Identification of Protein Phosphatase 1 Regulatory Subunit 3 as a Prognostic Biomarker in Patients with Gastric Cancer

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Abstract

Background: The study aimed to determine: (1) The potential application of the protein phosphatase 1 regulatory subunit 3 (*PPP1R3B*) gene as a prognostic marker in gastric cancer (GC) and (2) The possible role of *PPP1R3B* in biological processes and pathways.

Methods: The complete RNA-sequencing (RNA-seq) data and other relevant clinical and survival information was acquired from The Cancer Genome Atlas (TCGA). The univariate survival analysis with Cox regression model and Kaplan-Meier analysis was used to investigate the association between clinical pathologic features and *PPP1R3B* gene expression. A genome-wide gene set enrichment analysis (GSEA) was also conducted to define the underlying molecular mechanism of the *PPP1R3B* gene in GC development.

Results: The Log rank test and Cox regression identified the prognostic application of *PPP1R3B* expression in GC patients. Comprehensive survival analysis suggested that *PPP1R3B* might be an independent predictive factor for the survival time in patients with GC. The prognostic relationship between *PPP1R3B* and GC was also verified by the Kaplan–Meier plotter (KM plotter). Patients with a high expression of *PPP1R3B* were associated with a shorter clinical survival time. Additionally, GSEA demonstrated that *PPP1R3B* might be involved in multiple biological processes and pathways.

Conclusions: Our findings demonstrated that the *PPP1R3B* gene could be used as a potential molecular marker for GC prognosis.

Introduction

Gastric cancer (GC) accounted for approximately 5.7% of the new cancer cases reported in 2018 alone. This makes it the fifth most commonly diagnosed cancer according to the Global Cancer Statistics Report. However, GC related deaths accounted for about 8.2%, ranking it the third leading cause of cancer related mortality worldwide (1). In recent years, interventions including: early diagnostic technologies, improvement in surgical treatment, radiotherapy, chemotherapy, targeted drugs and immunotherapy as well as other comprehensive treatment methods in clinical practice, have significantly improved the management of GC. However, the overall effect of these interventions remains unsatisfactory since the 5-year mortality rate of advanced GC is still considerably high at 30%-50% (2). Postoperative recurrence and metastasis are still the primary causes of poor prognosis in GC patients. Therefore, it is necessary to study in detail the pathogenesis and factors influencing the growth, invasion and metastasis of GC. This is of great significance in finding therapeutic targets and improving the therapeutic effect and prognosis of GC.

Recent studies have found that almost all tumor cells are able to synthesize and utilize glycogen. Furthermore, glycogen metabolism is a common metabolic pathway in tumor cells and is one of the important markers of the malignant phenotype in tumor cells (3–7). Protein phosphatase 1 regulatory subunit 3 (*PPP1R3B*), which regulates liver glycogen metabolism, may be associated with tumor progression. Therefore, this investigation aimed to explore the potential prognostic application of *PPP1R3B* in GC patients.

Methods

Data mining

The complete RNA-seq data and relevant clinical and survival information was obtained from the TCGA database (<https://portal.gdc.cancer.gov/>; accessed May 15, 2019). The raw data was normalized using the DESeq package. A genome-wide dataset on TCGA is freely available to the public and was used according to the publication guidelines

and access policies of the database. Therefore, our investigation did not require further approval from an ethical review committee.

Comprehensive survival analysis of *PPP1R3B* gene

The GC patients were divided into two groups according to the median values for the gene expression profile. Unpaired t-test was used to investigate the differential expression of *PPP1R3B* in GC patients based on age, gender, stage, cancer status, histologic grade and *Helicobacter pylori* (HP) infection.

The univariate survival analysis was used to investigate the correlation between the clinical pathological parameters and clinical overall survival time (OS). Multivariate Cox survival analysis was also performed to identify the prognostic factors by adjusting for the OS-related clinical variables. Additionally, the clinical pathological parameters were analyzed by subgroup analysis to further investigate the potential application of *PPP1R3B* in gastric carcinoma patients with specific clinical characteristics. Furthermore, we analyzed the combined effect of *PPP1R3B* and a few clinical parameters, including histologic grade, radiation therapy, radical resection, targeted molecular therapy and stage.

