

Protein Phosphatase I Regulatory Subunit 3: A Prognostic Biomarker in Stomach Adenocarcinoma

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Purpose: This study aimed to determine the potential application of the protein phosphatase 1 regulatory subunit 3 (*PPP1R3B*) gene as a prognostic marker in stomach adenocarcinoma (STAD), as well as its potential mediating biological processes and pathways.

Materials and Methods: Differential expression analyses were performed using the TIMER2.0 and UALCAN databases. Complete RNA-seq data and other relevant clinical and survival data were acquired from The Cancer Genome Atlas (TCGA). Univariate survival analyses, Cox regression modelling, and Kaplan–Meier curves were implemented to investigate the associations between *PPP1R3B* gene expression and clinical pathologic features. A genome wide gene set enrichment analysis (GSEA) was conducted to define the underlying molecular mechanisms mediating the observed associations between the *PPP1R3B* gene and STAD development.

Results: We found that *PPP1R3B* was overexpressed in STAD tissues, and that higher *PPP1R3B* expression correlated with worse prognoses in patients with STAD. Comprehensive survival analyses suggested that *PPP1R3B* might be an independent predictive factor for survival time in patients with STAD. The prognostic relationship between *PPP1R3B* and STAD was also verified using Kaplan–Meier curves. Patients with higher *PPP1R3B* levels had a shorter clinical survival time on average. Additionally, a GSEA demonstrated that *PPP1R3B* might be involved in multiple biological processes and pathways.

Conclusion: Our findings demonstrate that the *PPP1R3B* gene has utility as a potential molecular marker for STAD prognoses.

Keywords: *PPP1R3B*, mRNA, stomach adenocarcinoma, prognosis, prognostic biomarkers

Introduction

Gastric cancer (GC) ranks fifth in terms of global cancer incidence and third in terms of cancer-associated mortality.¹ Globally, more than one million new cases of GC are diagnosed each year, and more than 782,685 people die due to GC each year according to annual Global Cancer Statistics Reports.² Stomach adenocarcinoma (STAD) is the most common GC subtype. In recent years, a number of interventions, including early diagnostic technologies, improvements in surgical treatment, and advances in radiotherapy, chemotherapy, targeted drugs, immunotherapy, and other comprehensive treatment methods in clinical practice have significantly improved the clinical management of STAD. However, the overall effects of these interventions remain unsatisfactory, as demonstrated by the high five-year mortality rate for advanced STAD (30–50%).³ Postoperative recurrence and metastases are the primary causes of poor prognoses in patients with STAD. Therefore, it is necessary to conduct detailed studies evaluating the pathogenesis of STAD as well as the factors influencing growth, invasion, and metastasis. This research is of great significance in identifying therapeutic targets as well as improving therapeutic effects and prognoses with regard to STAD.

Recent studies have found that almost all tumour cells synthesise and utilise glycogen. Glycogen metabolism is a common metabolic pathway in tumour cells and is an important marker of malignant tumour cell phenotypes.^{4–8}

Protein phosphatase 1 regulatory subunit 3 (*PPP1R3B*) has been reported to play a role in activating glycogen synthetase (GYS) and in inactivating glycogen phosphorylase in prior studies, thereby resulting in abnormal glycogen metabolism, and

PPP1R3B may be associated with tumour cell growth and development. Nevertheless, the potential relationship between the *PPP1R3B* gene and STAD prognoses remains unclear. Therefore, this investigation was conducted with the aim of exploring the potential prognostic application value and mechanisms of *PPP1R3B* in STAD.

Materials and Methods

Data Mining

TIMER2.0 (<http://timer.cistrome.org/>), a comprehensive and user-friendly web resource for analysing tumour-infiltrating immune cells,^{9–11} was implemented in the current study in order to explore differences in *PPP1R3B* mRNA expression levels between tumour and adjacent normal tissues in The Cancer Genome Atlas (TCGA) program cohorts. First, the “exploration component” was selected. Second, we entered the Gene_DE module. Finally, we input the *PPP1R3B* gene into the search box and obtained the expression distribution of *PPP1R3B* in multiple cancers. UALCAN (<http://ualcan.path.uab.edu/index.html>), a web server for comprehensive and interactive analyses of cancer data,¹² was used to examine *PPP1R3B* expression across all cancer types in the TCGA cohort.

The complete RNA-seq data and relevant clinical and survival data were obtained from the TCGA database (<https://portal.gdc.cancer.gov/>). Raw data were normalised using the DESeq package. This study received ethics approval from the ethics review board of our institution.

Comprehensive Survival Analysis of the *PPP1R3B* Gene

STAD patients were divided into two groups based on the median values of their gene expression profiles. Unpaired t-tests were used to investigate the differential expression of *PPP1R3B* in STAD patients based on age, sex, tumour stage, cancer status, histologic grade, and *Helicobacter pylori* (HP) infection status. We obtained *PPP1R3B* protein immunohistochemical staining in STAD patients from the Human Protein Atlas database (<https://www.proteinatlas.org/>).

Univariate survival analysis was used to investigate the correlation between clinical pathological parameters and overall clinical survival time (OS). Multivariate Cox survival analysis was also performed to identify prognostic factors by adjusting for OS-associated clinical variables. Additionally, clinical pathological parameters were analysed in subgroup analyses in order to investigate the potential application of *PPP1R3B* in STAD patients with specific clinical characteristics. We analysed the combined effects of *PPP1R3B* and several clinical parameters, including histologic grade, radiation therapy, radical resection, targeted molecular therapy, and tumour stage.

We then plotted a survival risk map and conducted a time-dependent receiver operating characteristic (ROC) curve analysis with R statistical software (The R Project for Statistical Computing, Vienna, Austria) using survival receiver operating characteristic curve (ROC) evaluations (<https://cran.rproject.org/web/packages/survivalROC/index.html>)¹³ to test the prediction effectiveness of *PPP1R3B*. Finally, the prognostic relationships between the *PPP1R3B* gene and multiple clinical parameters in STAD patients were verified using the Kaplan-Meier plotter (KM plotter) (<http://kmplot.com/private>) database.

Genome-Wide Co-Expression of *PPP1R3B*

The corrpilot software package in R was used for genome-wide co-expression analyses. P values of <0.05, and associated $|r|$ values of >0.2 were deemed statistically significant. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were then carried out using the Metascape database (<http://metascape.org/>)¹⁴ to explore the biological and molecular mechanisms and pathways associated with the *PPP1R3B* gene.

GSEA Analyses

The GSEA (<http://www.broadinstitute.org/gsea/index.jsp>)^{15,16} applies the c2 (v6.2) and c5 (v6.2)¹⁷ categories in gene set analyses to uncover the potential molecular mechanisms and pathways in patients with different *PPP1R3B* expression levels. The enrichment results were considered statistically significant based on the following criteria: a normalised enrichment score (NES) of >1, a P-value of <0.05, and a false discovery rate (FDR) of <0.25.

