDNA3.1-TXNDC5 vector, and the high TXNDC5 expression gastric cancer tissues were purified according to the instructions for using MedChemExpress's (Newark, USA) protein A/G immunomagnetic beads. The antibody binding pretreatment was performed on the magnetic beads according to the instructions, and the first antibody was added to the 30-μL magnetic bead (immunoglobulin G and TXNDC5 first antibodies were added according to the grouping situation, and the final concentration was 10 μG/mL). The antibody was thoroughly mixed with the magnetic beads, flipped, and shaken well, and the magnetic beads were incubated at room temperature for 1 hour.

The magnetic beads were then cleaned and enriched. The extracted total protein samples of the cells and tissues were then added and mixed thoroughly with the magnetic beads, flipped and shaken well, and the magnetic beads were incubated at 4 ℃ overnight. The magnetic beads were cleaned four times with binding/washing buffer, centrifuged at 8,000 r/min for 30 seconds, and naturally precipitated for about 2 minutes, after which the supernatant was discarded. Subsequently, the above sediment was cleaned twice with Tris-buffered saline solution (20 times the volume of the magnetic beads), and the supernatant was discarded. The above magnetic beads were then washed again with 0.1 mol/L glycine HCl (hydrochloric acid) (pH 3.5) (the two

were mixed for no more than 20 minutes), the supernatant was discarded, and 30 μL 5× sample buffer was added, the mixture was denatured in a metal bath for 10 minutes, and finally Western blot detection was performed.

Human Protein Atlas (HPA) database data extraction and analysis

A search was conducted of the *PRDX4* gene in the HPA database, which is a global authoritative biological protein database. Relevant data were collected under several sub-menus, such as Tissue, Single cell, Pathology, and Subcellular, to analyze the *PRDX4* gene's expression in human normal tissues, subcellular localization, expression in gastric histiocyte, and immunohistochemical staining results in normal gastric and gastric cancer tissues.

UCSC Xena (University of California Santa Cruz xenabrowser) database extraction and data analysis

UCSC Xena is a bioinformatics platform that combines data collection, analysis, and visualization. In total, 1,098 public data sets with 91 queues, including The Cancer Genome Atlas (TCGA), ICGC (International Cancer Genome Consortium), TARGET (Therapeutically Applicable Research to Generate Effective Treatments), GTEx (Genotype-Tissue Expression), and CCLE (Cancer Cell Line Encyclopedia), have been standardized. First, the Genomic Data Commons (GDC) TCGA stomach adenocarcinoma (STAD) data set of 544 gastric cancer patients was selected for analysis. Second, the TCGA STAD data set of 580 gastric cancer patients was then selected for analysis and confirmation. The analysis was performed using PRDX4 as the selected gene and messenger RNA (mRNA) expression as the analysis indicator, while the other grouping factors included tumor pathological staging and Tumor Node Metastasis (TNM) staging.

Analysis of the relationship between the PRDX4 gene and survival in gastric cancer patients

The Kaplan-Meier Plotter database was used to analyze and plot the survival curves of gastric cancer patients with high and low expressions of the *PRDX4* gene. At the same time, a further screening and an analysis were conducted of the survival curves of the gastric cancer patients with high and low expressions of the *PRDX4* gene across different Lauren subtypes, tumor stages, patient gender, and human epidermal growth factor receptor 2 (HER-2) positive and negative conditions to analyze the effect of this gene on disease prognosis. The Gene Expression Profiling Interactive Analysis (GEPIA) database and Kaplan-Meier pancancer database were also used to analyze and plot the survival curves of gastric cancer patients with high and low expressions of the *PRDX4* gene. RNA-sequencing expression profiles and corresponding clinical information for gastric cancer were downloaded from TCGA data set (https://portal.gdc.com). The log-rank test was used to compare differences in survival between these groups. For the Kaplan-Meier curves, P values and hazard ratios (HRs) with 95% confidence intervals (CIs) were generated by log-rank tests. All the analysis methods and R packages were implemented by R (foundation for statistical computing 2020) version 4.0.3. A P value <0.05 was considered statistically significant. Additionally, we used the survival curve of PRDX4 and gastric cancer in Park *et al.*'s study as an auxiliary explanation (12).

Analysis of the functional significance of the PRDX4 gene in gastric cancer

RNA-sequencing (level 3) expression profiles and associated clinical information were downloaded from TCGA gastric cancer data set (http://portal.gdc.com). The GSVA (Gene Set Variation Analysis) package of R was used for the analysis, with the parameter method = 'ssgsea'. A Spearman correlation analysis was conducted to analyze the correlations between the genes and pathway scores. The analysis methods and R packages were all implemented in R version 4.0.3. A P value <0.05 was considered statistically significant.

STRING database analysis of PRDX4 interacting proteins

The STRING database was used to analyze the potential interacting proteins of the PRDX4 molecules. The following conditions were set: map, organization, select the *PRDX4* gene, and draw a wiring diagram of the molecular interactions and reaction relationships.

KEGG PATHWAY, CST Pathway, Pathway Commons, and Reactome Pathway Database analyses of the PRDX4 molecular signaling pathway

The tumor-related molecular signaling pathways in which the PRDX4 molecules may participate were analyzed using

the KEGG PATHWAY, CST Pathway, Pathway Commons, and Reactome Pathway Database. These databases have a number of advantages, including that the molecular pathway diagrams are clear, intuitive, aesthetically pleasing, and comprehensive. Each online platform mentioned above was analyzed separately until the relevant molecular signaling pathways could be matched.

Analysis of the relationship between PRDX4 expression and TIME and immune checkpoint (IC) using the TIMER2.0 platform

The TIMER2.0 platform was used for the analysis (13,14). In the "Gene" module under the "immune association" menu, STAD was selected from the tumor types, and PRDX4 was selected as the target gene. The analysis item was immune infiltration. The TIMER2.0 platform analyzed the infiltration of six immune effector cells, including B-lymphocytes, cluster of differentiation (CD)8$^+$ T lymphocytes, CD4$^+$ T lymphocytes, macrophages, neutrophils, and dendritic cells. In the correlation module, STAD was selected as the cancer species, and PRDX4 was selected as the variable for the vertical axis for gene expression, while PDCD1 (programmed cell death 1), CTLA4 (cytotoxic T lymphocyte-associated antigen-4), BLTA (B and T lymphocyte attenuation factor), LAG3 (lymphocyte activation gene-3), HAVCR2 (hepatitis A virus cellular receptor 2), TIGIT (T cell immunoreceptor with Ig and ITIM domains), VISTA (C10ORF54), SIRPA (signal regulatory protein alpha), SIGLEC7 (sialic acid-binding Ig-like lectin 7), and LILRB2 (leukocyte immunoglobulin-like receptor subfamily B member 2) were selected as the variables for the horizontal axis to map and analyze their correlation in expression.

Statistical methods

SPSS 22.0 software (Statistical Product and Service Solutions Software Development Co., Ltd, USA) was used to process the data. The measurement data were compared by a one-way analysis of variance and Welch's *t*-test, and are presented as the mean ± standard deviation. The count data were compared by Chi-squared (χ^2) tests, and are expressed as the percentage (%). The significance level of the test was α=0.05.

Results

Validation of the TXNDC5-PRDX4 protein interaction using the co-IP method

The co-IP experiment results showed an interaction between TXNDC5 and PRDX4 in the gastric cancer tissues with high expressions of TXNDC5 (*Figure 1A*), and an interaction between TXNDC5 and PRDX4 in gastric cancer cell lines with high expressions of TXNDC5 (*Figure 1B*).

Expression of the PRDX4 gene in normal human tissues

The expression of the *PRDX4* gene was analyzed in normal human tissue from both the protein and mRNA perspectives, and the results indicated that the PRDX4 protein expression levels in various organ tissues of the human body were inconsistent. Notably, it was expressed at medium to low levels in brain, muscle, blood system, gastrointestinal tract, respiratory system, urogenital system, and immune system tissues, but at high levels in pancreatic tissues. The mRNA and protein levels were basically consistent, with medium to low levels of expression in various tissues, including gastrointestinal tissues, while pancreatic tissues had the highest expression level (*Figure 2*).

Subcellular localization of the PRDX4 protein, expression of various cell types in gastric tissues, and immunohistochemical detection of normal gastric tissues and gastric cancer tissues

The analysis results showed that the subcellular localization of the PRDX4 protein was in the cytoplasm (*Figure 3*). The expression level of the *PRDX4* gene differed in each cell type of the normal human gastric tissue; that is, the expression of various plasma cells was high, while the expression of gastric epithelial secretory cells was low (*Figure 4*). The immunohistochemical staining results of the normal gastric tissues showed a brownish-yellow positive signal in the cytoplasm of the gastric mucosal epithelial cells, and the sample staining intensity was low to moderate. The immunohistochemical staining results of the gastric cancer tissue showed positive staining in the cytoplasm of gastric cancer cells, and the sample staining intensity was strongly positive (*Figure 5*).

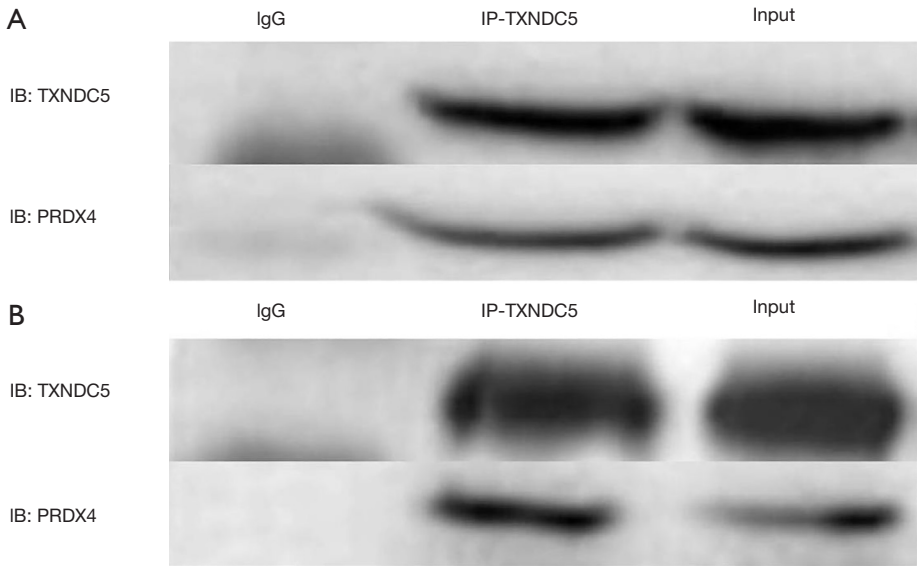

Figure 1 The co-IP method validation results of the interaction between TXNDC5 and PRDX4 in the gastric cancer. (A) The co-IP method validation results of the interaction between TXNDC5 and PRDX4 in the gastric cancer tissues, indicating the presence of an interaction between TXNDC5 and PRDX4 in the gastric cancer tissues. (B) The co-IP method validation results of the interaction between TXNDC5 and PRDX4 in gastric cancer cell lines with high expressions of TXNDC5 showed that there was an interaction between TXNDC5 and PRDX4 in the gastric cancer cells. co-IP, coimmunoprecipitation; IB, immunoblot.

