PLXDC1 May Serve As A Target For Combining Antiangiogenic Therapy and Immunotherapy In Gastric Adenocarcinoma

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Abstract

Introduction Based on the immunosuppression of traditional antiangiogenic agents in the treatment of tumors and the newly proposed concept of antiangiogenic therapy combined with immunotherapy, this paper will mainly explore the prospects of PLXDC1 in stomach adenocarcinoma (STAD) regarding antiangiogenic therapy and immunotherapy.

Methods First, the transcriptional and translational levels of PLXDC1 in STAD were analyzed using the Oncomine, The Cancer Genome Atlas (TCGA) and Human Protein Atlas databases and then univariate and multivariate Cox regression analyses were performed using TCGA data. Next, we explored the correlation between PLXDC1 and STAD immunity from multiple aspects. Finally, based on the acquisition of immunomodulators associated with PLXDC1 expression from TISIDB, we constructed PLXDC1-related immune prognostic signatures of four genes (NT5E, CTLA, TGFBR1, and CSF1R) and constructed a nomogram for predicting survival to analyze the clinical utility of PLXDC1 in immunotherapy.

Results Our results demonstrated that PLXDC1 was highly expressed in STAD and that its high expression was associated with poor prognosis in STAD. Multivariate Cox analysis suggested that PLXDC1 could be used as an independent prognostic risk factor for STAD. The high-risk group for which we constructed PLXDC1-related immune prognostic signatures showed poorer prognosis compared to low-risk group, and the risk score of our model could be used as an independent risk factor for STAD prognosis. Moreover, the nomogram survival prediction system showed good accuracy of the constructed immune signatures.

Conclusions In conclusion, PLXDC1 can serve as a biomarker for the diagnosis and treatment of STAD and it may also be a new target for STAD immunotherapy. Therefore, PLXDC1 can combine antiangiogenic therapy with immunotherapy.

1. Introduction

Stomach cancer has the fifth highest incidence and the second highest mortality rate of all cancers worldwide¹. According to the pathological staging of gastric cancer, stomach adenocarcinoma (STAD) accounts for 95% of all gastric cancer cases². Patients with early STAD limited to the mucosa and submucosa have five-year survival rates of 70–95% with surgical treatment³. While western surgical and population-based series show five-year survival rates of 20–30% for most patients with advanced tumors that have penetrated the submucosa⁴. Therefore, searching for biomarkers for STAD, aiding in the earlier detection of cancer, is of great importance for patient prognosis.

Immunotherapy is a landmark discovery in cancer treatment that kills tumor cells mainly by modulating the immune system and altering the tumor immune microenvironment^{5,6}. Additionally, an increasing number of studies have shown that immune cell infiltration plays a crucial role in tumor prognosis^{7,8}. Currently, immunotherapy is widely practiced in the clinical treatment of non-small cell lung cancer, and

programmed death-ligand 1 (PD-L1) agents developed to target programmed death-1 (PD-1) on the tumor surface have improved the survival of tumor patients by disrupting the tumor's immune evasion mechanism and killing tumor cells⁹⁻¹¹. The remarkable success of immunotherapy in non-small cell lung cancer provides a new direction for immunotherapy in STAD¹². A study found that PD-L1 was expressed in tumor cells and immune stroma across all stages and histologies of STAD, while patients with higher CD8 + T-cell densities also had higher PD-L1 expression, suggesting the occurrence of adaptive immune resistance mechanisms¹³. However, immunotherapy targeting PD-1 is only applicable to some STAD patients, and effective targets of immunotherapy for most patients still need to be explored, so identifying suitable immunotherapy targets is of great significance for STAD patients.