Statistical Analyses

Hazard ratio (HR) analyses generating effect estimates with 95% confidence intervals (CI) were performed to describe relative risks between STAD groups with differential expression levels of *PPP1R3B*. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 23.0; IBM Corp., Armonk, NY, USA) and R statistical software (version 3.5.1). Statistical significance was set at a P-value of <0.05.

Results

Data Mining and Differential Expression Analyses

Differences in *PPP1R3B* expression between tumour and normal tissues arising from patients with different cancer types were analysed using the TIMER2.0 database. As a result, we observed upregulation in GBM, KIRC, LUAD, PCPG, STAD, and THCA expression, with reduced expression in BLCA, BRCA, CESC, CHOL, COAD, HNSC, KICH, LIHC, PRAD, READ, and UCEC. However, no statistically significant differences were observed between tumour and normal

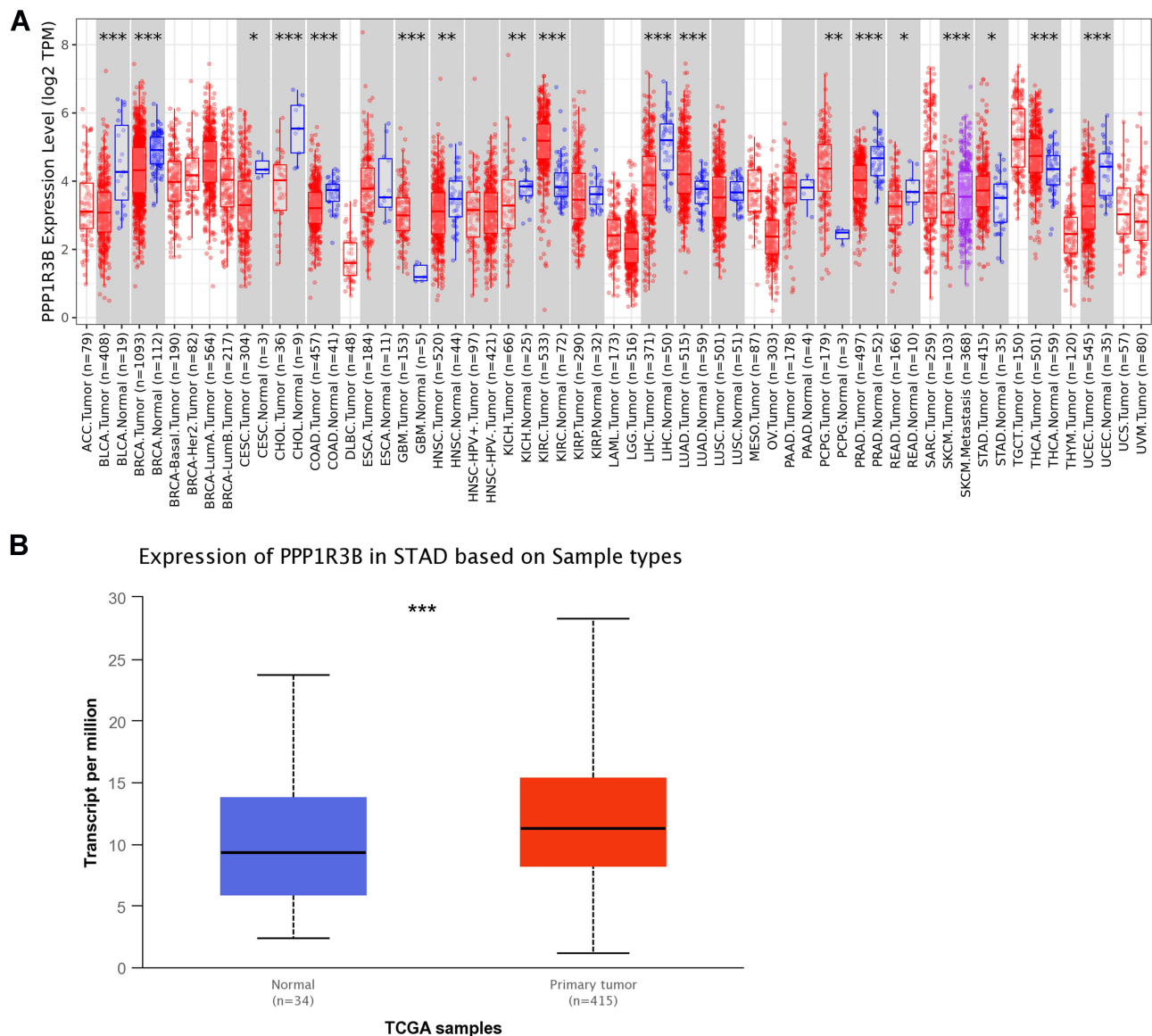


Figure 1 *PPP1R3B* was overexpressed in STAD tissues.

Notes: *PPP1R3B* mRNA expression levels in different cancer samples were explored using the Oncomine (A) and UALCAN databases (B). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