Differential expression of the PRDX4 gene in normal gastric tissues and gastric cancer tissues

The gastric cancer data in the UCSC Xena database were analyzed and visualized, and the results showed that the expression level of the *PRDX4* gene in the normal gastric tissues was significantly lower than that in the gastric cancer tissues (P=0.0002240, *t*=–4.127) in the 544 gastric cancer sample data set of GDC TCGA STAD. The refined analysis results showed that there was no significant difference in the expression level of the *PRDX4* gene in the gastric cancer tissues in terms of the tumor stage, tumor T group, tumor N group, and tumor M group (P=0.06432, f=1.733; P=0.05621, f=1.815; P=0.1520, f=1.579; P=0.4183, f=0.8733). Next, the data validation of the 580 gastric cancer samples from TCGA STAD were analyzed to verify the results. The results showed that the expression level of the *PRDX4* gene in normal gastric tissues was also significantly lower than that in the gastric cancer tissues (P=0.0002865, *t*=–4.719). The refined analysis results of this data set showed that there was no significant difference in the expression level of the *PRDX4* gene in the gastric cancer tissues in terms of the tumor staging, tumor T group, tumor N group, and tumor M group (P=0.2131, f=1.315; P=0.3535, f=1.109; P=0.5743, f=0.8161; P=0.8371, f=0.1779) (*Figures 6*,*7*).

The analysis results of the relationship between PRDX4 gene expression and survival time

The Kaplan-Meier Plotter database analysis results indicated that at the overall level of data from 875 gastric cancer patients, the survival time of the *PRDX4* gene high-expression group was significantly higher than that of the low-expression group, indicating that patients with high expressions of the *PRDX4* gene have a better prognosis than those with low expressions of the gene (P<0.05). A further analysis of the gastric cancer patients grouped according to Lauren classification, staging, gender, and HER-2 expression showed that the survival time of the *PRDX4* gene high-expression group was significantly higher than that of the low-expression group in the intestinal and diffuse gastric cancer groups (P<0.05); however, the sample size of the mixed type group is too small to meet statistical requirements. The survival time of patients with stage III and IV tumors was significantly higher in the *PRDX4* gene high-expression group than the low-expression group (P<0.05), but there were no statistically

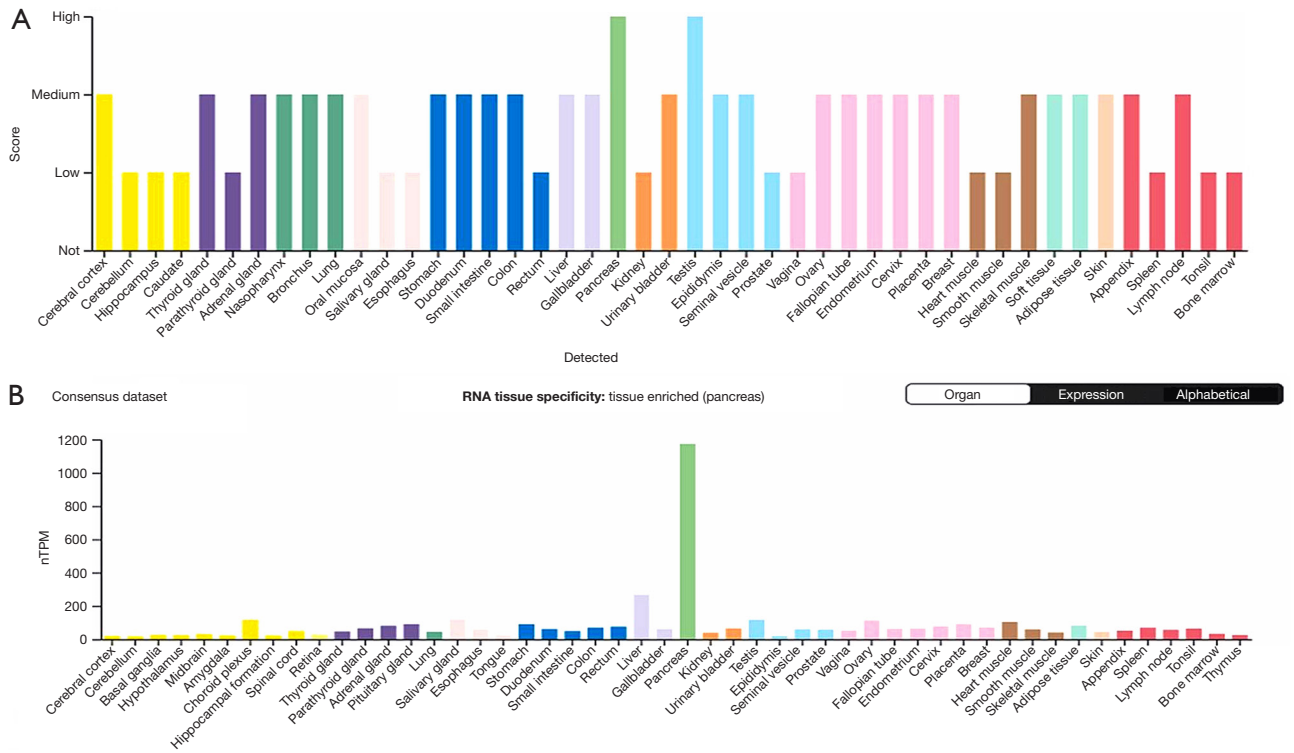

Figure 2 PRDX4 protein and RNA expression levels in various human tissues. (A) PRDX4 protein expression levels in various human tissues (the image is sourced from a tissue protein expression image from the HPA database website, https://www.proteinatlas.org/ENSG00000123131-PRDX4/tissue). (B) Results of PRDX4 RNA expression levels in human tissues from the consensus database (the image is sourced from a consensus database mRNA expression image from the HPA database website, https://www.proteinatlas.org/ENSG00000123131-PRDX4/tissue). nTPM, normalized transcript per million; HPA, Human Protein Atlas.

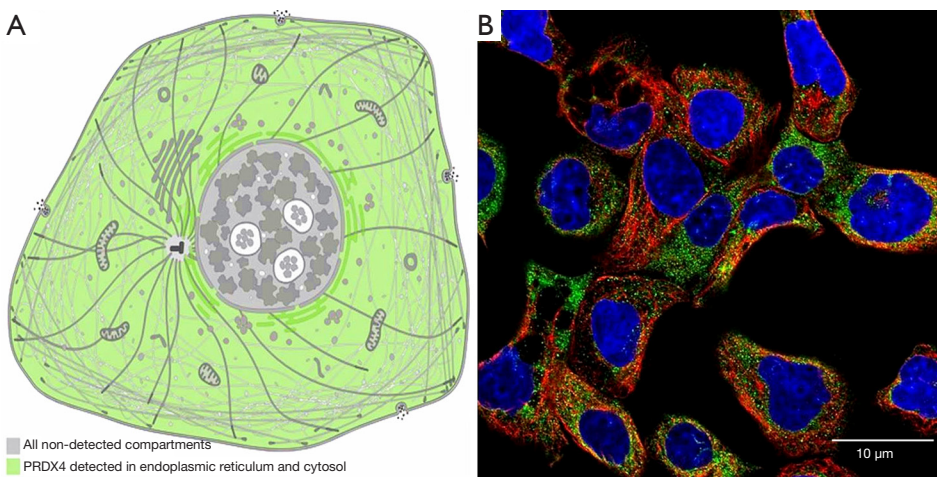

Figure 3 Subcellular localization of PRDX4 protein molecule. (A) Schematic diagram of subcellular localization of the PRDX4 protein; green indicates the distribution area of the target protein (the image is sourced from a schematic diagram of molecular subcellular expression from the HPA database website, https://www.proteinatlas.org/ENSG00000123131-PRDX4/subcellular). (B) Subcellular localization immunofluorescence detection of the PRDX4 protein; green fluorescence indicates the target protein; red fluorescence indicates the cell microtubule structure (×400) (the image is sourced from a cellular immunofluorescence staining image from the HPA database website, CAB027389: A-431, https://www.proteinatlas.org/ENSG00000123131-PRDX4/subcellular). HPA, Human Protein Atlas.

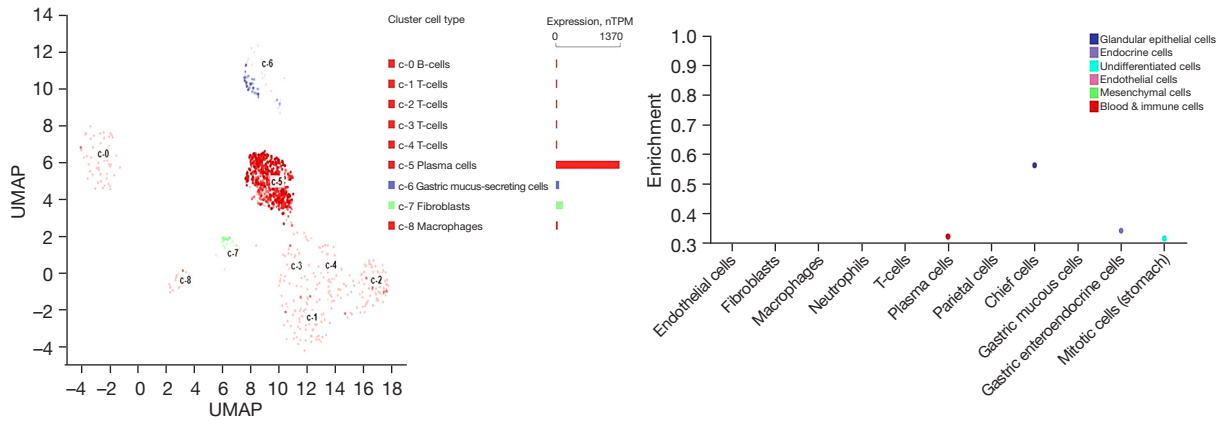

Figure 4 PRDX4 expression of various cell types in normal gastric tissues (the image is sourced from a molecular single cell expression analysis image from the HPA database website, https://www.proteinatlas.org/ENSG00000123131-PRDX4/single+cell+type/Stomach). UMAP, Uniform Manifold Approximation and Projection; nTPM, normalized transcript per million; HPA, Human Protein Atlas.