We then plotted a survival risk map and conducted a time-dependent receiver operating characteristic (ROC) curve analysis on the R software by survivalROC (<https://cran.r-project.org/web/packages/survivalROC/index.html>) (8) to test the prediction effectiveness of *PPP1R3B*. Finally, the prognostic relationship between the *PPP1R3B* gene and multiple clinical parameters in GC patients was verified using the Kaplan-Meier plotter (KM plotter) (<http://kmplot.com/private/>; June 15, 2019) database.

Genome-wide co-expression of *PPP1R3B*.

The corrpilot software package was used for genome-wide co-expression analysis and P values of < 0.05 and $|r| > 0.2$ were deemed statistically significant. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was then carried out using the Metascape (<http://metascape.org/>) (9) database to explore the biological and molecular mechanisms as well as pathways associated with the *PPP1R3B* gene.

GSEA analysis.

The GSEA (<http://www.broadinstitute.org/gsea/index.jsp>) (10, 11) applies c2 (v6.2) and c5 (v6.2) (12) in gene set analysis to uncover the potential molecular mechanisms and pathways in patients with different expression levels of the *PPP1R3B* gene. The enrichment results were considered significant based on the following criteria: |Normalized Enrichment Score (NES)| > 1 , P-value < 0.05 and false discovery rate (FDR) < 0.25 .

Statistical analysis

Hazard ratio (HR) analysis with 95% confidence intervals (CI) was performed to describe the relative risk between groups with different expression levels of *PPP1R3B* in GC patients. All statistical analyses were performed using SPSS v.23.0 (IBMCorp, Armonk, NY, USA) and R3.5.1. P values of < 0.05 were deemed statistically significant.

Results

Data mining and differential expression analysis

Following the expression of *PPP1R3B* mRNA by function Log2, 351 cases of GC and 32 cases of adjacent normal tissues were included in this experiment for further analysis. Expression of *PPP1R3B* was found to be up-regulated in

patients with tumor ($P < 0.001$; **Figure 1**). However, the expression of *PPP1R3B* in other clinical variables was not statistically significant.

Survival analysis

The clinical characteristics are outlined in **Table 1**. Clinicopathologic parameters such as age, TNM stage, cancer status, radiation and targeted therapy were remarkably associated with survival time in GC. After adjusting for these clinical characteristics, multivariate Cox regression found that the up-regulation of the *PPP1R3B* gene was related to poor prognosis among the 351 GC patients (high *PPP1R3B* vs low *PPP1R3B*; 22 months vs 60 months) and a higher mortality rate (Crude $P < 0.001$, Crude HR (95% CI) = 2.012(1.431-2.828), **Table 1, Figure 2**; adjusted $P = 0.005$, adjusted HR (95% CI) = 1.792(1.193-2.692), **Table 2**). Additionally, stratified analysis indicated that overexpression of the *PPP1R3B* gene increased the death rate in GC patients, except for stage I and cancer-free patients as shown in **Table 3**. Co-expression analysis also showed that *PPP1R3B* combined with specific clinical variables performed better than a single clinical parameter in GC survival time (**Figure 3(A–E)** and **Table 4**). The results of a survival risk map of *PPP1R3B* for low and high risk groups are constructed in **Figures 4A–4C**. The area under the 1-year, 2-year, 3-year, 4-year or 5-year curve (AUC) was 0.568, 0.620, 0.694, 0.680 or 0.649 in **Figure 4(D)**, respectively.

KM-plotter survival analyses

Finally, we used the KM-plotter database to verify the prognostic relationship between the *PPP1R3B* gene and GC patients. We witnessed worse OS rates with increasing *PPP1R3B* expression levels in all GC patients as outlined in **Figure 5** and **Table 5** (HR (95% CI) = 1.74 (1.4 – 2.17), $P = 5e-07$, **Figure 5 A**). We found that *PPP1R3B* could predict the prognosis of subjects in stage III (HR (95% CI) = 1.96 (1.34 – 2.86), $P = 0.00044$, **Figure 5 D**), HER2 negative status (HR (95% CI) = 1.62 (1.24 – 2.12), $P = 0.00036$, **Figure 5 G**), 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 6 A**). Nevertheless, the other clinical variables had no statistical significance on survival time at different expression levels of the *PPP1R3B* gene (**Figures 5B, 5C, 5E, 5F and 5I, Figures 6B–6F**).