Plexin domain containing 1 (PLXDC1) is a vascular protein associated with angiogenic states¹⁴. It was demonstrated that pigment epithelium derived factor binds to PLXDC1 on the cell surface through its extracellular structural domain, thus achieving anti-neoangiogenesis and inhibiting tumor growth¹⁵. Currently, PLXDC1 is reported to be involved in the development of various cancers. For example, glioblastoma endothelium drives bevacizumab-induced infiltrative growth through the regulation of PLXDC1¹⁶, and PLXDC1 promotes migration and invasion in gastric cancer¹⁷. However, there are no relevant studies on PLXDC1 in STAD and its immunity, and this study will focus on these aspects.

2. Methods And Materials

2.1 Acquisition of PLXDC1 expression profiles in gastric cancer

First, we used the Oncomine database (<https://www.oncomine.org/resource/login.html>.) and selected the "Gene Differential Expression" module to explore the expression of PLXDC1 across cancers. Then, we chose gastric cancer as the subject of our study and further analyzed the differential expression of PLXDC1 in gastric cancer and its subtypes with normal tissues using the dataset on the website (P-value < 0.05). Next, we downloaded the STAD data (normal = 32, cancer = 375) from TCGA database (<https://www.cancer.gov/>.) and compared the expression of PLXDC1 in STAD and normal tissues using the "limma" R package (P-value < 0.05; t test). Finally, using the Human Protein Atlas database (<https://www.proteinatlas.org/>), we analyzed the expression of PLXDC1 in STAD at the protein level.

2.2 Survival analysis of PLXDC1 in gastric cancer

Using the Kaplan-Meier Plotter online website (<https://kmplot.com/analysis/>), we selected the "Gastric Cancer" module to classify high- and low-risk groups according to the median value of PLXDC1 expression. Then, we analyzed the correlation of PLXDC1 expression with overall survival (OS) (n = 875), first progression (FP) (n = 640), and post-progression survival (PPS) (n = 498) in gastric cancer (P-value < 0.05; log-rank test). Next, we downloaded STAD data (normal: n = 32, tumor: n = 375) from TCGA and then performed univariate Cox analyses of PLXDC1 with clinical factors such as age, gender, grade, stage, T stage, N stage and M stage (P-value < 0.05). Then, we used receiver operating characteristic (ROC) curves

to assess the accuracy of the prognostic value of these clinical factors, and the larger the area under the curve (AUC) was, the more accurate the prediction. Finally, using multivariate Cox analysis, we identified independent risk factors for prognosis. (P-value < 0.05)

2.3 Enrichment of immune-related pathways

We used GSEA version 4.1.0, a method for the analysis of genome-wide expression profiling microarray data that compares genes with predefined gene sets, to enrich for immune pathways associated with PLXDC1 in STAD. The gene expression matrix in STAD was processed by Perl software to obtain the input file related to the target gene. Then, the upregulated gene set was defined as phenotype h (h = 3649/5093), the downregulated gene set was defined as phenotype l (l = 1444/5093), and the "h-versus-l" and "immune signature" patterns were selected to enrich for PLXDC1-related immune pathways.

2.4 Analysis of the immune microenvironment of PLXDC1 in STAD

First, we utilized the R package obtained from the Cell Type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) (<https://cibersort.stanford.edu/>) website, which can qualify and quantify 22 types of immune cells in the tissue, to visualize immune cell infiltration in STAD and to analyze the mRNA expression matrix for further differential analysis of immune cells infiltrating in STAD and normal tissues¹⁸ (STAD vs. normal = 375 vs. 32). Moreover, we performed correlation analysis of immune cells in the STAD immune microenvironment using the "corrplot" R package.

Then, we analyzed the correlation between the expression of PLXDC1 and immune cells in the STAD immune microenvironment using the TISIDB online website (P-value < 0.05) (<http://cis.hku.hk/TISIDB/index.php>), an integrated repository portal for tumor-immune system interactions¹⁹. Based on the "Subtype" panel, immune cells in STAD were classified into six subtypes, C1 (wound healing; n = 129), C2 (IFN-gamma dominant; n = 210), C3 (inflammatory; n = 36), C4 (lymphocyte depleted; n = 9), C5 (immunologically quiet), and C6 (TGF- β dominant; n = 7), to explore the correlation between PLXDC1 expression and the C1/2/3/4/6 subtypes.