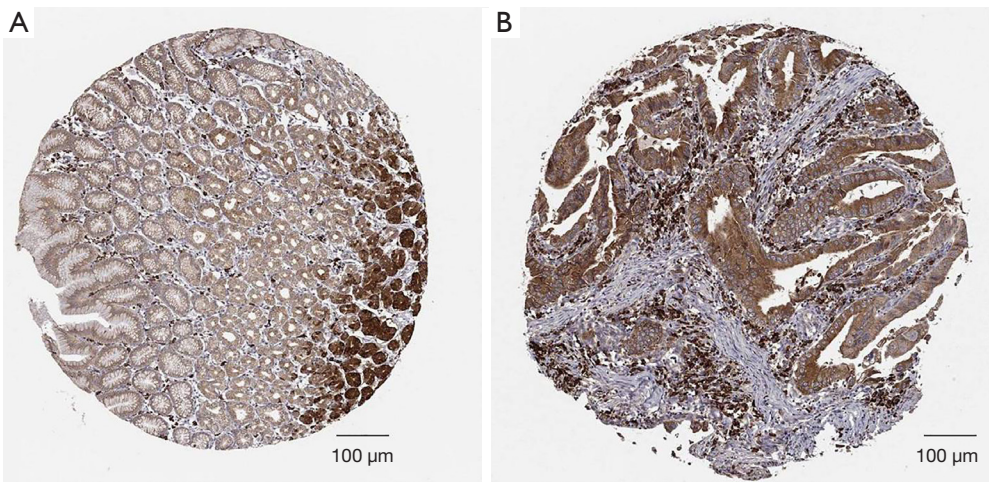

Figure 5 Immunohistochemical staining of the PRDX4 protein in normal gastric mucosal and gastric cancer tissues. (A) Immunohistochemical staining of the PRDX4 protein in normal gastric mucosal tissues adjacent to cancer showed that the gastric mucosal epithelial cell cytoplasm was positive (×200) (the image is sourced from an immunohistochemical staining image from the HPA database website, HPA067039, https://www.proteinatlas.org/ENSG00000123131-PRDX4/tissue/stomach#img). (B) Immunohistochemical staining of the PRDX4 protein in the gastric cancer tissues showed strong positive staining of tumor cell cytoplasm (×200) (the image is sourced from an immunohistochemical staining image from the HPA database website, HPA067039, https://www.proteinatlas.org/ENSG00000123131-PRDX4/tissue/stomach#img). HPA, Human Protein Atlas.

significant differences in other stages. The survival time of the *PRDX4* gene high-expression group was significantly higher than that of the low-expression group in both the male and female gastric cancer groups (P<0.05). The survival time of the HER-2 positive and negative gastric cancer patients in the *PRDX4* gene high-expression group was significantly higher than that in the low-expression group (P<0.05) (*Figure 8*).

In relation to the data from the GEPIA platform and Kaplan-Meier pancancer database, the survival time of patients with high expressions of the *PRDX4* gene was not significantly different from that of patients with low expressions of the gene (P>0.05). In relation to the data from TCGA database, the R language analysis results showed that the survival time of patients with high

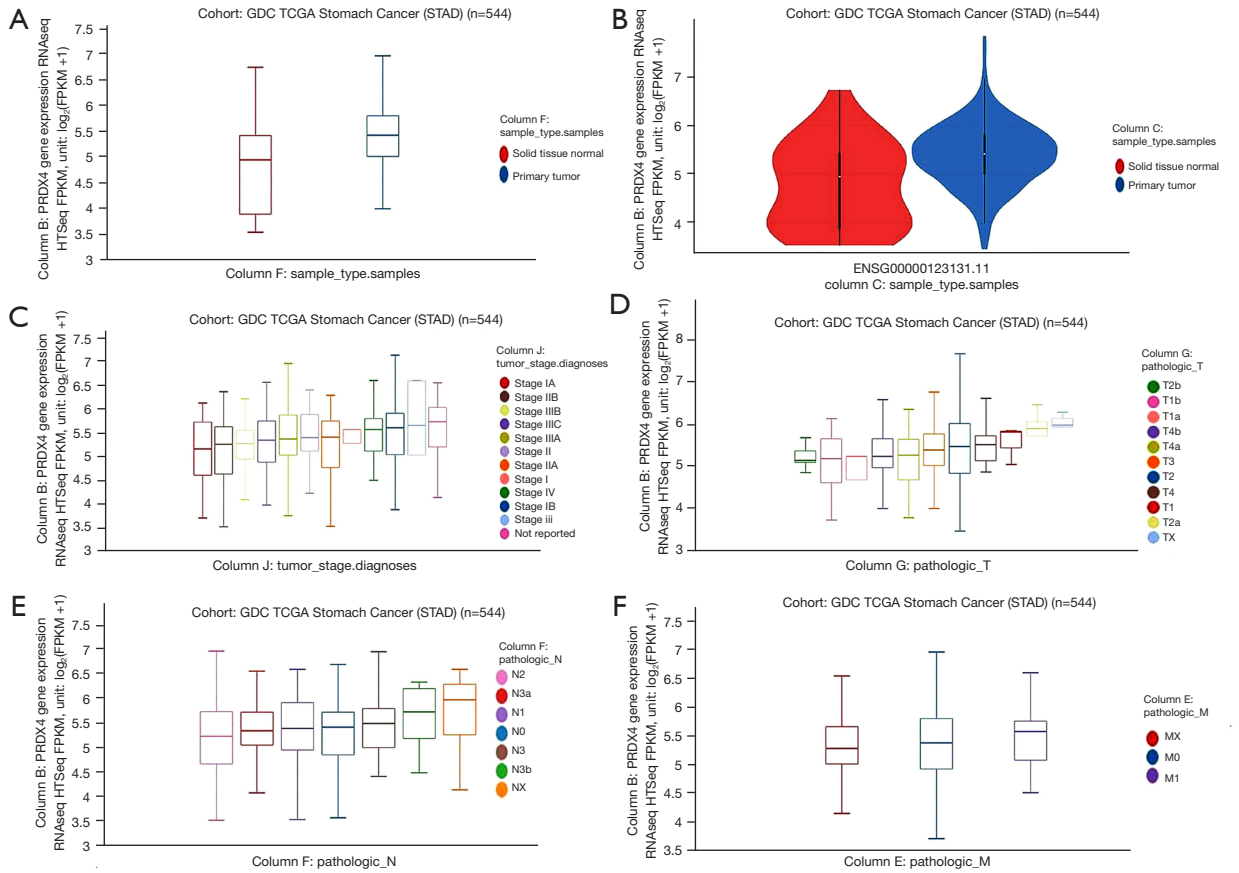

Figure 6 The analysis results of 544 gastric cancer samples from GDC TCGA STAD. (A) Comparison of the expression differences of PRDX4 in the normal gastric tissues and gastric cancer tissues using a column chart. (B) Comparison of the expression differences of PRDX4 between the normal gastric tissues and gastric cancer tissues on a violin plot. (C) Comparison of the differential expression of PRDX4 in the gastric cancer tissues, grouped by tumor staging. (D) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor T staging. (E) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor N staging. (F) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor M staging (all images are sourced from the UCSC Xena database website). RNAseq, RNA-sequencing; HTSeq, high-throughput sequencing; FPKM, fragments per kilobase of transcript per million fragments mapped; GDC, Genomic Data Commons; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma.

expressions of the *PRDX4* gene was not significantly different from that of patients with low expressions of the gene (P>0.05). Conversely, Park *et al.*'s research findings (12) suggest that the survival time of patients with high expressions of the *PRDX4* gene was significantly lower than that of patients with low expressions of the gene (P<0.05) (*Figure 9*).

Analysis results of the functional significance of the PRDX4 gene in gastric cancer

The R language analysis results showed that the *PRDX4* gene was significantly positively correlated with cellular DNA replication, DNA damage repair, epithelial-mesenchymal transition (EMT) markers, the G2/M checkpoint, the tumor proliferation signature, ferroptosis, and the cellular response to hypoxia in the main tumor-related cellular biological functions (P<0.05), but not with apoptosis or angiogenesis (P>0.05). These results suggest that the *PRDX4* gene may have the effect of promoting DNA replication and DNA damage repair in gastric cancer cells, as well as promoting tumor cell EMT, proliferation, ferroptosis, and cell response to hypoxia. It may also affect the cell cycle of gastric cancer cells and to some extent limit