Genome-wide co-expression analysis result.

The GO analysis indicated that the *PPP1R3B* gene was linked to transferase activity, mRNA 5'-UTR, folic acid and chromatin binding, ECM-receptor interaction, organic acid transmembrane transporter activity, Signaling pathways, regulating pluripotency of stem cells, Glycosaminoglycan biosynthesis, amino and nucleotide sugar metabolism (**Figure 7 A**). These results were consistent with those obtained from KEGG analysis (**Figure 7 B**).

GSEA

The GSEA data indicated that in the c2 category, high expression of *PPP1R3B* may be related to in hypoxia, HIF1A and SOX4 (**Figure 8A–C**). In the c5 category, down-regulation of *PPP1R3B* expression might be linked to cell-cell adhesion, T cell differentiation, homeostasis and receptor signaling pathway, antigen receptor mediated signaling pathway and lipopolysaccharide mediated signaling pathway (**Figure 8D–F**).

Discussion

The loss of alleles in 8p11.2-12, 8p21-22 and 8p23.1 in the human chromosome 8 is reported to be a common characteristic in esophageal(13) , colorectal (14, 15) and prostate cancers (16-20). This therefore suggests that there are multiple tumor suppressor genes (TSG) on the short arm of chromosome 8. Previous studies have shown that 8p loss also occurs in GC (21, 22). The *PPP1R3B* gene is located on chromosome 8p23.1. However, no study exists that

explores the relationship and importance of *PPP1R3B* in cancer patients, more so in gastric adenocarcinoma. Our study showed that the overexpression of *PPP1R3B* conferred a shorter survival time and higher mortality rates in GC patients. Therefore, *PPP1R3B* may be considered an oncogene in GC.

In this study, we analyzed the differential expression of the *PPP1R3B* gene in clinical variables and found that *PPP1R3B* was highly expressed in GC patients with tumor, thus increasing their risk of death. This was consistent with survival analysis, indicating that high expression of *PPP1R3B* confers a short survival time in GC patients. A comprehensive survival analysis including stratified analysis and co-analysis further investigated the clinical variables as well as the association between expression of *PPP1R3B* and OS in GC patients. Time-dependent ROC analysis showed that the *PPP1R3B* expression profile could reliably predict OS in GC patients. This finding was further verified in the KM plotter database. Therefore, *PPP1R3B* may be an oncogene in GC and plays a role in increased mortality in patients.

The gene is a serine/threonine kinase, regulating glycogen synthesis in the liver and skeletal muscles (23-25). It has previously been shown to be actively involved in the development of Alzheimer's disease (26), hepatic steatosis (27), and lipid levels in patients of European origin (28). Cancer is a kind of metabolic disease and metabolic disorders tend to play an integral role in the occurrence and development of the ailment (29). Hanahan et al highlighted metabolic changes as one of the ten characteristics of malignant tumors (30). A defect in lipid metabolism for instance, may affect the proliferation and differentiation of tumor cells and accelerate the occurrence and development of cancer(31). Actually, a decrease of choline and cholesterol in serum and tissues of patients with GC can be employed as a biomarker for early diagnosis of GC (32-34). This highlights the significance of defects in lipid metabolism and cell membrane biosynthesis in cancer progression.

In addition, *PPP1R3B* has been reported to play a role in activating glycogen synthetase (GYS) and inactivating glycogen phosphorylase resulting to abnormal metabolism of glycogen. GYS is the key enzyme and rate limiting enzyme in glycogen synthesis (35). Hypoxia inducible factor-1 (HIF-1) increases GYS levels in tumor cells, up-regulates the production of glycogen in cells and makes cells more tolerant to the hypoxia and nutritional deficiency. This suggests that glycogen synthesis caused by the expression of GYS plays a crucial role in the stressful growth environment of tumor cells (36, 37). Glycogen metabolism is an important process for energy generation in tumor cells (5, 38). Previous studies have shown glycogen accumulation to be a characteristic feature in many tumor cells and tissues (39-43). In addition, glycogen is involved in regulating the proliferation of tumor cells (44) and may therefore be an important signal molecule. High concentration of glycogen can activate AMPK, while continuous activation of AMPK can cause cell aging and inhibition of multiplication. Skwarski et al (45) reported that the risk of GC recurrence increased with prolonged operation time, which may be related to glycogen storage. Therefore, it is possible to artificially reduce the amount of glycogen and as a result inhibit the growth of cancer by maintaining the cell cycle at the G1 stop or G0 states.