Subsequently, we used TIMER 2.0 (<http://timer.cistrome.org/>), a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types, and selected the "Immune Association" panel to analyze the correlation between the copy number of PLXDC1 and the number of immune cells (P-value < 0.05). The results of the analysis were supplemented with the results of CIBERSORT for the missing data from TIMER 2.0.

2.5 Immunomodulators in the immune microenvironment of STAD

Using the "Immunomodulators" panel in the TISIDB, we analyzed the correlation between 23 immunoinhibitors and 44 immunostimulators produced in the STAD immune microenvironment and PLXDC1 expression (P-value < 0.05). Then, the Search Tool for the Retrieval of Interacting Genes/Proteins

(STRING) (<https://www.string-db.org/online>.) site was used to construct a protein interaction network of immunomodulators associated with PLXDC1 expression. Next, we performed Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of immunomodulators associated with PLXDC1 expression in the STAD immune microenvironment using the WebGestalt online tool (<http://www.webgestalt.org/>), a functional enrichment analysis web tool that supports three well-established and complementary methods for enrichment analysis, including overrepresentation analysis, gene set enrichment analysis, and network topology-based analysis.

2.6 Construction of PLXDC1-related immune prognostic signatures and performance evaluation

Based on 48 immunomodulators associated with PLXDC1 expression, we used the least absolute shrinkage and selection operator (LASSO) regression algorithm with penalty parameter tuning conducted by 10-fold cross-validation with the “glmnet” and “survival” packages. Then, the screened immunomodulators from LASSO were subjected to stepwise multivariate Cox proportional hazards regression analysis to obtain the optimal candidates and construct an immune prognostic model of immunity. The formula for calculating the risk score was as follows:

$$Riskscore = \sum_{i=1}^n coef_i \times X_i$$

where “coef_i” and “X_i” represent the coefficient and expression level of each PLXDC1-related immunomodulator, respectively. Patients with STAD were classified into high-risk and low-risk groups based on the median score as the risk cutoff point. The survival curves of the two groups were plotted using the Kaplan–Meier method and compared by the log-rank test using the “survival” and “survminer” packages in R, with a P-value < 0.05 indicating significance. ROC curve analysis was conducted, and the AUC values were obtained to evaluate the prognostic model’s predictability using the “survivalROC” package. Finally, we integrated the risk score obtained from the model with clinical data such as age, gender, grade, stage, T stage, N stage, and M stage downloaded from TCGA (n = 163) for univariate and multivariate Cox analyses to explore whether the risk score was an independent prognostic risk factor.

2.7 Construction a nomogram prognostic prediction model

Based on the results of the multivariate Cox analysis, we combined the risk score and clinical factors including age, gender, grade, stage, T stage, N stage, and M stage. Each factor was scored in the nomogram scoring system and then the scores were summed to obtain an overall score to predict survival at 1, 3, and 5 years. The concordance index (C-index) and calibration plot were used to assess the predictive performance and discrimination ability of the nomogram scoring system (C = 0.5, no predictive capability; C = 0.51 ~ 0.70, low accuracy; C = 0.71 ~ 0.90, medium accuracy; and C > 0.90, high accuracy).

2.8 Statistics

We have implemented all statistical analyses using R (version 4.1.0) and some online databases. Differences between two groups of continuous variables were analysed using t-tests, and differences in continuous variables over two groups were analysed using kruskal-wallis tests. Moreover, survival analysis between the two groups was performed using log-rank test. P value < 0.05 was considered statistically significant.