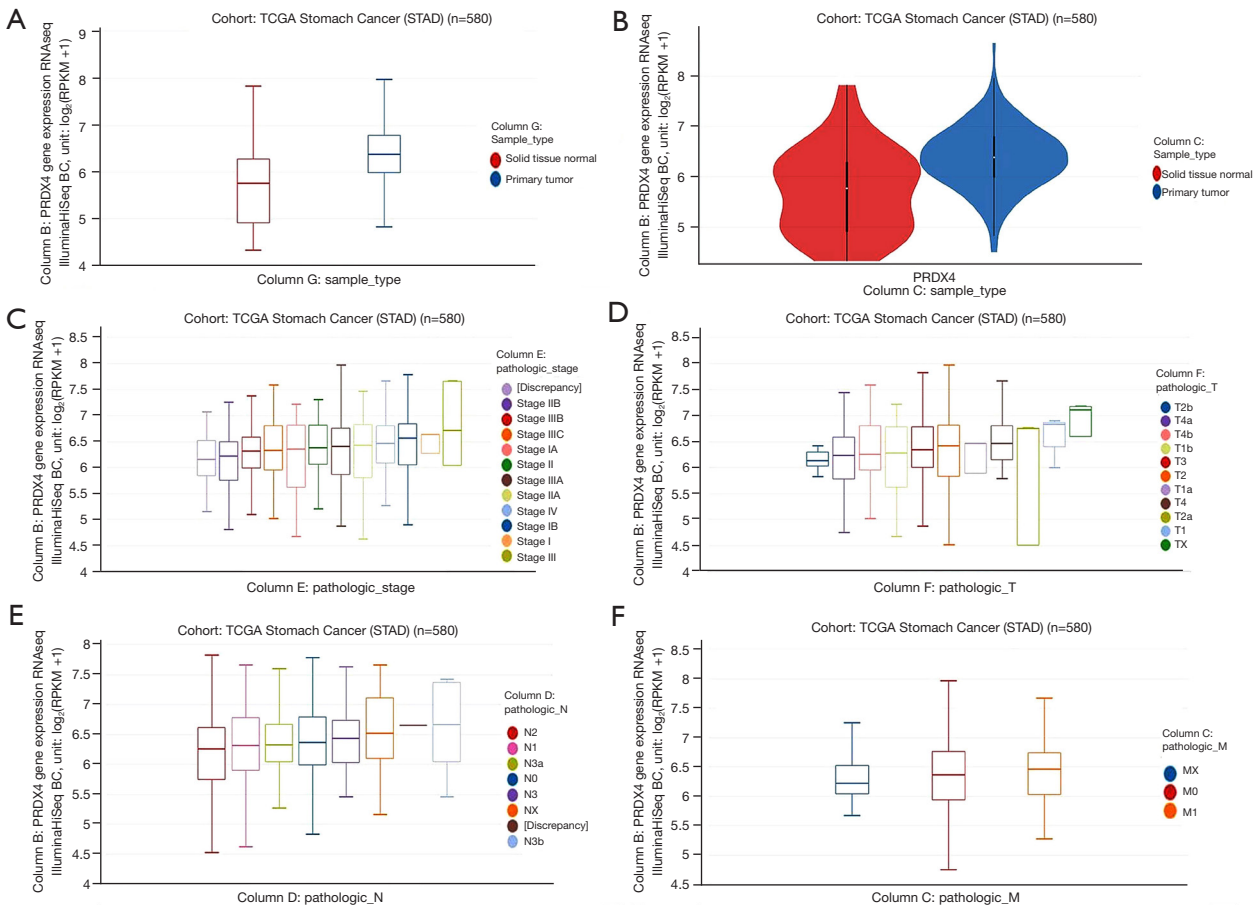

Figure 7 The analysis results of 580 gastric cancer samples from TCGA STAD: (A) Comparison of the expression differences of PRDX4 in the normal gastric tissues and gastric cancer tissues using a column chart. (B) Comparison of the expression differences of PRDX4 between the normal gastric tissues and gastric cancer tissues on a violin plot. (C) Comparison of differential expression of PRDX4 in the gastric cancer tissues, grouped by tumor staging. (D) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor T staging. (E) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor N staging. (F) Comparison of differences in the expression of PRDX4 in the gastric cancer tissues, grouped by tumor M staging. (All images are cited from the UCSC Xena database website). RNAseq, RNA-sequencing; RPKM, reads per kilobase of transcript per 1 million mapped reads; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma.

the entry into the mitotic phase. Conversely, the *PRDX4* gene may not significantly affect the apoptosis and tumor angiogenesis of gastric cancer cells (*Figure 10*).

Analysis results of the possible interacting proteins between PRDX4 and TXNDC5

We analyzed the possible interacting protein molecules of PRDX4 and TXNDC5 in the STRING database and listed the main 10 in the form of wiring diagrams (*Figure 11A*,*11B*). The analysis results indicated that PRDX4 and TXNDC5 interact with each other, further confirming the experimental validation results of the co-IP detection using the bioinformatics methods.

Analysis results of the PRDX4 molecular signaling pathway

Synonymous molecular searches, including searches for PRDX4, AOE372 (antioxidant enzyme 372 protein), and PRX-4 (peroxiredoxin 4) were conducted using the KEGG PATHWAY, Pathway Commons, and CST Pathway molecular signaling pathway databases; however, these searches did not reveal any tumor-related molecular

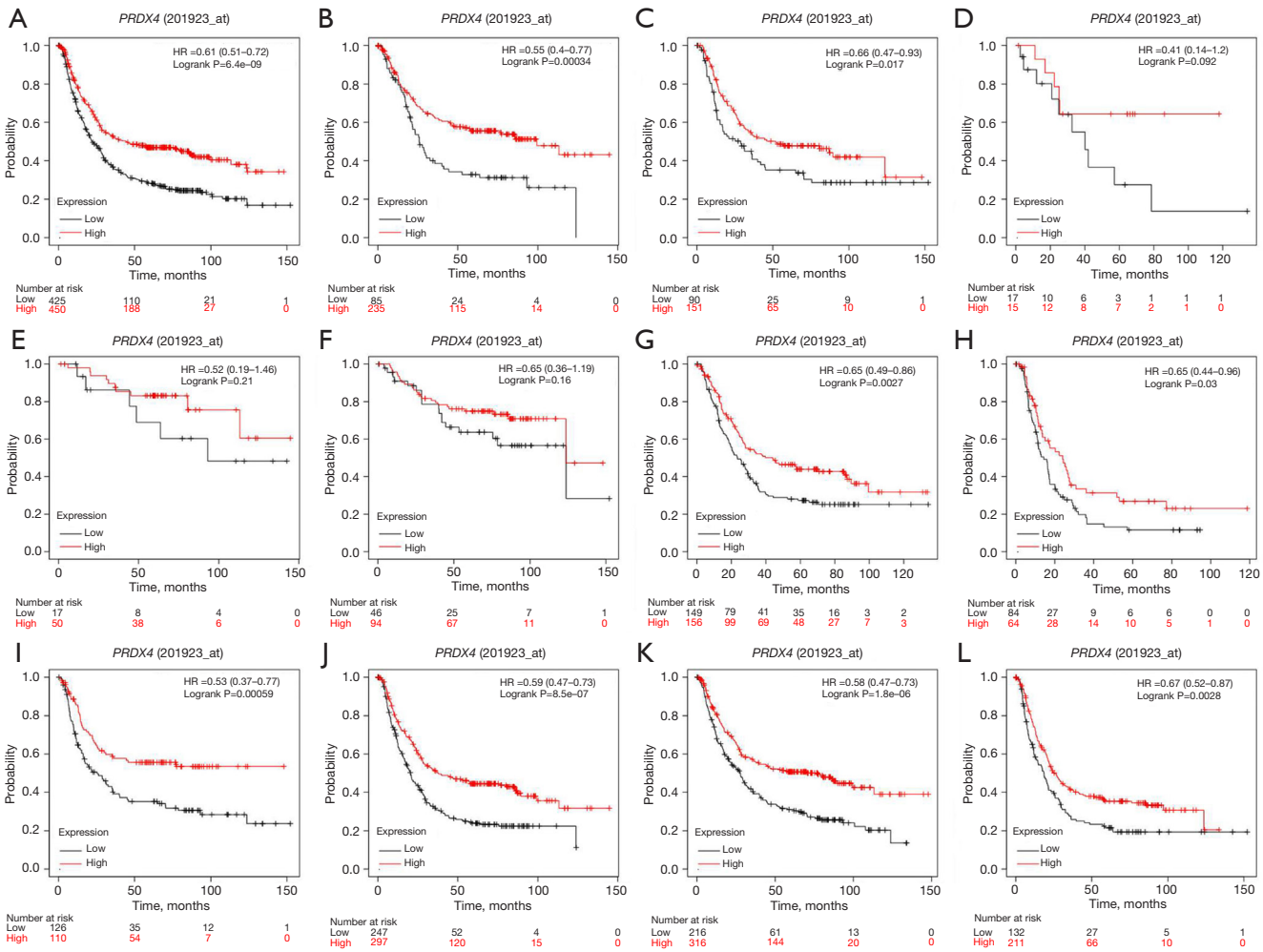

Figure 8 Analysis of *PRDX4* gene expression and the survival time data of cancer patients. (A) Comparison of the survival curves between gastric cancer patients with high and low expressions of PRDX4. (B) Comparison of the survival curves between intestinal gastric cancer patients with high and low expressions of PRDX4. (C) Comparison of the survival curves between diffuse gastric cancer patients with high and low expressions of PRDX4. (D) Comparison of the survival curves between mixed gastric cancer patients with high and low expressions of PRDX4. (E) Comparison of the survival curves between stage I gastric cancer patients with high and low expressions of PRDX4. (F) Comparison of the survival curves between stage II gastric cancer patients with high and low expressions of PRDX4. (G) Comparison of the survival curves between stage III gastric cancer patients with high and low expressions of PRDX4. (H) Comparison of the survival curves between stage IV gastric cancer patients with high and low expressions of PRDX4. (I) Comparison of the survival curves between female gastric cancer patients with high and low expressions of PRDX4. (J) Comparison of the survival curves between male gastric cancer patients with high and low expressions of PRDX4. (K) Comparison of the survival curves between HER-2 negative gastric cancer patients with high and low expressions of PRDX4. (L) Comparison of the survival curves between HER-2 positive gastric cancer patients with high and low expressions of PRDX4 (all images are cited from the Kaplan-Meier Plotter database website). HR, hazard ratio.