The GSEA analysis indicated that *PPP1R3B* may be related to hypoxia, HIF1A, SOX4, T cell receptor signaling pathway, differentiation and T cell homeostasis, lipopolysaccharide mediated signaling pathway and antigen receptor mediated signaling pathway. Genome-wide co-expression analysis indicated that the *PPP1R3B* gene plays important roles in metabolic processes and pathways.

Studies have shown that the expression of SOX4 is higher in gastric tumor tissues compared to those with no tumors. The high expression of SOX4 in gastric tumor tissues was largely linked to tumor development and metastasis (46). T cell immunity is an important part of the human immune system and an effective T cell response is necessary for sufficient control of viral infections and growth of tumors (47). Therefore, T cell immunity plays a significant role in many cancers, including, lung (48), colorectal cancer(49), breast cancer (50) and ovarian cancer(51). CD4 + T cells and CD8 + T cells, which influence the progression and prognosis of GC, are important cell types in T-cell immunity (52, 53).

Some studies have shown that beta-catenin/ T cytokine (TCF) mediated transcription (classical Wnt signaling) can lead to chromosomal instability (CIN) (54), leading to the development of GC (55). Our study suggests that the inhibition of T cell differentiation, homeostasis and signaling pathway may affect the prognosis of GC.

Despite the findings, our study has a number of limitations. First, the clinical information was only acquired from the TCGA database hence it is incomprehensive. We therefore need to study multiple databases in order to make our findings more conclusive. Secondly, in the TCGA database, the expression data for only 32 adjacent tissues was available. The insufficient sample size might therefore underrepresent the outcomes of survival analyses. Thirdly, TCGA is an open database hence the underlying molecular mechanisms through which *PPP1R3B* affects the occurrence and prognosis of GC needs to be further interrogated.

Although our study had a few limitations, to the best of our knowledge, it was the first one to explore the prognostic value of *PPP1R3B* in gastric adenocarcinoma. Comprehensive survival analysis suggests that *PPP1R3B* might be a potential independent prognostic determinant for the OS of GC patients. Moreover, GSEA were used to reveal the cancer-related biological processes and pathways. Once these findings are validated, we expect *PPP1R3B* to be used in the diagnosis and treatment for GC.

Conclusions

The *PPP1R3B* gene might be an independent predictive marker for GC. However, further studies are needed to validate these findings.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

YZ, XL, YL, XM, WT and SL designed this manuscript, conducted the study, collected and analyzed the data. YZ wrote this manuscript; XL and YL contributed to the acquisition of data and sample; XM, YL and WT assisted with the data interpretation, and SL guided the writing. All authors read and approved the final manuscript.