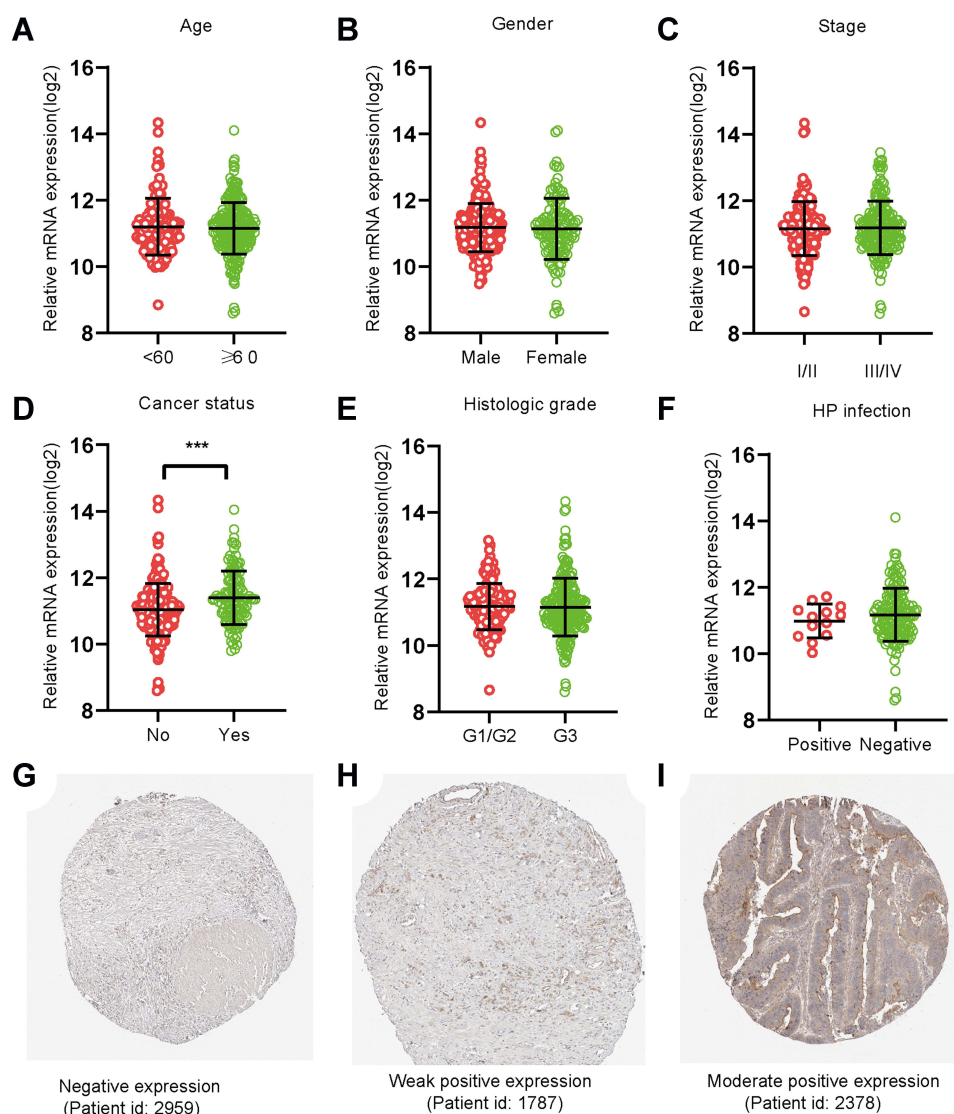


Figure 2 *PPP1R3B* expression in STAD tissues. (A–F) Expression of *PPP1R3B* in STAD in terms of clinical variables. (G–I) Images of immunohistochemical staining showing *PPP1R3B* protein expression in STAD from The Human Protein Atlas. *** $P < 0.001$.

tissues in patients with ESCA, KIRP, LUSC, and PAAD (Figure 1A). These common tumour types are summarised in [Supplementary Material 1 \(S1\)](#). In addition, UALCAN was used to analyse *PPP1R3B* expression in STAD and adjacent normal tissues (Figure 1B). We found that *PPP1R3B* expression levels were overexpressed in STAD.

Following evaluations of *PPP1R3B* expression (mRNA) via the Log2 function, 351 cases of STAD and 32 cases providing normal adjacent tissues were included in this experiment for additional analyses. *PPP1R3B* expression was found to be upregulated in patients with STAD tumours ($P < 0.001$; Figure 2D). However, the expression of *PPP1R3B* was not statistically significantly associated with the other evaluated clinical variables (Figure 2A–C, E and F). Images of immunohistochemical staining showing *PPP1R3B* protein expression in STAD are provided in Figure 2G–I.

Survival Analyses

The clinical characteristics of the enrolled patients are outlined in Table 1. Clinicopathologic parameters such as age, TNM tumour stage, cancer status, and information on radiation and targeted therapies were statistically significantly

Table I Correlation Between OS and Clinicopathologic Features of GC Patients

Variables	Events/Total (n=351)	MST (Months)	HR (95% CI)	Log-Rank P-value
PPPIR3B	144/351	29		<0.001
Low	57/175	60	1	
High	87/176	22	2.012(1.431–2.828)	
Age (years)	144/348	29		0.022
<60	36/108	60	1	
≥60	108/240	26	1.549(1.061–2.263)	
Missing	3			
Gender	144/351	29		0.178
Male	100/226	29	1	
Female	44/125	35	0.784(0.551–1.118)	
Missing	0			
Tumor location	138/337	29		0.919
Cardia	36/84	26	1	
Body	50/123	28	0.916(0.596–1.407)	
Antrum	52/130	35	0.936(0.611–1.432)	
Missing	14			
HP infection	66/161	43		0.304
Positive	6/18	58	1	
Negative	60/143	43	1.552(0.667–3.614)	
Missing	190			
Histologic type	144/350	29		0.057
Intestinal	64/160	38	1	
Diffuse	24/61	60	1.001(0.625–1.603)	
Signet ring cell	8/11	13	2.515(1.204–5.251)	
Others	48/118	26	1.287(0.884–1.875)	
Missing	1			
Histologic grade	140/342	29		0.169
G1	2/9	NA	1	
G2	48/127	43	1.666(0.405–6.862)	
G3	90/206	26	2.217(0.546–9.008)	
Missing	9			
MSI status	144/350	29		0.225
MSI-H	99/240	28	1	
MSI-L	22/51	29	1.263(0.794–2.007)	
MMS	23/59	35	0.756(0.481–1.191)	
Missing	1			
Pathological M	138/336	29		0.012
M1	13/23	12	1	
M0	125/313	35	0.486(0.274–0.861)	
Missing	15			
Pathological N	139/341	29		0.004
N0	28/103	60	1	
N+	111/238	25	1.826(1.206–2.764)	
Missing	10			
Pathological T	140/347	31		0.009
T1/T2	28/91	70	1	
T3/T4	112/256	26	1.730(1.138–2.269)	
Missing	4			

(Continued)

Table 1 (Continued).

Variables	Events/Total (n=351)	MST (Months)	HR (95% CI)	Log-Rank P-value
TNM stage	136/338	29		<0.001
Stage I	11/47	73	1	
Stage II	34/109	56	1.608(0.813–3.182)	
Stage III	69/147	26	2.435(1.286–4.610)	
Stage IV	22/35	16	3.789(1.836–7.819)	
Missing	13			
Cancer status	121/324	37		<0.001
No	35/206	NA	1	
Yes	86/118	17	5.526(3.695–8.264)	
Missing	27			
Radiation therapy	135/328	31		0.001
Yes	19/62	NA	1	
No	116/266	26	2.322(1.418–3.802)	
Missing	23			
Targeted therapy	134/326	31		0.022
Yes	56/151	43	1	
No	78/175	26	1.489(1.055–2.100)	
Missing	25			

Abbreviations: *PPP1R3B*, protein phosphatase 1 regulatory subunit 3B; HR, hazard ratio; MST, median survival time; OS, overall survival; GC, gastric cancer; G1, highly differentiated; G2, moderately differentiated; G3, poorly differentiated; MSI-H, microsatellite instability-high; MSI-L, Microsatellite instability-low.

associated with survival time in patients with STAD. After adjusting for these clinical characteristics, multivariate Cox regression demonstrated that the upregulation of the *PPP1R3B* gene was associated with poor prognoses in the 351 enrolled STAD patients (high *PPP1R3B* vs low *PPP1R3B*; 22 months vs 60 months), as well as with a higher mortality rate (Crude $P < 0.001$, Crude HR [95% CI] = 2.012 [1.431–2.828], Table 1, Figure 3B; adjusted $P = 0.005$, adjusted HR [95% CI] = 1.792 [1.193–2.692], Table 2). The results of survival risk mapping of *PPP1R3B* expression in low-and high-risk groups are shown in Figure 3A. The areas under the curve (AUC) for one-year, two-year, three-year, four-year, and five-year survival were 0.568, 0.620, 0.694, 0.680, and 0.649, Figure 3C, respectively.