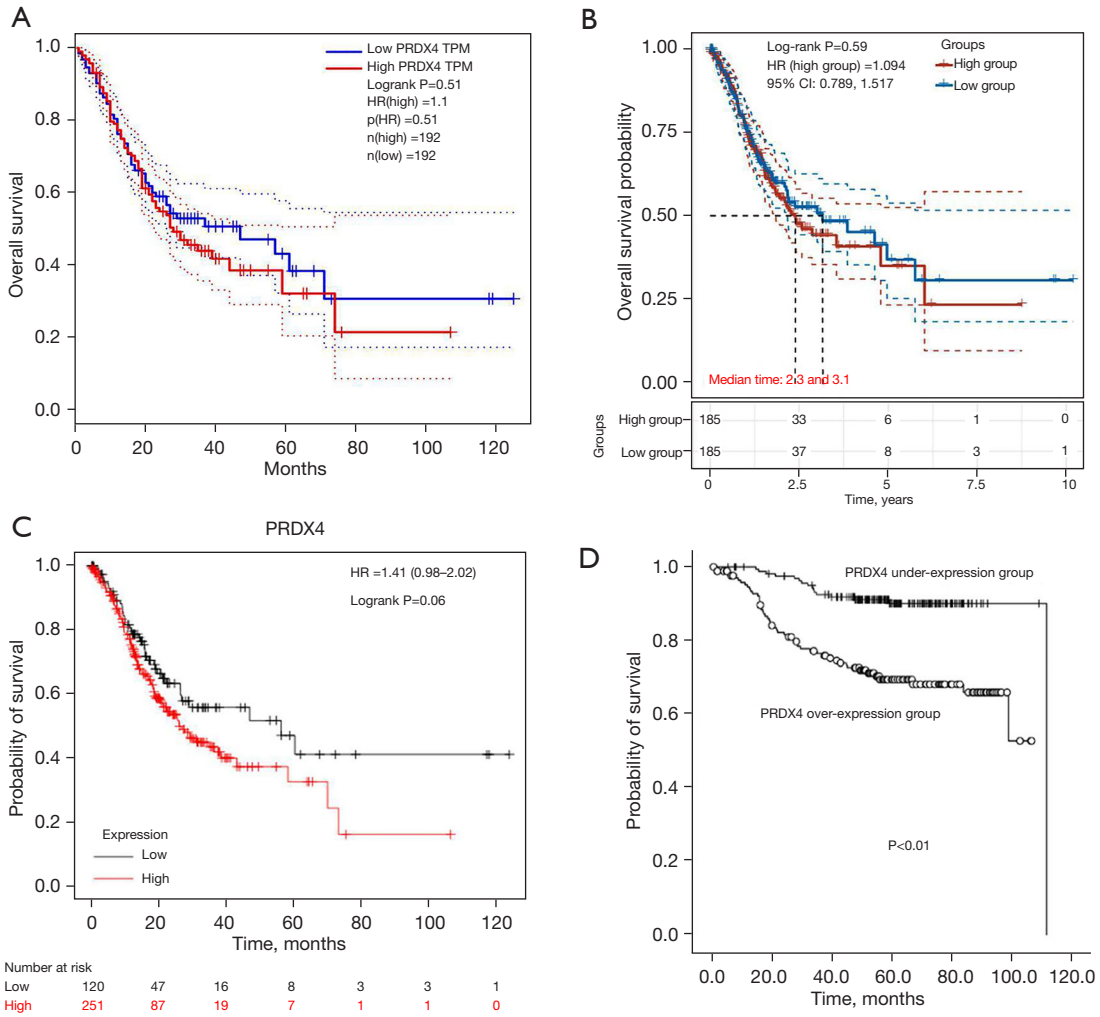

Figure 9 Comparative analysis of the impact of PRDX4 on the survival curve of gastric cancer patients. (A) Comparison of the survival curves between gastric cancer patients with high and low expressions of PRDX4 from the GEPIA platform (the image is sourced from the GEPIA platform database website). (B) Comparison of the survival curves between gastric cancer patients with high and low expressions of PRDX4 from TCGA database, analyzed by R language. (C) Comparison of the survival curves between gastric cancer patients with high and low expressions of PRDX4 from the Kaplan Meier pan cancer database (the image is sourced from the Kaplan-Meier Plotter database website). (D) Comparison of the survival curves between gastric cancer patients with high and low expressions of PRDX4 from Park *et al.*'s research (12). HR, hazard ratio; CI, confidence interval; GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas.

signaling pathways in which PRDX4 may be involved. The analysis results of the Reactome Pathway database suggest that PRDX4 is mainly involved in the molecular signaling pathway related to neutrophil degranulation. Neutrophil degranulation is one of the main steps after cell phagocytosis, which is initiated before ingestion is completed to release content from specific particles. This process is of great significance for the phagocytic and killing function of neutrophils against microorganisms and foreign objects, as well as their participation in anti-tumor immune responses. In this way, PRDX4 acts directly on the transport and release of particles rich in collagen/fibrinogen domain containing protein 1 (Ficolin1), and the importance of such particles may be to provide rapid release of pattern recognition molecules to activate the lectin complement pathway and thus affect immune function (*Figure 12*).

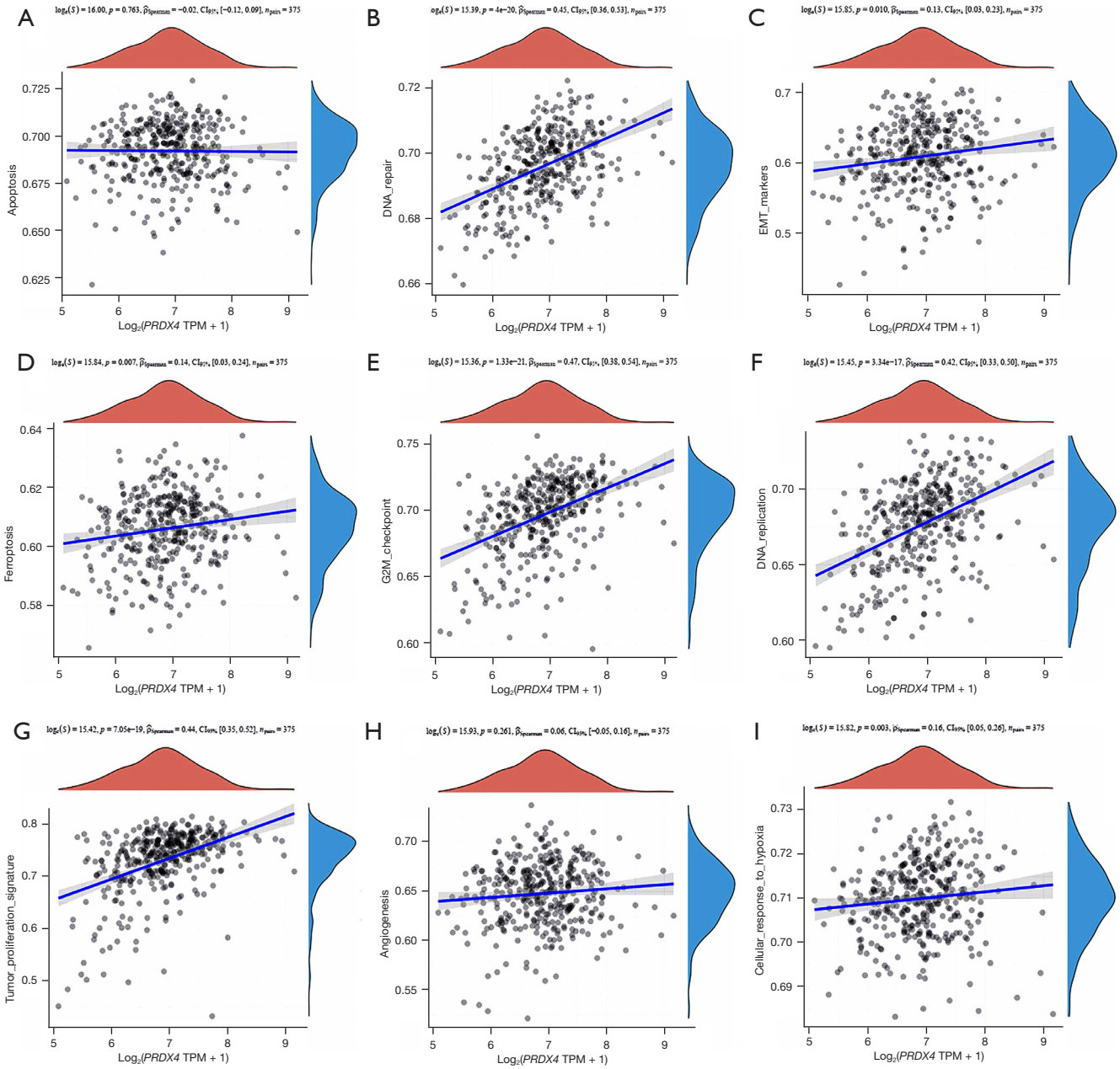

Figure 10 Analysis results of the functional significance of the PRDX4 gene in gastric cancer. (A) There was no significant correlation between *PRDX4* gene expression and cell apoptosis (P>0.05). (B) The *PRDX4* gene was significantly positively correlated with DNA damage repair (P<0.05). (C) The *PRDX4* gene was significantly positively correlated with EMT markers (P<0.05). (D) The *PRDX4* gene was significantly positively correlated with ferroptosis (P<0.05). (E) The *PRDX4* gene was significantly positively correlated with the G2/M checkpoint (P<0.05). (F) The *PRDX4* gene was significantly positively correlated with DNA replication (P<0.05). (G) The *PRDX4* gene was significantly positively correlated with the tumor proliferation signature (P<0.05). (H) There was no significant correlation between *PRDX4* gene expression and angiogenesis (P>0.05). (I) The *PRDX4* gene was significantly positively correlated with the cellular response to hypoxia (P<0.05). TPM, transcript per million; EMT, epithelial-mesenchymal transition.

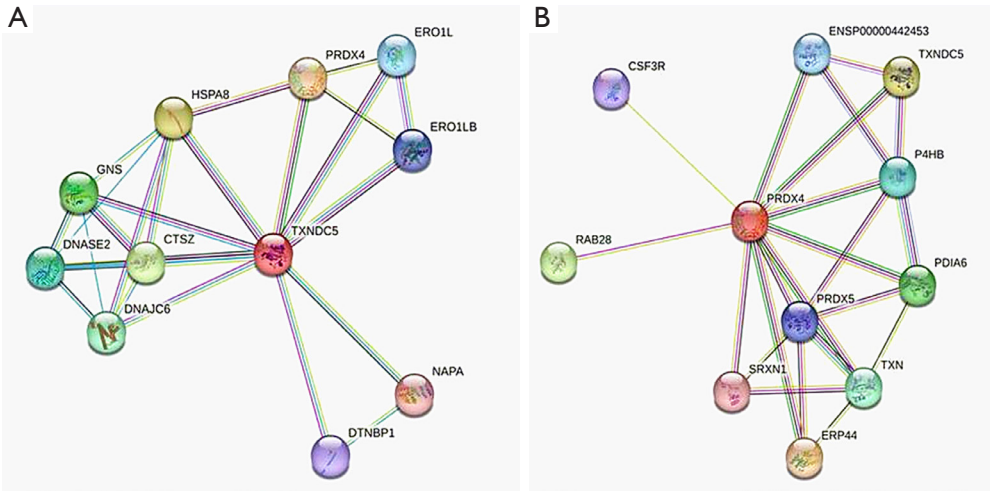

Figure 11 Analysis results showing the possible interacting proteins of PRDX4 and TXNDC5. (A) STRING database analysis results showing the possible interacting protein molecules of TXNDC5. (B) STRING database analysis results showing the possible interacting protein molecules of PRDX4.