3. Results

3.1 Expression of PLXDC1 in gastric cancer

First, the pan-cancer analysis in Oncomine showed that PLXDC1 was highly expressed in multiple cancers, including gastric cancer (Fig. 1a). Then, analysis of gastric cancer and its subtypes showed that PLXDC1 was overexpressed in gastric cancer compared to normal tissue according to the data of Wang²⁰. Based on the data of Cho²¹, PLXDC1 was highly expressed in diffuse gastric adenocarcinoma, gastric mixed adenocarcinoma and gastric intestinal type adenocarcinoma (Table 1). Next, we downloaded the data of STAD in TCGA, and the analysis revealed that PLXDC1 was overexpressed in STAD compared to normal tissue (P-value < 0.05; t test) (Fig. 1b, Fig. 1c). Moreover, we used the Human Protein Atlas to confirm the conclusion that PLXDC1 was highly expressed in STAD at the protein level (Fig. 1D). Overall, PLXDC1 is always highly expressed in gastric cancer and its subtypes.

Table 1. Expression of PLXDC1 in subtypes of gastric cancer

Types of gastric cancer vs. normal tissues	Fold change P value		t-Test	Reference
Gastric Cancer vs. Normal	2.048	7.16E-7	6.306	Wang ²⁰
Diffuse Gastric Adenocarcinoma vs. Normal	1.621	0.023	2.511	Eur J ²¹
Gastric Mixed Adenocarcinoma vs. Normal	2.040	0.025	3.022	Eur J ²¹
Gastric Intestinal Type Adenocarcinoma vs. Normal	1.335	0.004	2.784	Eur J ²¹

3.2 Survival analysis of PLXDC1 in gastric cancer

First, the survival analysis on the Kaplan-Meier Plotter online site showed that high expression of PLXDC1 in gastric cancer was associated with OS, FP, and PPS (P-value < 0.05; log-rank test), representing poor prognosis (hazard ratio (HR) > 1) (Fig. 2a). Then, we analyzed the prognosis of PLXDC1 in STAD. Univariate Cox analysis showed correlations of PLXDC1, age, gender, grade, stage, T stage, N stage and M stage with OS (P-value < 0.05), representing poor prognosis (HR > 1) (Table 2). Furthermore, the ROC curves showed high predictive accuracy for age, grade, stage, N stage and M stage in the univariate Cox prognostic analysis (Fig. 2b). Finally, multivariate Cox analysis indicated that PLXDC1 and age were independent prognostic risk factors for STAD (P-value < 0.05) (Fig. 2c). Overall, high expression of

PLXDC1 is associated with poor prognosis in gastric cancer and STAD. Moreover, it may serve as an independent prognostic risk factor in STAD.

Table 2. Univariate Cox analysis of the correlation between clinical factors and OS

ID	HR	HR.95L	HR.95H	P value
PLXDC1	1.15	1.03	1.29	0.0171 *
Age	1.03	1.01	1.05	0.0056 *
Gender	1.48	0.98	2.25	0.0624
Grade	1.37	0.95	1.98	0.0954
Stage	1.54	1.22	1.93	0.0002 *
T	1.30	1.02	1.65	0.0315 *
M	2.05	1.10	3.83	0.0246 *
N	1.27	1.07	1.50	0.0064 *

*: p-value<0.05

3.3 Enrichment analysis of PLXDC1-related immune pathways in STAD

Using nominal (NOM) P-value < 0.05, false discovery rate (FDR) Q-value < 0.05 and familywise error rate (FWER) P-value < 0.05 as screening conditions for the results of GSEA immune enrichment analysis, we obtained 67 upregulated immune pathways (Supplementary Materials 1, 2) and 28 downregulated immune pathways (Supplementary Materials 3) by PLXDC1 in STAD. Finally, we plotted the top five paths that were upregulated or downregulated based on the combined assessment of the normalized enrichment score (NES), P-value < 0.05, FDR Q-value < 0.05 and FWER P-value < 0.05 (Fig. 3a, Fig. 3b). Overall, PLXDC1 may be involved in STAD immune regulation.