Table 2 Multivariate Analyses of the *PPP1R3B* in the Prediction of Gastric Cancer Overall Survival

Variables	HR (95% CI)	P-value
<i>PPP1R3B</i> (high vs low)	1.792(1.193–2.692)	0.005
Age (years) (≥ 60 vs < 60)	1.574 (1.023–2.422)	0.039
Pathological M (M0 vs M1)	1.168 (0.418–3.266)	0.767
Pathological T (T3/4 vs T1/2)	1.454 (0.743–2.847)	0.275
Pathological N (N+ vs N0)	1.889 (0.885–4.031)	0.100
TNM stage		
Stage II vs Stage I	1.400 (0.453–4.328)	0.559
Stage III vs Stage I	1.142 (0.286–4.568)	0.851
Stage IV vs Stage I	1.574 (0.359–6.903)	0.548
Radiation therapy (No vs Yes)	1.215 (0.637–2.319)	0.554
Targeted molecular therapy (No vs Yes)	1.667 (1.049–2.648)	0.030
Cancer status (Yes vs No)	5.319 (3.320–8.523)	<0.001

Abbreviations: *PPP1R3B*, protein phosphatase 1 regulatory subunit 3B; HR, hazard ratio; CI, confidence interval; T, tumor; N, node; M, metastasis.

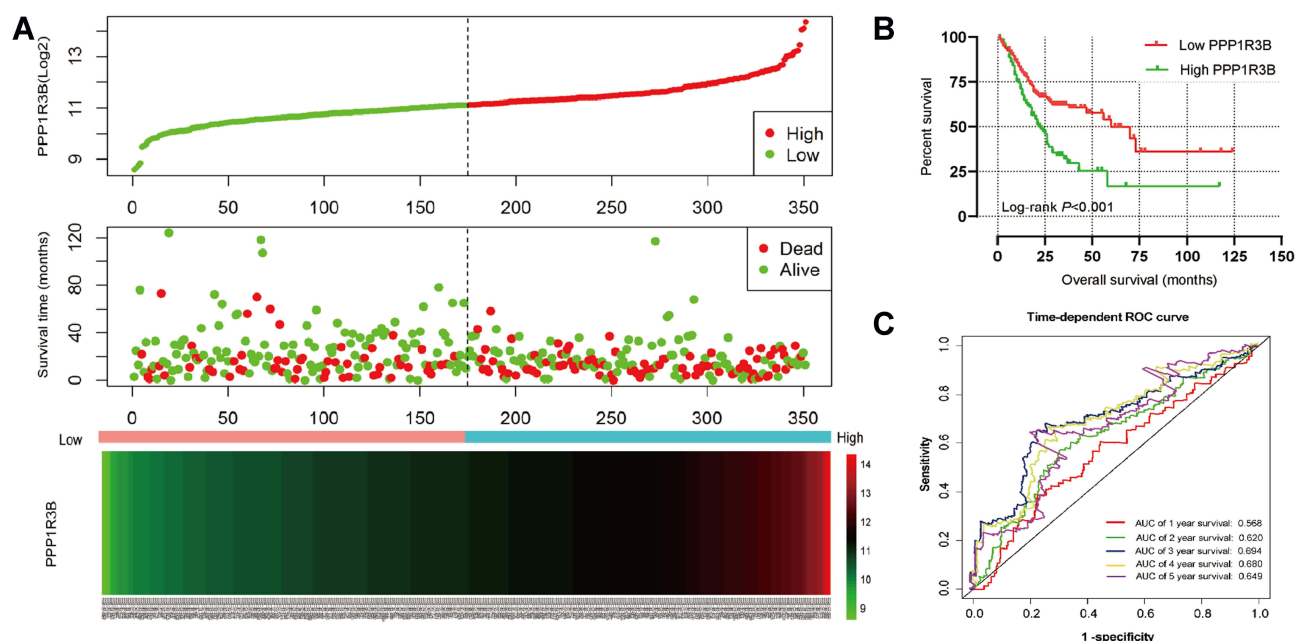


Figure 3 Prognostic model of *PPP1R3B* in patients with STAD.

Notes: (A) From up to down are risk score plot, survival status scatter plot, and heat map of the expression of *PPP1R3B* for low-and high-risk groups. (B) Kaplan–Meier survival curves for *PPP1R3B* gene in STAD of TCGA cohort. (C) Receiver operating characteristic curve for predicting overall survival in STAD patients by the *PPP1R3B*.

Additionally, stratified analyses indicated that overexpression of the *PPP1R3B* gene increased the mortality rate in STAD patients, with the exception of Stage I and cancer-free patients (Table 3). Co-expression analyses also showed that *PPP1R3B* expression combined with specific clinical variables performed better than any single clinical parameter in terms of predicting STAD survival time (Figure 4A–E and Table 4).

KM-Plotter Survival Analyses

Finally, we used the KM-plotter database to verify the prognostic relationships between *PPP1R3B* and STAD outcomes. We witnessed worse OS rates with increasing *PPP1R3B* expression levels in all STAD patients, as outlined in Figure 5A and Table 5 (HR [95% CI] = 1.74 [1.4 – 2.17], $P = 5e-07$). We found that *PPP1R3B* expression could predict prognoses in subjects with Stage III (HR [95% CI] = 1.96 [1.34 – 2.86], $P = 0.00044$, Figure 5D), HER2 negative (HR [95% CI] = 1.62 [1.24 – 2.12], $P = 0.00036$, Figure 5G), 5-FU treatment (HR [95% CI] = 3.94 [1.39 – 11.17], $P = 0.0058$, Figure 5H), and intestinal (HR [95% CI] = 2.51 (1.72 – 3.68), $P = 9.3e-07$, Figure 5J) presentations. Nevertheless, no statistically significant differences were observed with respect to other clinical variables, including Stage I, II, and IV presentations (Figure 5B, C and E), HER2 positive status (Figure 5F), surgery (Figure 5I), diffuse type cancers (Figure 5K), mixed type cancers (Figure 5L), and differentiation (Figure 5M–O).

Genome-Wide Co-Expression Analyses

The GO analysis demonstrated that the *PPP1R3B* gene was linked to transferase activity, mRNA 5'-UTR, folic acid and chromatin binding, extracellular matrix (ECM)-receptor interactions, organic acid transmembrane transporter activity, signalling pathways, regulatory pathways with respect to the pluripotency of stem cells, glycosaminoglycan biosynthesis, and amino and nucleotide sugar metabolism (Figure 6A). These results were consistent with the findings obtained in the KEGG analysis (Figure 6B).