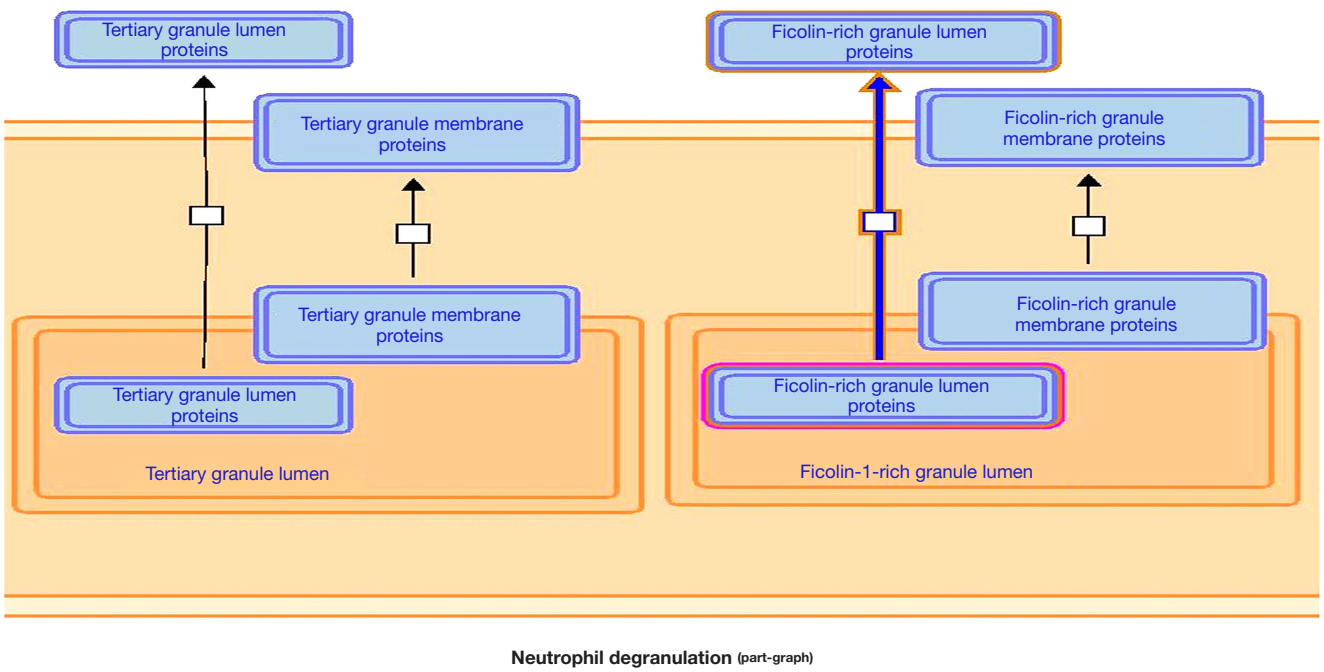

Figure 12 The analysis results of the Reactome Pathway platform indicate that PRDX4 is involved in the molecular signaling pathway related to neutrophil degranulation. The red circle indicates the location of the target molecule participating in the reaction (image sourced from the Reactome Pathway database website).

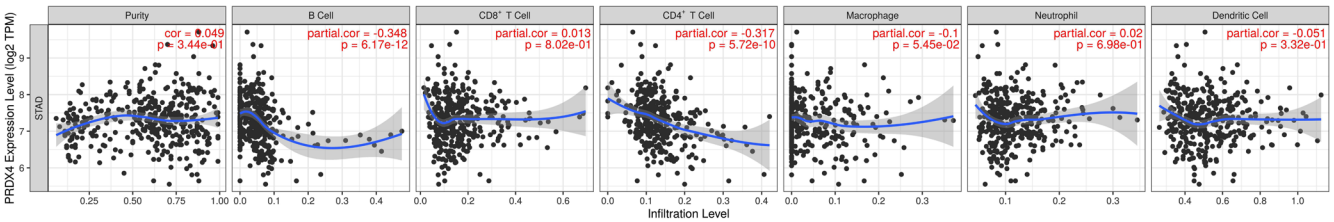

Figure 13 The TIMER2.0 platform analysis results suggested a negative correlation between PRDX4 expression and the infiltration of B lymphocytes or CD4+ T lymphocytes (P<0.05). (Image cited from the TIMER2.0 website). TPM, transcript per million; STAD, stomach adenocarcinoma.

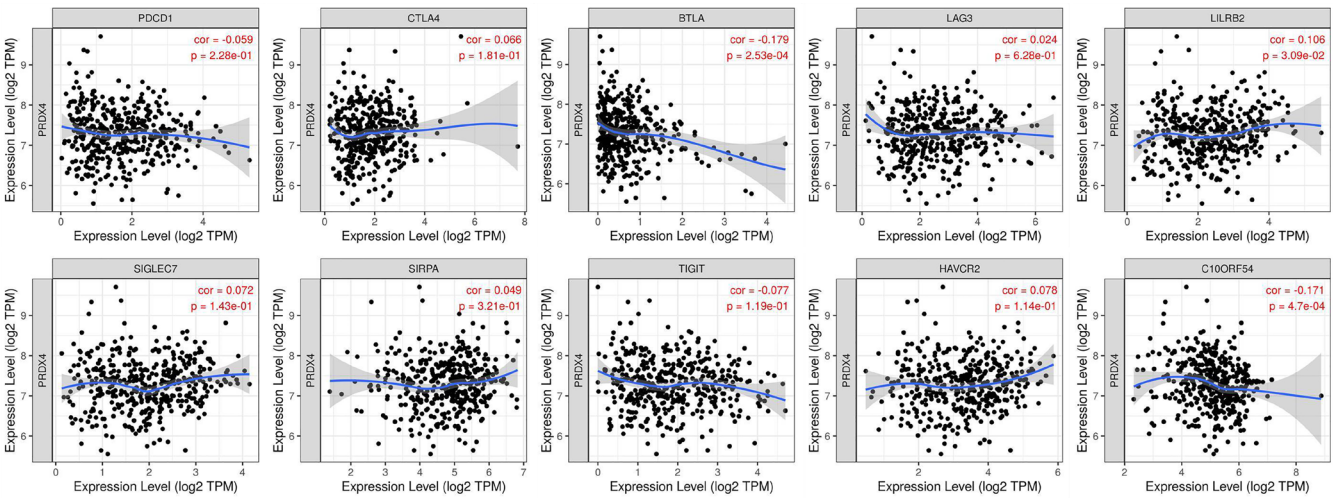

Figure 14 The TIMER2.0 platform analysis results indicated that the expression levels of PRDX4 and LILRB2 were positively correlated, while the expression levels of PRDX4 and BLTA or VISTA were negatively correlated respectively (P<0.05) (image cited from the TIMER2.0 website). TPM, transcript per million; BLTA, B and T lymphocyte attenuation factor; VISTA, V-type immunoglobulin domain-containing suppressor of T cell activation.

Effects of PRDX4 expression on the immune microenvironment in gastric cancer

TIMER2.0 online on the TIME analysis platform was used for the analysis, and the results showed that the expression level of PRDX4 had a certain relationship with the TIME. Among the six main immune cells, there was a negative correlation between PRDX4 expression and the infiltration of B lymphocytes and CD4+ T lymphocytes (P<0.05), but there was no statistically significant correlation with other immune cells (P>0.05) (*Figure 13*).

The effects of PRDX4 expression on the expression of IC molecules in gastric cancer

TIMER2.0 online on the TIME analysis platform was used to analyze the co-expression relationship between PRDX4 and 10 major ICs, and the results showed that the expression level of PRDX4 was positively correlated with the expression level of LILRB2 and negatively correlated with the expression levels of BLTA and VISTA (P<0.05), but no statistically significant correlations were found with the expression of other ICs (P>0.05) (*Figure 14*).

The effects of PRDX4 expression on the infiltration of B lymphocyte and CD4+ T lymphocyte subsets in the immune microenvironment in gastric cancer

The analysis of the effects of PRDX4 expression on the immune microenvironment in gastric cancer (STAD) suggested that the up-regulation of PRDX4 expression

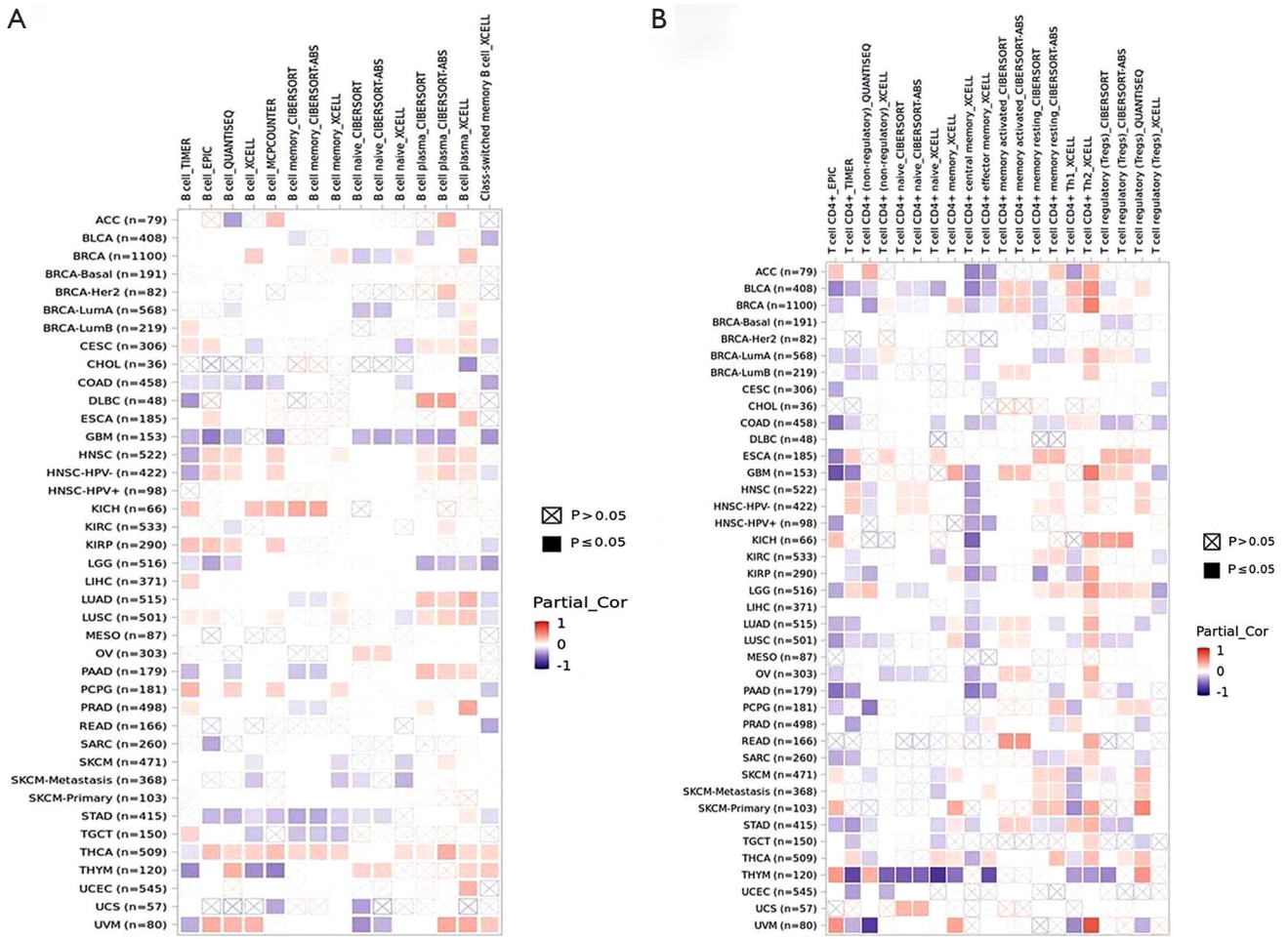

Figure 15 The TIMER2.0 platform analysis results suggested a relationship between the PRDX4 expression level and the infiltration of the B lymphocyte and CD4$^+$ T lymphocyte subsets in the immune microenvironment. (A) The relationship between the expression level of PRDX4 and the infiltration of B lymphocytes in various subpopulations in pan cancer; STAD represents gastric cancer; red represents a positive correlation; blue represents a negative correlation; white represents no correlation. (B) The relationship between the expression level of PRDX4 and the infiltration of CD4$^+$ T lymphocytes in various subpopulations in pan cancer; STAD represents gastric cancer (image cited from the TIMER2.0 website).