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Abbreviations

PPP1R3B: Protein Phosphatase 1 Regulatory Subunit 3; TCGA: The Cancer Genome Atlas; OS: overall survival; RNA-Seq: RNA-sequencing; KM plotter: Kaplan–Meier plotter; HP: *Helicobacter Pylori*; GSEA: Gene set enrichment analysis; FDR: false discovery rate; AUC: area under curve; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; NES: Normalized Enrichment Score; FDR: false discovery rate; HR: hazard ratio; CI: confidence interval; TSG: tumor suppressor genes; GYS: glycogen synthetase; HIF-1: Hypoxia inducible factor-1; CIN: chromosomal instability.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2018 Nov;68(6):394-424. PubMed PMID: 30207593.
2. Hamashima C, Shabana M, Okada K, Okamoto M, Osaki Y. Mortality reduction from gastric cancer by endoscopic and radiographic screening. *Cancer science*. 2015 Dec;106(12):1744-9. PubMed PMID: 26432528. Pubmed Central PMCID: 4714659.
3. Brahimi-Horn MC, Bellot G, Pouyssegur J. Hypoxia and energetic tumour metabolism. *Current opinion in genetics & development*. 2011 Feb;21(1):67-72. PubMed PMID: 21074987.
4. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. *Nature*. 2012 Nov 15;491(7424):364-73. PubMed PMID: 23151579.
5. Zois CE, Favaro E, Harris AL. Glycogen metabolism in cancer. *Biochemical pharmacology*. 2014 Nov 1;92(1):3-11. PubMed PMID: 25219323.
6. Zois CE, Harris AL. Glycogen metabolism has a key role in the cancer microenvironment and provides new targets for cancer therapy. *Journal of molecular medicine*. 2016 Feb;94(2):137-54. PubMed PMID: 26882899. Pubmed Central PMCID: 4762924.
7. Ritterson Lew C, Guin S, Theodorescu D. Targeting glycogen metabolism in bladder cancer. *Nature reviews Urology*. 2015 Jul;12(7):383-91. PubMed PMID: 26032551. Pubmed Central PMCID: 4678000.
8. Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics*. 2005 Mar;61(1):92-105. PubMed PMID: 15737082.
9. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature communications*. 2019 Apr 3;10(1):1523. PubMed PMID: 30944313. Pubmed Central PMCID: 6447622.
10. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*. 2005 Oct 25;102(43):15545-50. PubMed PMID: 16199517. Pubmed Central PMCID: 1239896.
11. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature genetics*. 2003 Jul;34(3):267-73. PubMed PMID: 12808457.

12. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov Jill P, Tamayo P. The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Systems*. 2015;1(6):417-25.
13. Ishii H, Baffa R, Numata SI, Murakumo Y, Rattan S, Inoue H, et al. The FEZ1 gene at chromosome 8p22 encodes a leucine-zipper protein, and its expression is altered in multiple human tumors. *Proceedings of the National Academy of Sciences of the United States of America*. 1999 Mar 30;96(7):3928-33. PubMed PMID: 10097140. Pubmed Central PMCID: 22397.
14. Fujiwara Y, Emi M, Ohata H, Kato Y, Nakajima T, Mori T, et al. Evidence for the presence of two tumor suppressor genes on chromosome 8p for colorectal carcinoma. *Cancer research*. 1993 Mar 1;53(5):1172-4. PubMed PMID: 8439963.
15. Farrington SM, Cunningham C, Boyle SM, Wyllie AH, Dunlop MG. Detailed physical and deletion mapping of 8p with isolation of YAC clones from tumour suppressor loci involved in colorectal cancer. *Oncogene*. 1996 Apr 18;12(8):1803-8. PubMed PMID: 8622901.
16. Bova GS, Carter BS, Bussemakers MJ, Emi M, Fujiwara Y, Kyprianou N, et al. Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer. *Cancer research*. 1993 Sep 1;53(17):3869-73. PubMed PMID: 7689419.
17. Jenkins R, Takahashi S, DeLacey K, Bergstrahl E, Lieber M. Prognostic significance of allelic imbalance of chromosome arms 7q, 8p, 16q, and 18q in stage T3N0M0 prostate cancer. *Genes, chromosomes & cancer*. 1998 Feb;21(2):131-43. PubMed PMID: 9491325.
18. Macoska JA, Trybus TM, Benson PD, Sakr WA, Grignon DJ, Wojno KD, et al. Evidence for three tumor suppressor gene loci on chromosome 8p in human prostate cancer. *Cancer research*. 1995 Nov 15;55(22):5390-5. PubMed PMID: 7585607.
19. Suzuki H, Emi M, Komiya A, Fujiwara Y, Yatani R, Nakamura Y, et al. Localization of a tumor suppressor gene associated with progression of human prostate cancer within a 1.2 Mb region of 8p22-p21.3. *Genes, chromosomes & cancer*. 1995 Jul;13(3):168-74. PubMed PMID: 7669736.
20. Kagan J, Stein J, Babaian RJ, Joe YS, Pisters LL, Glassman AB, et al. Homozygous deletions at 8p22 and 8p21 in prostate cancer implicate these regions as the sites for candidate tumor suppressor genes. *Oncogene*. 1995 Nov 16;11(10):2121-6. PubMed PMID: 7478532.
21. Emi M, Fujiwara Y, Nakajima T, Tsuchiya E, Tsuda H, Hirohashi S, et al. Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. *Cancer research*. 1992 Oct 1;52(19):5368-72. PubMed PMID: 1356616.
22. Yustein AS, Harper JC, Petroni GR, Cummings OW, Moskaluk CA, Powell SM. Allelotype of gastric adenocarcinoma. *Cancer research*. 1999 Apr 1;59(7):1437-41. PubMed PMID: 10197609.
23. Gasa R, Jensen PB, Berman HK, Brady MJ, DePaoli-Roach AA, Newgard CB. Distinctive regulatory and metabolic properties of glycogen-targeting subunits of protein phosphatase-1 (PTG, GL, GM/RGI) expressed in hepatocytes. *The Journal of biological chemistry*. 2000 Aug 25;275(34):26396-403. PubMed PMID: 10862764.
24. Moorhead G, MacKintosh C, Morrice N, Cohen P. Purification of the hepatic glycogen-associated form of protein phosphatase-1 by microcystin-Sepharose affinity chromatography. *FEBS letters*. 1995 Apr 3;362(2):101-5. PubMed PMID: 7720853.
25. Doherty MJ, Cadefau J, Stalmans W, Bollen M, Cohen PT. Loss of the hepatic glycogen-binding subunit (GL) of protein phosphatase 1 underlies deficient glycogen synthesis in insulin-dependent diabetic rats and in adrenalectomized starved rats. *The Biochemical journal*. 1998 Jul 15;333 (Pt 2):253-7. PubMed PMID: 9657963. Pubmed Central PMCID: 1219580.