Table 3 Stratified Analysis of *PPP1R3B* Gene and OS in GC Patients

Group	Low	High	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
Age(years)						
<60	55	53	2.701(1.335–5.465)	0.006	5.000(2.012–12.425)	0.001
≥60	118	122	1.711(1.161–2.521)	0.007	2.029(1.324–3.109)	0.001
Pathological T						
T 1/2	40	51	3.012(1.224–7.413)	0.016	3.175(1.004–10.040)	0.049
T 3/4	134	122	1.918(1.313–2.800)	0.001	2.062(1.372–3.098)	0.001
Pathological N						
N0	51	52	1.881(0.859–4.118)	0.114	2.371(1.007–5.583)	0.048
N+	122	116	1.978(1.345–2.909)	0.001	2.063(1.356–3.139)	0.001
Pathological M						
M1	12	11	1.976(0.626–6.234)	0.245	12.766(1.719–94.803)	0.013
M0	158	155	1.950(1.356–2.803)	<0.001	2.051(1.390–3.030)	<0.001
TNM stage						
Stage I	19	28	1.886(0.496–7.172)	0.352	20.000(0.791–505.604)	0.069
Stage II	59	50	2.258(1.109–4.597)	0.025	2.217(1.079–4.556)	0.030
Stage III	73	74	1.986(1.220–3.232)	0.006	2.025(1.205–3.403)	0.008
Stage IV	18	17	1.987(0.814–4.848)	0.131	3.799(1.064–13.572)	0.040
Radiation therapy						
Yes	39	23	4.406(1.709–11.358)	0.002	4.078(1.369–12.147)	0.012
No	125	141	1.725(1.177–2.528)	0.005	2.037(1.352–3.070)	0.001
Targeted therapy						
Yes	74	77	3.912(2.160–7.084)	<0.001	3.573(1.903–6.708)	<0.001
No	88	87	1.408(0.894–2.217)	0.140	1.694(1.024–2.804)	0.040
Cancer status						
No	120	86	1.641(0.840–3.205)	0.147	1.995(0.967–4.116)	0.062
Yes	45	73	1.681(1.052–2.685)	0.030	1.873(1.144–3.066)	0.013

Notes: ^aAdjusted for age, TNM stage, radiation therapy and targeted molecular therapy; The missing patients of these clinical parameters are the same as Table 1.

Abbreviations: *PPP1R3B*, protein phosphatase 1 regulatory subunit 3B; HR, hazard ratio; OS, overall survival; GC, gastric cancer.

GSEA

The GSEA data indicated that in the c2 category, high *PPP1R3B* expression levels may be associated with hypoxia and with hypoxia-inducible factor-1 (HIF1A) and SRY-box transcription factor 4 (SOX4) expression (Figure 7A-C). In the c5 category, downregulation of *PPP1R3B* expression may be linked with cell-cell adhesion, T cell differentiation, homeostasis and receptor signalling pathways, antigen receptor-mediated signalling pathways, and lipopolysaccharide-mediated signalling pathways (Figure 7D-I).

Discussion

The loss of alleles in 8p11.2–12, 8p21–22, and 8p23.1 in human chromosome 8 are characteristic of oesophageal,¹⁸ colorectal,¹⁹ and prostate cancers, suggesting the presence of multiple tumour suppressor genes (TSG) on the short arm of chromosome 8. Previous studies have shown that 8p loss also occurs in STAD.²⁰ The *PPP1R3B* gene is located on chromosome 8p23.1.²¹ However, to our knowledge, no study whatsoever has explored the importance of *PPP1R3B* in cancer processes and prognoses. Our investigation showed that the overexpression of *PPP1R3B* was associated with a shorter survival time and with higher mortality rates in STAD patients. Therefore, *PPP1R3B* may be considered an oncogene in STAD.

The evaluated *PPP1R3B* gene is a serine/threonine kinase that regulates glycogen synthesis in the liver and in skeletal muscles.^{22,23} This gene has previously been shown to be actively involved in the development of Alzheimer's disease,²⁴

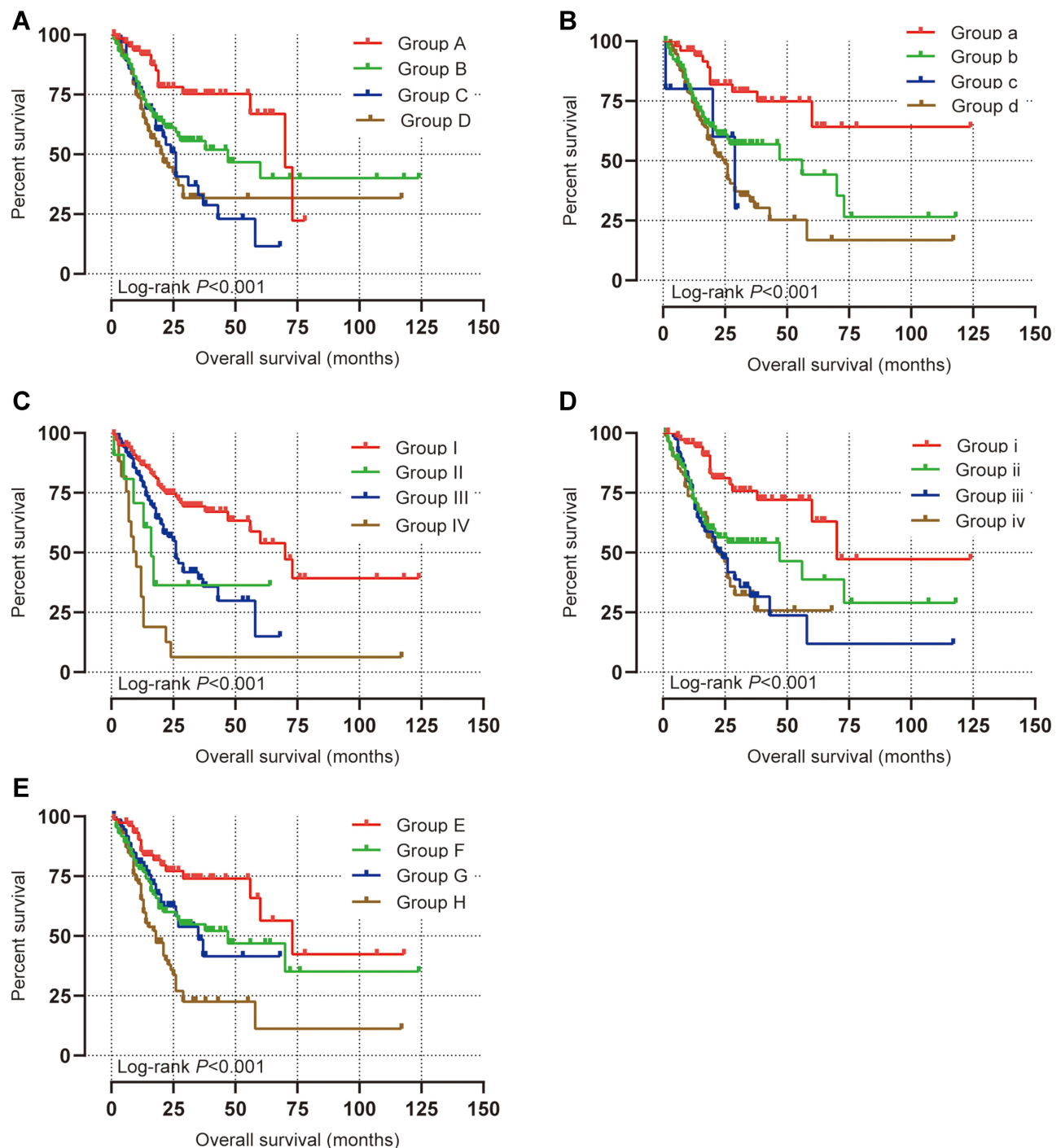


Figure 4 Joint effects analysis of overall survival stratified by *PPP1R3B* and STAD clinical parameters.