was related to the decreased infiltration of B lymphocytes and CD4$^+$ T lymphocytes. Based on this, the correlation between the infiltration of these two immune cells and their subpopulations was analyzed. Due to the inability of the TIMER2.0 platform to display single cancer data separately from pancancer data, the results are presented in pancancer charts. The results showed that in gastric cancer, the expression of PRDX4 was only positively correlated with the infiltration of plasma cells and was negatively correlated with the infiltration of memory B lymphocytes, natural B lymphocytes, and other B lymphocytes (P<0.05) (*Figure 15A*). The expression of PRDX4 was positively correlated with the infiltration of CD4$^+$ activated memory T lymphocytes, CD4$^+$ Th1 type T lymphocytes, and CD4$^+$ Th2 type T lymphocytes, but negatively correlated with the infiltration of CD4$^+$ central memory T lymphocytes, CD4$^+$ effector memory T lymphocytes, CD4$^+$ resting memory T lymphocytes, CD4$^+$ native T lymphocytes, and CD4$^+$ regulatory T lymphocytes (P<0.05) (*Figure 15B*).

Discussion

The *TXNDC5* gene is a member of the epithelial PDI gene family. The product of this gene is the TXNDC5 protein

molecule. The subcellular location of the TXNDC5 protein is in the mitochondria in the cytoplasm. The current research results suggest that it has a cancer promoting effect in tumors (15-18), but the specific molecular signaling pathway involved is still unclear. In a previous study (5), we screened and analyzed the main interacting protein molecules of TXNDC5 in gastric cancer cells using tandem affinity coupled proteomics technology. In this study, we further verified the interaction between the TXNDC5 protein and the PRDX4 protein in gastric cancer cells and tissues through a co-IP experiment, and we speculated that part of the tumor promoting effect of TXNDC5 in gastric cancer may be achieved through the signaling pathway related to the PRDX4 molecule. Therefore, we analyzed the expression, prognosis, molecular signaling pathway, and immune microenvironment related to gastric cancer of the selected PRDX4 molecule.

This study used bioinformatics data mining methods to analyze two large sample databases of 1,124 gastric cancer cases in TCGA database. The results confirmed that there was a significant difference in the expression of PRDX4 between gastric cancer tissues and normal tissues, and the expression level in the tumor tissues was significantly higher than that in the normal tissues. A previous study (19) also showed that PRDX4 is abnormally overexpressed in prostate cancer, breast cancer, colon cancer, lung cancer, and many other malignant tumors. Based on these results, the abnormal overexpression of PRDX4 may be a common in malignant tumors and may play a significant role in the occurrence and development of tumors.

In a further analysis of the relationship between PRDX4 expression and patient prognosis in gastric cancer using the Kaplan-Meier Plotter platform, we found that the survival time of the *PRDX4* gene high-expression gastric cancer group was significantly higher than that of the low-expression group and was not affected by gastric cancer staging factors. Moreover, in terms of the intestinal type, diffuse type gastric cancer, male and female patients, and HER-2 negative and positive expression patients, the prognosis of the *PRDX4* gene high-expression group was significantly better than that of the low-expression group. These findings contradict the findings of Park *et al.* (12).

In Park *et al.*'s study, which examined the relationship between PRDX4 expression and patient prognosis in gastric cancer, immunohistochemical methods were used to evaluate the level of PRDX4 expression in gastric cancer tissues. Survival curves were the drawn based on this grouping to analyze the relationship between PRDX4 expression and patient prognosis. The results showed that patients with high PRDX4 expression had a poorer prognosis than those with low PRDX4 expression. Due to these contradictory research results, we analyzed the relationship between the survival of more clinical gastric cancer cases and the *PRDX4* gene using data from the GEPIA online platform database, TCGA cancer database, and Kaplan-Meier pancancer database. The results showed that there was no significant difference in the survival curves of patients with high or low *PRDX4* gene expression in these gastric cancer cases.

Based on the current data, it is difficult to determine the relationship between *PRDX4* gene expression and the prognosis of gastric cancer patients. The reasons for this divergence are relatively complex and could be related to the sample size and sample control factors. It is also possible that PRDX4 itself has a dual effect on tumor prognosis. At present, there is limited research available on the relationship between the *PRDX4* gene and the prognosis of gastric cancer patients.

In the future, our researchers will collect clinical and pathological data of gastric cancer patients for related research to further clarify the relationship between the *PRDX4* gene and the prognosis of gastric cancer patients through experimental research. Our large sample data analysis suggests that its expression in gastric cancer tissues is higher than that in normal tissues, but the effect of the high expression of this gene on patient prognosis is still uncertain. Therefore, it is our view that PRDX4 cannot be simply classified as an oncogenic or tumor suppressor gene, and it may have a dual effect in different situations.

The analysis of the functional significance of the *PRDX4* gene in gastric cancer cells suggests that the *PRDX4* gene may have the effect of promoting DNA replication and DNA damage repair in gastric cancer cells, as well as promoting tumor cell EMT, proliferation, ferroptosis, and the cell response to hypoxia. It may also affect the cell cycle of gastric cancer cells and to some extent, limit the entry into the mitotic phase. Conversely, the *PRDX4* gene may not significantly affect the apoptosis and tumor angiogenesis of gastric cancer cells. These results further demonstrate the pro-cancer effects of the *PRDX4* gene in gastric cancer.

In functional studies, Park *et al.* (12). used short hairpin RNA (shRNA) to knock down PRDX4 expression in AGS and MKN28 gastric cancer cells, and the overexpression of PRDX4 in the PRDX4-depleted cells was used in knock-in experiments. PRDX4 expression was knocked down by shRNA *in vitro*, resulting in a significant decrease in cancer

invasion. Conversely, PRDX4 overexpression promoted invasion and migration in the PRDX4-depleted cancer cells. Our analysis and Park's research suggest that PRDX4 plays a role in EMT-mediated cancer cell migration and invasion.

Previous studies have also provided some insights into the tumor-related molecular signaling pathway of PRDX4 in gastric cancer. Wang *et al.* (20) found that PRDX4 is involved in the β-catenin/ID2 (inhibitor of DNA binding 2) pathway that inhibits apoptosis and enhances the growth and metastasis of liver cancer cells. The research results of Simula *et al.* (21) suggest that PRDX4 plays an important role in the tissue damage process of celiac disease by participating in the PPAR (peroxisome proliferators activated receptor) signaling pathway. Aihaiti *et al.* (22) found that PRDX4 regulates the tumor cell-like characteristics of rheumatoid arthritis fibroblast-like synovial cells through the PI3K/Akt (phosphatidylinositol 3-kinase/ protein kinase B) signaling pathway.

In the present study, we conducted a more in-depth analysis of PRDX4 tumor-related molecular signaling pathways using the classic Reactome Pathway database. The main tumor-related molecular pathways were directed toward the molecular signaling pathways related to neutrophil degranulation. However, other molecular signaling pathways were not identified, which indicates that the data of few studies have been entered into the main molecular pathway database. PRDX4 may affect tumor immune-related conditions by participating in the neutrophil degranulation pathway, thereby affecting tumor occurrence and development.

In this study, we analyzed the relationship between PRDX4 and TIME, and the expression of ICs in gastric cancer, and our research results are similar to those of Zheng *et al.* (23). Specifically, he results of this study suggest that PRDX4 is negatively related to B lymphocytes and $CD4^+$ T-lymphoid infiltration in the immune microenvironment of gastric cancer. A further in-depth analysis revealed that the expression of PRDX4 was only positively correlated with the infiltration of plasma cells, and was negatively correlated with the infiltration of memory B lymphocytes, natural B lymphocytes, and other B lymphocytes. PRDX4 was positively correlated with the infiltration of $CD4^+$ activated memory T lymphocytes, $CD4^+$ Th1 type T lymphocytes, and $CD4^+$ Th2 type T lymphocytes, but PRDX4 was negatively correlated with the infiltration of $CD4^+$ central memory T lymphocytes, $CD4^+$ effector memory T lymphocytes, $CD4^+$ resting memory T lymphocytes, $CD4^+$ native T lymphocytes, and $CD4^+$ regulatory T lymphocytes. These results suggest that the effects of PRDX4 on the TIME is very complex.

The up-regulation of PRDX4 expression, the decrease of the B lymphocyte infiltration, and the increase of $CD4^+$ Th2 T lymphocyte infiltration may inhibit the anti-tumor immune response. Conversely, the up-regulation of PRDX4 expression, the increase of $CD4^+$ activated memory T lymphocytes, the increase of $CD4^+$ Th1 T lymphocyte infiltration, and the decrease of regulatory T lymphocyte infiltration may enhance anti-tumor immunity. Therefore, the influence of PRDX4 expression on the TIME may result in the enhancement or weakening of anti-tumor immunity, which may depend on the degree of the influence of the gene expression on the immune cell of each subpopulation in different circumstances.

In addition, the results of this study suggest that the expression level of PRDX4 is positively correlated with the expression of the IC LILRB2, and negatively correlated with the expression levels of BLTA and VISTA. In solid tumors, LILRB2 can interact with its ligands HLA-G (human leucocyte antigen-G), ANGPTLs (angiopoietin-like proteins), SEMA4A (semaphorin 4A), and CD1d (cluster of differentiation 1d) to induce a tolerance phenotype in myeloid cells, thereby inhibiting T cell activation and leading to tumor immune escape (24). Based on these findings, it can be inferred that PRDX4 may exert a cancer promoting effect by promoting LILRB2 expression.