3.4 Analysis of the immune microenvironment in STAD

First, we visualized the immune infiltration of 22 immune cells in STAD using CIBERSORT (Fig. 4a). Differential analysis of infiltrated immune cells revealed that “B cells naive, T cells CD4 naive, Macrophages M0 and Macrophages M1” showed high infiltration levels in STAD compared to normal tissues, while “B cells memory, Plasma cells, T cells CD8, T cells CD4 memory resting, T cells gamma delta, Monocytes and Mast cells resting” showed low infiltration levels (P-value < 0.05) (Fig. 4b). “Macrophages M0” and “T cells CD8” were the most significantly negatively correlated in the STAD immune cell infiltration correlation analysis, while “T cells CD4 memory activated” and “T cells CD8” were the most positively correlated (Fig. 4c). Overall, there is a complex immune microenvironment in STAD.

3.5 Correlation analysis of PLXDC1 and the STAD immune microenvironment

TISIDB online website analysis revealed that the expression of PLXDC1 was closely associated with the immune microenvironment across cancers (Fig. 5a). In the immune microenvironment of STAD, we found that PLXDC1 expression was positively correlated with Tcm_CD8, Tem_CD8, Tcm_CD4, Tem_CD4, Tfh, Tgd, Th1, Th17, NK, MDSC, NKT, Act_DC, pDC, iDC, macrophages, eosinophils, mast, and neutrophil ($\rho > 0$, P-value < 0.05) (Fig. 5b), while it was negatively correlated with Act_CD4 (Fig. 5b) ($\rho < 0$, P-value < 0.05). In addition, STAD immune subtype phenotyping (C1/2/3/4/6) was correlated with PLXDC1 expression, and the highest gene expression was found in the C6 phenotype (P-value < 0.05) (Fig. 5c). Finally, we explored the correlation between PLXDC1 copy number and the number of immune cells in the STAD immune microenvironment using the TIMER 2.0 database. The results indicated that there was a correlation between PLXDC1 copy number and multiple immune cells (P-value < 0.05) (Fig. 5d). In summary, our analysis from multiple perspectives revealed a close relationship between PLXDC1 and the STAD immune microenvironment.

3.6 Immunomodulators associated with PLXDC1 expression in STAD

TISIDB analysis revealed 13 immunoinhibitors (ADORA2A, BTLA, CD96, CSF1R, CTLA4, HAVCR2, IL10, KDR, PDCD1LG2, PVRL2, TGFB1, TGFBR1, and TIGIT) (Table 3) (P-value < 0.05) and 35 immunostimulators (C10orf54, CD27, CD28, CD40, CD40LG, CD48, CD80, CD86, CD267, CXCL12, CXCR4, ENTPD1, ICOS, ICOSLG, IL2RA, IL6, KLRC1, KLRK1, LTA, NT5E, PVR, TMEM173, TNFRSF4, TNFRSF8, TNFRSF9, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF17, TNFRSF25, TNFSF4, TNFSF13B, TNFSF14, TNFSF15, and TNFSF18) (Table 4) (P-value < 0.05) in the STAD immune microenvironment associated with PLXDC1 expression. Then, the protein-protein interaction network constructed by STRING revealed that 48 immunomodulators associated with PLXDC1 expression have complex interactions (Fig. 6a). Finally, based on the WebGestalt online tool, GO functional annotation and KEGG pathway enrichment analysis of 48 immunomodulators demonstrated that PLXDC1-related immunomodulators were extensively involved in the body's immune processes (Fig. 6b, Fig. 6c, Fig. 6d). Overall, PLXDC1-related immunomodulators may play an important role in the immune microenvironment of STAD.