Notes: Joint effects analysis stratified by *PPP1R3B* and following clinical parameters: histologic grade (A), radiation therapy (B), radical resection (C), targeted molecular therapy (D), stage (E). Group A, B, a, b, I, II, I, ii, E, F *PPP1R3B* Low expression. Group C, D, c, d, III, IV, iii, iv, G, H *PPP1R3B* High expression.

hepatic steatosis,²⁵ and lipid levels in patients of European origin.²⁶ Cancer is a metabolic disease, and metabolic disorders tend to play an integral role in the occurrence and development of a variety of ailments.²⁷ Hanahan et al highlighted metabolic changes as one of the ten characteristics of malignant tumours.²⁸ A defect in lipid metabolism, for instance, may affect the proliferation and differentiation of tumour cells and may accelerate the occurrence and development of a range of cancer types.²⁹ In fact, a decrease in choline and cholesterol levels in the serum and tissues

Table 4 Joint Effects Survival Analysis of Clinical Factors and the *PPP1R3B* Expression with OS

Group	<i>PPP1R3B</i>	Variables	Events/ Total	MST (Months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
Histologic grade								
A	Low expression	G1 + G2	15/65	70	1		1	1
B	Low expression	G3 + G4	41/108	47	2.069(1.144–3.742)	0.016	1.647(0.798–3.397)	0.177
C	High expression	G1 + G2	35/71	24	3.139(1.706–5.774)	<0.001	3.409(1.639–7.090)	0.001
D	High expression	G3 + G4	49/98	20	3.402(1.894–6.108)	<0.001	3.277(1.610–6.667)	0.001
Radiation therapy								
A	Low expression	Yes	7/39	NA	1	1		1
B	Low expression	No	46/125	56	3.429(1.541–7.629)	0.003	3.233(1.236–8.458)	0.017
C	High expression	Yes	12/23	43	4.149(1.630–10.564)	0.003	4.456(1.592–12.473)	0.004
D	High expression	No	70/141	21	5.945(2.693–13.126)	<0.001	6.831(2.724–17.130)	<0.001
Radical resection								
I	Low expression	R0	39/144	70	1	1		1
II	Low expression	R1+R2	6/11	16	2.975(1.256–7.048)	0.013	2.879(1.061–7.814)	0.038
III	High expression	R0	61/144	26	2.123(1.410–3.197)	<0.001	2.571(1.641–4.029)	<0.001
IV	High expression	R1+R2	15/17	10	6.355(3.468–11.644)	<0.001	4.379(2.016–9.511)	<0.001
Targeted molecular therapy								
I	Low expression	Yes	16/74	70	1	1		1
II	Low expression	No	36/88	47	2.564(1.421–4.627)	0.002	1.859(0.864–4.002)	0.113
III	High expression	Yes	40/77	22	3.605(2.008–6.472)	<0.001	3.381(1.702–6.717)	0.001
IV	High expression	No	42/87	23	3.791(2.115–6.795)	<0.001	3.564(1.669–7.612)	0.001
Stage								
E	Low expression	I + II	18/78	73		1		1
F	Low expression	III+IV	37/91	47	1.878(1.068–3.300)	0.029	2.858(1.373–5.950)	0.005
G	High expression	I + II	27/78	35	1.946(1.066–3.553)	0.030	3.047(1.432–6.458)	0.004
H	High expression	III+IV	54/91	18	3.813(2.226–6.531)	<0.001	5.965(2.895–12.290)	<0.001

Note: ^aAdjusted for histologic grade, radiation therapy, radical resection, targeted molecular therapy and stage.

Abbreviations: *PPP1R3B*, protein phosphatase 1 regulatory subunit 3B; GC, gastric cancer; HR, hazard ratio; MST, median survival time; OS, overall survival; CI, confidence interval.

Table 5 The Prognostic Value of the mRNA Expression of *PPP1R3B* in Various Clinical Features from KM-Plotter

Group	Low	High	HR (95% CI)	P-value
All	317	314	1.74 (1.4 – 2.17)	5e–07
Histology				
Intestinal	134	135	2.51 (1.72 – 3.68)	9.3e–07
Diffuse	120	120	1.28 (0.91 – 1.8)	0.16
Mixed	14	15	0.43 (0.14 – 1.32)	0.13
TNM stage				
Stage I	31	31	2.25 (0.67 – 7.51)	0.18
Stage II	69	66	1.85 (0.97 – 3.52)	0.058
Stage III	99	98	1.96 (1.34 – 2.86)	0.00044
Stage IV	70	70	1.33 (0.89 – 1.98)	0.16
Treatment				
Surgery	190	190	1.14 (0.86 – 1.52)	0.37
5-FU	17	17	3.94 (1.39 – 11.17)	0.0058
HER2 status				
Negative	215	214	1.62 (1.24 – 2.12)	0.00036
Positive	101	101	1.31 (0.9 – 1.91)	0.15
Differentiation				
Poorly	60	61	1.27 (0.78 – 2.05)	0.34
Moderately	34	33	0.84 (0.44 – 1.6)	0.59
Well	2	3	NA	0.41

Abbreviations: *PPP1R3B*, protein phosphatase 1 regulatory subunit 3B; HR, hazard ratio; OS, overall survival; GC, gastric cancer; T, tumour; N, node; M, metastasis.

of patients with STAD can be employed as a biomarker for the early diagnosis of STAD.^{30–32} These findings highlight the importance of defects in lipid metabolism and in cell membrane biosynthesis with respect to cancer progression.

In addition, *PPP1R3B* has been reported to play a role in activating glycogen synthetase (GYS) and in inactivating glycogen phosphorylase, thus resulting in abnormal glycogen metabolism. GYS is a key and rate-limiting enzyme in glycogen synthesis.³³ Hypoxia inducible factor-1 (HIF-1) increases GYS levels in tumour cells, upregulates glycogen production, and causes cells to be more tolerant to hypoxia and nutritional deficiency. This suggests that glycogen synthesis triggered by the expression of GYS plays a crucial role in the stressful growth environment found within tumour cells.^{34,35} Glycogen metabolism is an important process for energy generation in tumor cells,^{6,36} and previous studies have shown glycogen accumulation to be a characteristic feature of many tumour cells and tissues.^{37,38} In addition, glycogen is involved in regulating the proliferation of tumour cells³⁹ and may therefore be an important signalling molecule. For example, a high concentration of glycogen can activate AMP-activated protein kinase (AMPK), and the continuous activation of AMPK may cause cell aging and inhibit multiplication. Skwarski et al⁴⁰ reported that the risk of STAD recurrence increased with prolonged operation time, which may be associated with glycogen storage during surgery. We note that it is possible to artificially reduce the amount of glycogen, thereby inhibiting the growth of cancer by maintaining the cell cycle at the G1 stop or G0 states.

GSEA analyses indicated that *PPP1R3B* may be associated with hypoxia, HIF1A and SOX4 expression, T cell receptor signalling pathways, cell differentiation, and T cell homeostasis, lipopolysaccharide-mediated signalling pathways, and antigen receptor-mediated signalling pathways. Genome-wide co-expression analyses in the current study indicated that the *PPP1R3B* gene may play an important role in metabolic processes and pathways.