BTLA (CD272) is an important co inhibitory receptor with structural similarities to PD-1 and CTLA-4 (cytotoxic T lymphocyte-associated protein 4). BTLA signal transduction includes the phosphorylation of ITIMs (immunoreceptor tyrosine-based activation motif) and the binding of SHP-1/SHP-2 (SH2 domain-containing protein tyrosine phosphatase 1/2), thereby inhibiting T cell proliferation and cytokine production (25). VISTA is an important regulator of immune homeostasis and anti-tumor immunity. In immune cells, VISTA is mainly expressed in myeloid cells (neutrophils, monocytes, macrophages, and dendritic cells), and its main function is to reduce the activation status of dendritic cells (26). Therefore, PRDX4 expression may also activate T lymphocytes and dendritic cells to some extent by inhibiting the expression of BLTA and VISTA, thereby enhancing anti-tumor immunity. Therefore, the effects of PRDX4 on IC molecules are also relatively complex, and the resulting enhanced or weakened anti-tumor immune effects may differ from its effects in increasing or decreasing the expression levels of related IC molecules under different conditions.

Our research results suggest that the expression of the *PRDX4* gene is mainly negatively correlated with the infiltration of $CD4^+$ T lymphocytes and B lymphocytes, which are the main immune effector cells in the TIME, but that there are no other significant correlations with other types of immune cells. In each IC molecule, it is mainly positively correlated with LILRB2 and negatively correlated with BLTA and VISTA. Overall, the $CD4^+$ T cell subpopulation controls pathogen infection, assists B cells, regulates tissue balance and repair, or performs immune regulation through MHC-II peptide complexes.

$CD4^+$ T cells, such as $CD8^+$ T cells, play equally important roles in the body's anti-tumor immunity. The role of $CD4^+$ T cells in tumor immunity is mainly reflected in the following aspects: (I) $CD4^+$ T cells can provide assistance and guidance by interacting with other immune cells to enhance the immune response. They can interact with antigen presenting cells (such as dendritic cells) to promote the presentation and presentation of tumor antigens, thereby activating the reactions of other immune cells; (II) $CD4^+$ T cells can secrete various cytokines, such as interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), interleukin-2 (IL-2), and other cytokines can directly kill tumor cells or indirectly activate other immune cells, such as cytotoxic T lymphocytes and natural killer cells, enhancing their killing ability against tumor cells; and (III) $CD4^+$ T cells have memory properties, and once activated, they can persist for a long time and quickly respond to re-exposure to the same antigen. This allows $CD4^+$ T cells to have a long-lasting immune response to tumor formation and quickly launch attacks when needed.

Conversely, B lymphocytes are key mediators of humoral immunity. In cancer, B cells can exert anti-tumor effects through antibody dependent cytotoxicity and complement activation. B cells can exist in the tertiary lymphoid structure in tumors, where they promote T cell activation through antigen presentation. Due to the important role of $CD4^+$ T lymphocytes and B lymphocytes in the anti-tumor immune response, overall, the *PRDX4* gene may have a certain inhibitory effect in the immune microenvironment of gastric cancer. The inhibitory effect of the *PRDX4* gene expression on immune cells may mainly be achieved through the IC LILRB2.

These research findings may be helpful for immunotherapy strategies for gastric cancer. Supplementing these two therapeutic immune effector cells to increase $CD4^+$ T and B lymphocyte infiltration in the immune microenvironment of gastric cancer with generally high expressions of the *PRDX4* gene may be an important strategy for adoptive immune cell therapy. The targeted inhibition of LILRB2 may be an important choice for IC blockade therapy in gastric cancer patients with high expressions of the *PRDX4* gene.

This study had a number of limitations. Due to the use of bioinformatics methods, the accuracy of the results needs to be further verified in future experiments. We used the UCSC Xena data secondary analysis platform for alternative data mining, which may have resulted in incomplete data. In the next study, we will further optimize the analysis conditions, improve the data platforms, and further verify the downstream molecular signaling pathways of the target molecules and the effects of the TIME through experiments to lay an important foundation for further elucidating the molecular mechanism of the TXNDC5-PRDX4 pathway in gastric cancer.

Conclusions

This study experimentally verified the interaction between the TXNDC5 and PRDX4 molecules in gastric cancer. The results suggested that the *PRDX4* gene is highly expressed in gastric cancer and has the effect of promoting the malignant biological characteristics of gastric cancer, which in turn exerts a certain inhibitory effect on anti-tumor immunity in the immune microenvironment.

Acknowledgments

We sincerely thank our colleagues from the Radiology Department, Pathology Department, and General Surgery Department of The Eighth Medical Center of PLA General Hospital for their great support in the study. We also wish to thank the Fourth Military Medical University of PLA for providing the means for the current investigation.

Funding: This work was supported by the Capital Health Development Research Special Fund (No. 2011-5007-01).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-904/rc

Data Sharing Statement: Available at https://tcr.amegroups.

com/article/view/10.21037/tcr-23-904/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-904/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-904/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Medical Ethics Committee of The Eighth Medical Center of PLA General Hospital (No. 309202309121507). Informed consent was obtained from the patient. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Zhang L, Hou YH, Wu K, et al. Proteomic analysis reveals molecular biological details in varioliform gastritis without Helicobacter pylori infection. World J Gastroenterol 2010;16:3664-73.
2. Sullivan DC, Huminiecki L, Moore JW, et al. EndoPDI, a novel protein-disulfide isomerase-like protein that is preferentially expressed in endothelial cells acts as a stress survival factor. J Biol Chem 2003;278:47079-88.
3. Zhang L, Hou Y, Li N, et al. The influence of TXNDC5 gene on gastric cancer cell. J Cancer Res Clin Oncol 2010;136:1497-505.
4. Wu Z, Zhang L, Li N, et al. An immunohistochemical study of thioredoxin domain-containing 5 expression in gastric adenocarcinoma. Oncol Lett 2015;9:1154-8.
5. Zhang L, Hou Y, Li C, et al. Screening of TXNDC5 interacting protein molecules in gastric cancer cells. J Gastroenterol Hepatol 2021;30:140-4.
6. Zito E. PRDX4, an endoplasmic reticulum-localized peroxiredoxin at the crossroads between enzymatic oxidative protein folding and nonenzymatic protein oxidation. Antioxid Redox Signal 2013;18:1666-74.
7. Yamada S, Guo X. Peroxiredoxin 4 (PRDX4): Its critical in vivo roles in animal models of metabolic syndrome ranging from atherosclerosis to nonalcoholic fatty liver disease. Pathol Int 2018;68:91-101.
8. Lipinski S, Pfeuffer S, Arnold P, et al. Prdx4 limits caspase-1 activation and restricts inflammasome-mediated signaling by extracellular vesicles. EMBO J 2019;38:e101266.
9. Zhang B, Li X, Liu G, et al. Peroxiredomin-4 ameliorates lipotoxicity-induced oxidative stress and apoptosis in diabetic cardiomyopathy. Biomed Pharmacother 2021;141:111780.
10. Shao C, Wang R, Kong D, et al. Identification of potential core genes in gastric cancer using bioinformatics analysis. J Gastrointest Oncol 2021;12:2109-22.
11. Zhang L, Hou Y, Li X, et al Verification of immune coprecipitation of TXNDC5 protein interacting protein in gastric cancer cells and tissues. J Gastroenterol Hepatol 2022;31:861-6.
12. Park SY, Lee YJ, Park J, et al. PRDX4 overexpression is associated with poor prognosis in gastric cancer. Oncol Lett 2020;19:3522-30.
13. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res 2017;77:e108-10.
14. Li B, Severson E, Pignon JC, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol 2016;17:174.
15. Nissom PM, Lo SL, Lo JC, et al. Hcc-2, a novel mammalian ER thioredoxin that is differentially expressed in hepatocellular carcinoma. FEBS Lett 2006;580:2216-26.
16. Claudio JO, Masih-Khan E, Tang H, et al. A molecular compendium of genes expressed in multiple myeloma. Blood 2002;100:2175-86.
17. Wei Q, Li M, Fu X, et al. Global analysis of differentially expressed genes in androgen-independent prostate cancer. Prostate Cancer Prostatic Dis 2007;10:167-74.
18. Wang X, Li H, Chang X. The role and mechanism of TXNDC5 in diseases. Eur J Med Res 2022;27:145.
19. Jia W, Chen P, Cheng Y. PRDX4 and Its Roles in Various Cancers. Technol Cancer Res Treat 2019;18:1533033819864313.

20. Wang W, Shen XB, Huang DB, et al. Peroxiredoxin 4 suppresses anoikis and augments growth and metastasis of hepatocellular carcinoma cells through the β-catenin/ID2 pathway. Cell Oncol (Dordr) 2019;42:769-81.
21. Simula MP, Cannizzaro R, Canzonieri V, et al. PPAR signaling pathway and cancer-related proteins are involved in celiac disease-associated tissue damage. Mol Med 2010;16:199-209.
22. Aihaiti Y, Tuerhong X, Zheng H, et al. Peroxiredoxin 4 regulates tumor-cell-like characteristics of fibroblast-like synoviocytes in rheumatoid arthritis through PI3k/Akt signaling pathway. Clin Immunol 2022;237:108964.
23. Zheng J, Guo X, Nakamura Y, et al. Overexpression of PRDX4 Modulates Tumor Microenvironment and Promotes Urethane-Induced Lung Tumorigenesis. Oxid Med Cell Longev 2020;2020:8262730.
24. Chen HM, van der Touw W, Wang YS, et al. Blocking immunoinhibitory receptor LILRB2 reprograms tumor-associated myeloid cells and promotes antitumor immunity. J Clin Invest 2018;128:5647-62.
25. Ning Z, Liu K, Xiong H. Roles of BTLA in Immunity and Immune Disorders. Front Immunol 2021;12:654960.
26. Huang X, Zhang X, Li E, et al. VISTA: an immune regulatory protein checking tumor and immune cells in cancer immunotherapy. J Hematol Oncol 2020;13:83.

Cite this article as: Zhang L, Wu K, Hou Y, Li X. Validation of the interaction between PRDX4 and TXNDC5 in gastric cancer and the significance of the *PRDX4* gene in gastric cancer based on a data mining analysis. Transl Cancer Res 2024;13(1):81-101. doi: 10.21037/tcr-23-904