Table 3. Correlations between the expression of PLXDC1 and immunoinhibitors

Immunoinhibitors	Rho	P-value
ADORA2A	0.363	2.98e-14 *
BTLA	0.19	0.000104 *
CD96	0.206	2.42e-05 *
CD160	0.086	0.0809
CD244	0.088	0.0735
CD274	0.045	0.361
CSF1R	0.446	2.2e-16 *
CTLA4	0.107	0.0287 *
HAVCR2	0.337	2.48e-12 *
IDO1	0.041	0.403
IL10	0.386	1.43e-16 *
IL10RB	0.055	0.263
KDR	0.533	2.2e-16 *
LAG3	0.038	0.442
LGALS9	-0.085	0.0833
PDCD1	0.062	0.206
PDCD1LG2	0.398	2.2e-16 *
PVRL2	-0.173	0.000412 *
TGFB1	0.444	2.2e-16 *
TGFBR1	0.287	3.11e-09 *
TIGIT	0.157	0.00133 *
VTCN1	0.008	0.873

*p-value<0.05

3.7 Establishment of PLXDC1-related immune prognostic signatures and predictive evaluation

Immunomodulators are an important component of the tumor immune microenvironment, and their alterations are closely related to the prognosis of patients²². Based on 48 immunomodulators associated with PLXDC1 expression in the STAD immune microenvironment, we used LASSO regression (Fig. 7a, Fig. 7b), and stepwise multivariate Cox proportional hazards regression analyses were performed to construct

a risk model for survival prediction (Fig. 7c-f). Then, PLXDC1-related immune prognostic signatures were constructed based on the expression levels of four immunomodulator target genes (NT5E, CTLA, TGFBR1, and CSF1R) (Fig. 7c). The multivariate Cox proportional hazards model had the following computational formula: risk score = (coefficient × expression of NT5E) + (coefficient × expression of CTLA4) + (coefficient × expression of TGFBR1) + (coefficient × expression of CSF1R). Based on the median value of the risk score, we divided the training set into high- and low-risk groups. We found that patients with STAD in the low-risk group had better survival outcomes than those in the high-risk group (P-value < 0.05) (Fig. 7e). The AUC of the ROC curve for the multivariate Cox proportional hazards model was 0.722, indicating the high accuracy of our model's risk prediction capability (Fig. 7f). Finally, the independent prognostic analysis of the multivariate Cox proportional hazards model's risk score combined with clinical factors such as gender, grade, stage, T stage, N stage, and M stage suggested that the risk score could be used as a prognostic risk indicator independent of these clinical factors (P-value < 0.05) (Fig. 7h, Fig. 7i). Overall, these results demonstrated that the immune prognostic signatures based on the four PLXDC1-related immunomodulators has good survival predictive efficacy.

3.8 Construction and validation of a nomogram for the survival prediction of patients with STAD

To improve the model's clinical applicability, we constructed a statistical nomogram model in the training set to predict 1-, 3-, and 5-year patient survival by integrating the model's risk score and clinical factors such as age, gender, grade, stage, T stage, N stage, and M stage (Fig. 8a). To assess the accuracy of the clinical prediction model, we plotted the calibration curves of the 3-year and 5-year survival rates of STAD patients based on the model (Fig. 8b). At the same time, we assessed the overall prediction accuracy of the model using the C-index, and the results indicated that the prediction accuracy of our nomogram reached 0.722 (Fig. 8c).

4. Discussion

In 1971, Folkman et al. proposed a hypothesis that tumors, to promote tumor cell growth, provide nutrition to their cells by creating new blood vessels²³. Subsequently, this hypothesis was confirmed as one of the hallmarks of tumors²⁴. Until now, the antiangiogenic agents developed have achieved great success in treating many types of cancer, such as breast cancer^{25–27}, lung cancer^{28,29}, colorectal cancer³⁰ and gastric cancer³¹. Previous studies found that PLXDC1 was closely associated with angiogenesis¹⁴. In this study, we used the Oncomine, TCGA and Human Protein Atlas databases and found that PLXDC1 was highly expressed in STAD in terms of gene transcription and translation. Simultaneously, survival analysis showed that high expression of PLXDC1 in STAD was associated with poor prognosis. Moreover, multivariate Cox analysis showed that PLXDC1 could be an independent prognostic risk factor for STAD. These findings suggest that PLXDC1 can be used as a biomarker for the diagnosis and treatment of STAD.