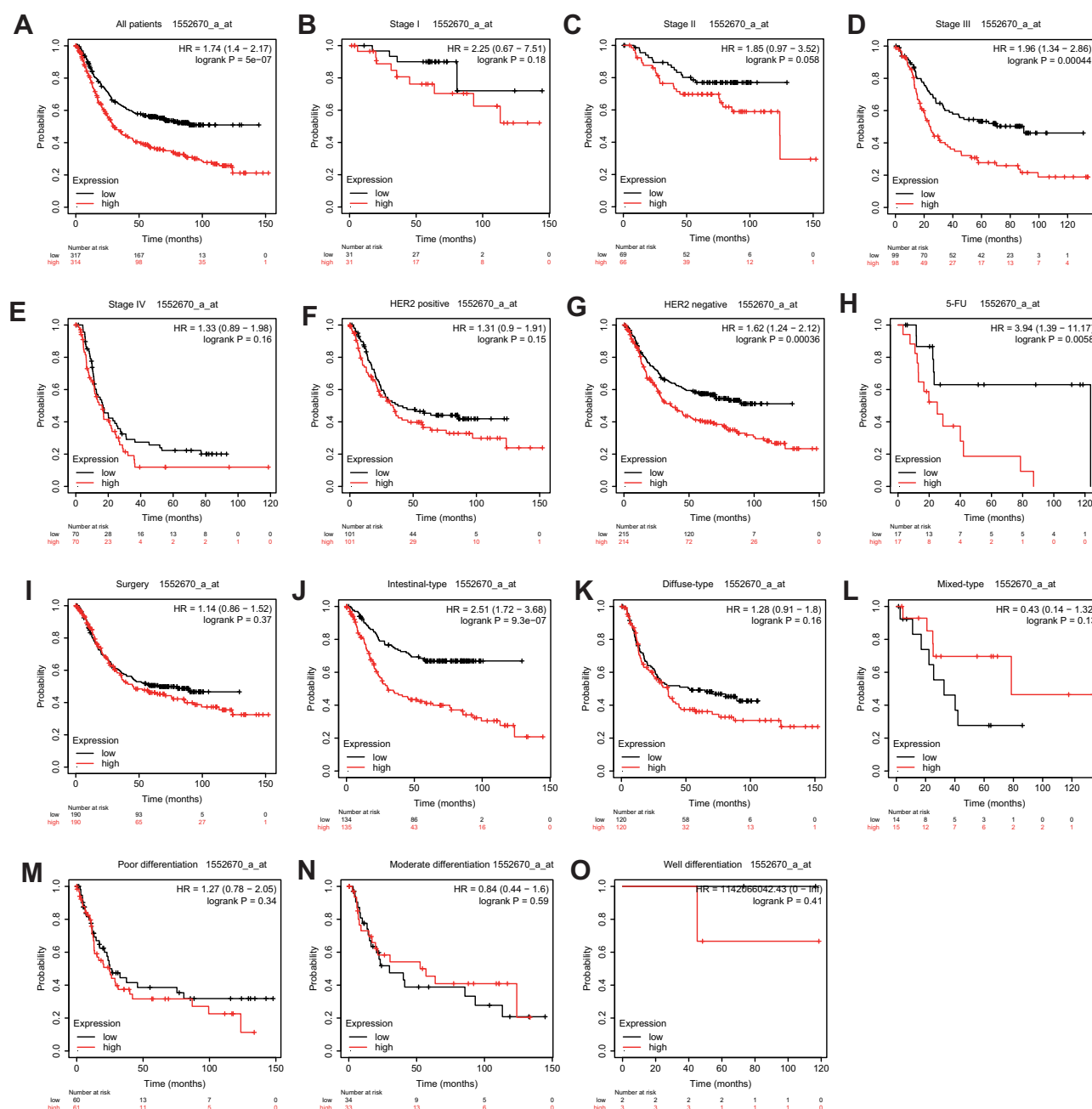
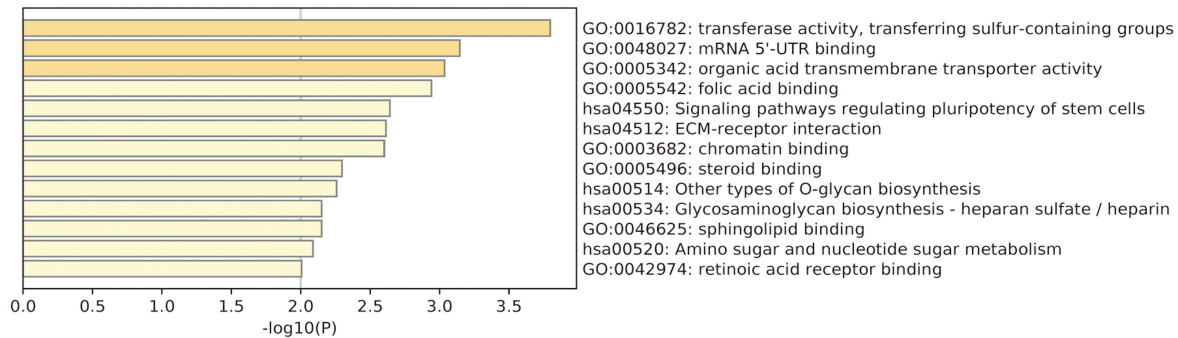
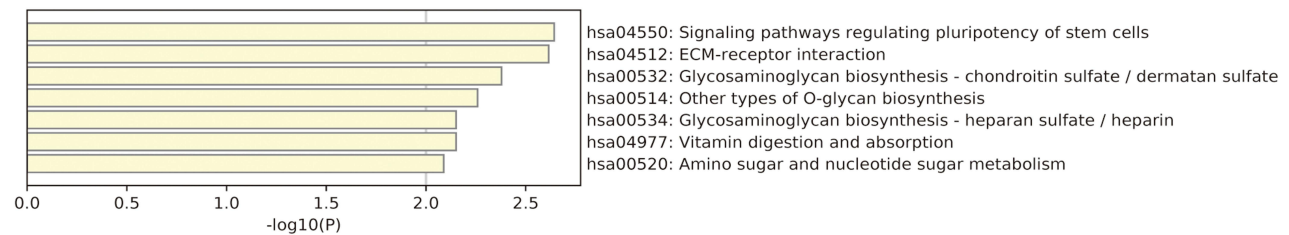


Figure 5 Prognostic value of *PPP1R3B* (1552670_a_at) expression determined by Kaplan-Meier plotter database.

Notes: Overall survival curves for all patients (**A**), stage I (**B**), stage II (**C**), stage III (**D**), stage IV (**E**), HER2 positive (**F**), HER2 negative (**G**), 5-FU treatment (**H**), surgery treatment (**I**), intestinal-type (**J**), diffuse-type (**K**), mixed-type (**L**), poor differentiation (**M**), moderate differentiation (**N**), well differentiation (**O**).