Despite the achievements of antiangiogenic drugs in the treatment of gastric cancer, some intractable problems have emerged³². Inhibition of angiogenesis provides only a transient survival benefit to patients because of the presence of escape mechanisms in tumors, including the upregulation of compensatory pathways³³, vasculogenic mimicry³⁴ and the recruitment of bone marrow-derived cells³⁵. Moreover, several studies have found that angiogenesis inhibitors increase the aggressiveness and metastasis of tumors^{36,37}. For example, treatment with short-term sunitinib led to accelerated tumor metastasis in a mouse model³⁸. Notably, scientists have discovered that the use of antiangiogenic agents may cause tumors to develop immunosuppression^{39,40}. This is because the infiltration and function of immune cells depend on normal vascular channels and their provision of oxygen and nutrients^{41–44}. However, antiangiogenic agents disrupt the blood vessels of the tumor, preventing immune cells from infiltrating and functioning. Based on the immunosuppression and multiple deficiencies of antiangiogenic agents, researchers recently proposed a program that combines antiangiogenic agents with immunotherapy^{39,45,46}, which may bring a breakthrough in tumor treatment.

In this study, we found that PLXDC1 is closely associated with the immune microenvironment of STAD from immune pathway enrichment analysis, immune cell infiltration analysis and correlation analysis of immune cells, immune subtypes, and copy number with PLXDC1. In addition, we found 48 immunomodulators in the immune microenvironment of STAD associated with PLXDC1 expression through the TISIDB website. Immunomodulators are an essential component of the tumor microenvironment, and changes in them have a significant impact on patient survival²². Then, we constructed PLXDC1-related immune prognostic signatures containing four genes (NT5E, CTLA, TGFBR1, and CSF1R) based on these prognosis-related immunomodulators using LASSO regression and stepwise multivariate Cox proportional hazards regression analyses. The results indicated that the high-risk group of our constructed model was associated with the poor prognosis of patients, and the accuracy of the model reached 0.722. Furthermore, multivariate Cox analysis showed that the risk score of the model was an independent risk factor for the prognosis of STAD patients. Therefore, we can conclude that PLXDC1 may be a new target for STAD immunotherapy.

In summary, PLXDC1 can be used as a biomarker for the diagnosis and treatment of STAD, while immune analysis suggests that it may serve as a new target for STAD immunotherapy. Because PLXDC1 can inhibit tumor angiogenesis while playing a role in immunotherapy, we speculate that PLXDC1 might represent a breakthrough in tumor therapy³⁹. However, there are some shortcomings in our study. Most of our data came from authoritative databases on the web, and the authenticity of the data relies heavily on these open databases. For this reason, we used multiple databases for validation to verify our findings from multiple perspectives simultaneously, which supports the robustness of our findings.

Abbreviations

STAD: Stomach Adenocarcinoma; TCGA: The Cancer Genome Atlas; ROC: Receiver Operating Characteristic; AUC: Area Under the Curve; PD-L1: Programmed Death-ligand 1; PD-1: Programmed Death-

1; OS: Overall Survival; FP: First Progression; PPS: Post Progression Survival; GSEA: Gene Set Enrichment Analysis; CIBERSORT: Cell Type Identification by Estimating Relative Subsets of RNA Transcripts; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LASSO: Least Absolute Shrinkage and Selection Operator.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed in the current study can be found in the following databases. TCGA: <https://www.cancer.gov/>; Oncomine: <https://www.oncomine.org/resource/login.html>; The Human Protein Atlas: <https://www.proteinatlas.org/>; Kaplan-Meier Plotter: <https://kmplot.com/analysis/>; CIBERSORT <https://cibersort.stanford.edu/>; TISIDB: <http://cis.hku.hk/TISIDB/index.php>; WebGestalt: <http://www.webgestalt.org/>.

Competing interests

TCGA, Oncomine, The Human Protein Atlas, Kaplan-Meier Plotter, CIBERSORT, TISIDB and WebGestalt are public databases. The patients involved in the databases provided ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues or other conflicts of interest.

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Authors' contributions

XL and YF codesigned the project. XL contributed to the data compilation, literature search and manuscript writing. FS and ZW refined the project proposal and revised the manuscript. All authors reviewed the manuscript and approved the manuscript for publication.

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Figures

Figure 1

The expression of PLXDC1 in tumors. (a) The expression of PLXDC1 in pan-cancer in the Oncomine database. Figures represent the number of studies. Red represents high expression. Blue represents low expression. (b), (c) The expression of PLXDC1 in STAD in the TCGA database. (d) The protein expression of PLXDC1 in STAD in the Human Protein Atlas database.

Figure2a

Figure2b

Figure2c

Figure 2

Prognostic analysis of PLXDC1 in STAD. (a) Correlation analysis between PLXDC1 expression and OS, FP and PPS in gastric cancer in Kaplan-Meier Plotter. (b) The accuracy of ROC curves to assess prognostic analysis in STAD in univariate COX analysis. (c) Multivariate COX analysis between PLXDC1 and clinical factors in TCGA data.

Figure3a

Figure3b

Figure 3

Enrichment analysis of PLXDC1 immune-related pathways in STAD. (a) The top five up-regulated immune pathways of PLXDC1 in STAD. (b) The top five down-regulated immune pathways of PLXDC1 in STAD.

Figure 4

Analysis of the immune microenvironment in STAD. (a) Visualization of immune cell infiltration in STAD. (b) Differential analysis of infiltrated immune cells in STAD with normal tissue. (c) Correlation analysis among infiltrated immune cells in STAD.

Figure 5

Correlation analysis of PLXDC1 and STAD immune microenvironment. (a) Immunological correlation of PLXDC1 in pan-cancer. (*:P-value<0.05) (b) Immunological correlation between PLXDC1 expression and immune cells in STAD. (c) Correlation of PLXDC1 expression with immune cell typing in STAD. (d) Correlation of PLXDC1 copy number with immune cells in STAD.

Figure 6

Analysis of immunomodulators associated with PLXDC1. (a) Protein-protein network of PLXDC1-associated immunomodulators in STAD constructed by STRING online website. (b) GO annotation and (c), (d) KEGG pathway enrichment for 48 PLXDC1-associated immunomodulators in STAD.

Figure7a

Figure7b

Figure7c

Figure7e

Figure7f

Figure7d

Figure7h

Figure7i

Figure 7

Establishment of PLXDC1-related immune prognostic signatures. (a), (b), (c) LASSO and stepwise multivariate Cox proportional hazards regression analysis to obtain candidate genes and construct prognostic models. (d) Relationship between PLXDC1-associated immune prognostic signatures and patient survival. (e) Kaplan-Meier survival curves of patients in the high- and low-risk groups separated by risk score of the model. (f) Evaluation of PLXDC1-related immune prognostic signatures' predictive

performance of OS in patients with SATD. (h), (i) Univariate and multivariate Cox analysis of the risk score and clinical factors for the independent prognostic significance in STAD.

Figure8a

Figure8b

Figure8c

Model Tests		Discrimination Indexes	
Obs	162	LR chi2	42.41
Events	62	d.f.	13
Center	7.5792	Pr(> chi2)	0.0001
		Score chi2	53.95
		Pr(> chi2)	0.0000
		R2	0.239
		Dxy	0.440
		g	1.263
		gr	3.537

Figure 8

Construction and evaluation of a nomogram for survival prediction of patients with STAD based on risk score and clinical variables. (a) Construction a nomogram prognostic prediction system in STAD. (b) Calibration curve for Nomogram 3-year and 5-year survival prediction. (c) Evaluation of Nomogram prediction model accuracy using C-index.

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