Studies have likewise shown that *SOX4* expression is higher in gastric tumour tissues than in adjacent normal tissues. The high expression of *SOX4* in gastric tumour tissues is linked to tumour development and metastasis.⁴¹ T cell immunity is an important component of the human immune system, and an effective T cell response is necessary for the sufficient control of viral infections and tumour growth.⁴² Therefore, T cell immunity plays a significant role in many cancers, including, lung cancer,⁴³ colorectal cancer,⁴⁴ breast cancer⁴⁵ and ovarian cancer.⁴⁶ CD4⁺ T cells and CD8⁺

A Go term enrichment**B** KEGG enrichment**Figure 6** GO and KEGG analysis of genes co-expressed with *PPP1R3B*.**Notes:** (A) GO enrichment analysis. (B) KEGG enrichment analysis.**Abbreviations:** *PPP1R3B*, protein phosphatase 1 regulatory subunit 3. GO, Gene Ontology. KEGG, Kyoto Encyclopedia of Genes and Genomes.

T cells, which influence the progression and prognosis of STAD, are important cell types in T-cell immunity as well.^{47,48} Some studies have shown that beta-catenin/T cytokine-mediated transcription (ie, classical Wnt signalling) may lead to chromosomal instability (CIN),⁴⁹ leading to the development of STAD.⁵⁰ Our study suggests that the inhibition of T cell differentiation, homeostasis, and associated signalling pathways may affect STAD prognoses.

Despite these important and novel findings and the overall substantial strengths of our investigation, our study has several limitations. First, the evaluated clinical information was acquired only from the TCGA database. Hence, future studies are needed to comprehensively validate and confirm our findings within study multiple databases. Second, in the TCGA database, expression data was only available for 32 adjacent tissues. Therefore, the insufficient sample size might lead to an underrepresentation of the outcomes of survival analyses. Third, the TCGA is an open database; hence, the underlying molecular mechanisms through which *PPP1R3B* affects the occurrence and prognosis of STAD should be further investigated.

Nevertheless, to the best of our knowledge, ours is the first study to explore the prognostic value of *PPP1R3B* in gastric adenocarcinoma. Comprehensive survival analyses suggest that *PPP1R3B* may be a potential independent prognostic determinant of OS in STAD patients. In the current study, GSEA was used to reveal cancer-associated biological processes and pathways. Once these findings are validated, we expect that *PPP1R3B* will be used in the diagnosis and treatment of STAD in clinical practice.

Conclusions

To the best of our knowledge, no study whatsoever has explored the importance of *PPP1R3B* in cancer progression and prognoses. Hence, ours is the first study to explore the prognostic value of *PPP1R3B* in any cancer. We focused on gastric adenocarcinoma specifically. Our results demonstrate that the *PPP1R3B* gene may be an independent predictive

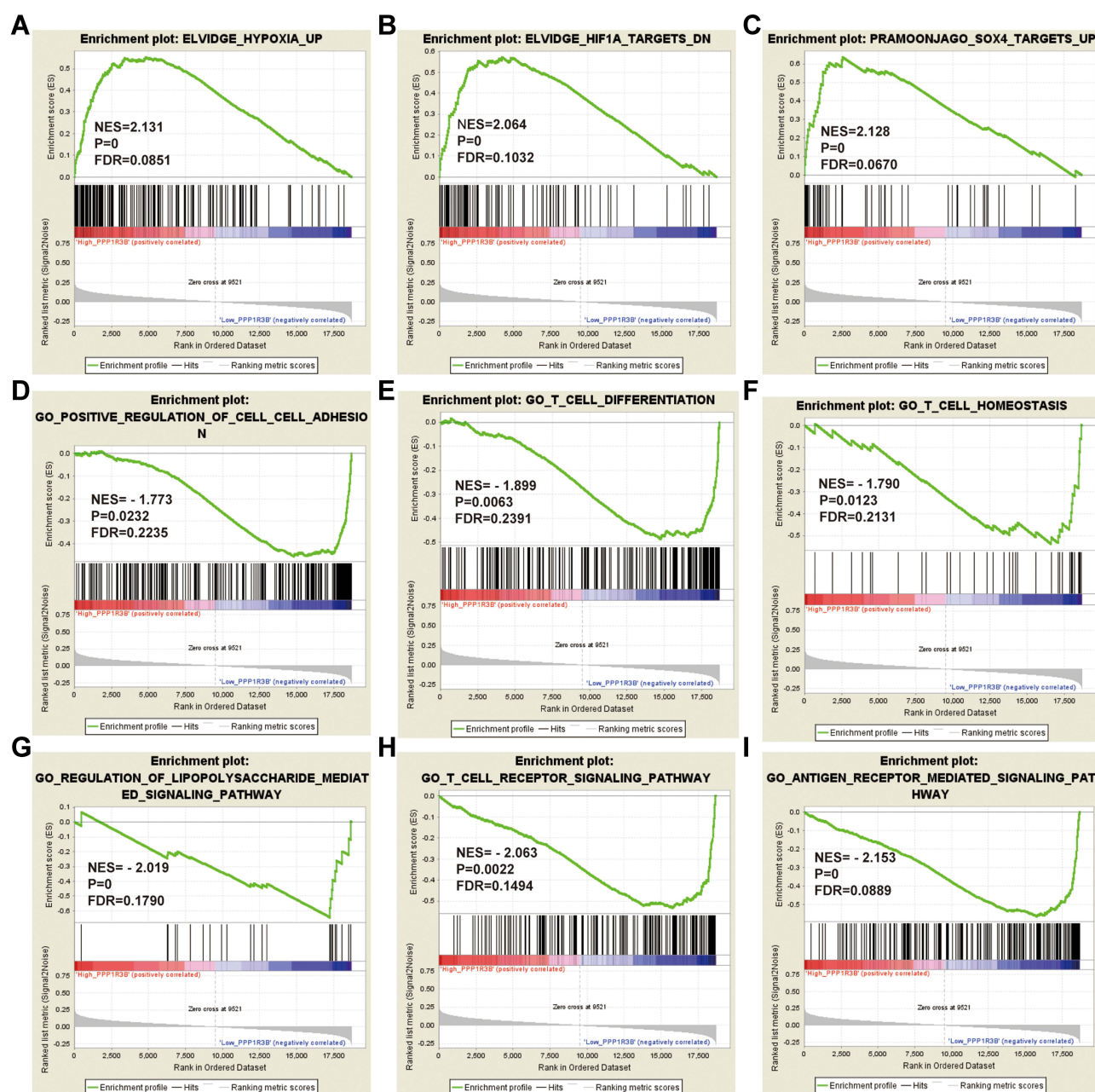


Figure 7 GSEA analysis of *PPP1R3B* in STAD patients using the TCGA database.

Notes: (A–C) GSEA results of C2 reference gene sets for high *PPP1R3B* expression groups; (D–I) GSEA results of C5 reference gene sets for low *PPP1R3B* expression groups.

Abbreviations: NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; *PPP1R3B*, protein phosphatase 1 regulatory subunit 3.

marker for STAD. However, additional highly-powered studies are needed to comprehensively validate these findings in the future. Our results guide future research directions and medical guidelines.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Our study has been approved by the Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest relevant to this work.

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