26. Kamboh MI, Demirci FY, Wang X, Minster RL, Carrasquillo MM, Pankratz VS, et al. Genome-wide association study of Alzheimer's disease. *Translational psychiatry*. 2012 May 15;2:e117. PubMed PMID: 22832961. Pubmed Central PMCID: 3365264.
27. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS genetics*. 2011 Mar;7(3):e1001324. PubMed PMID: 21423719. Pubmed Central PMCID: 3053321.
28. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010 Aug 5;466(7307):707-13. PubMed PMID: 20686565. Pubmed Central PMCID: 3039276.
29. Beger RD. A review of applications of metabolomics in cancer. *Metabolites*. 2013 Jul 5;3(3):552-74. PubMed PMID: 24958139. Pubmed Central PMCID: 3901293.
30. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011 Mar 4;144(5):646-74. PubMed PMID: 21376230.
31. Nie S, Zhao Y, Qiu X, Wang W, Yao Y, Yi M, et al. Metabolomic Study on Nude Mice Models of Gastric Cancer Treated with Modified Si Jun Zi Tang via HILIC UHPLC-Q-TOF/MS Analysis. Evidence-based complementary and alternative medicine : eCAM. 2019;2019:3817879. PubMed PMID: 31341492. Pubmed Central PMCID: 6612382.
32. Song H, Peng JS, Dong-Sheng Y, Yang ZL, Liu HL, Zeng YK, et al. Serum metabolic profiling of human gastric cancer based on gas chromatography/mass spectrometry. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas*. 2012 Jan;45(1):78-85. PubMed PMID: 22124703. Pubmed Central PMCID: 3854141.
33. Wang H, Zhang H, Deng P, Liu C, Li D, Jie H, et al. Tissue metabolic profiling of human gastric cancer assessed by (1)H NMR. *BMC cancer*. 2016 Jun 29;16:371. PubMed PMID: 27356757. Pubmed Central PMCID: 4928316.
34. Zhang H, Cui L, Liu W, Wang Z, Ye Y, Li X, et al. (1)H NMR metabolic profiling of gastric cancer patients with lymph node metastasis. *Metabolomics : Official journal of the Metabolomic Society*. 2018;14(4):47. PubMed PMID: 29541009. Pubmed Central PMCID: 5840249.
35. Zeqiraj E, Sicheri F. Getting a handle on glycogen synthase - Its interaction with glycogenin. *Molecular aspects of medicine*. 2015 Dec;46:63-9. PubMed PMID: 26278983. Pubmed Central PMCID: 5839463.
36. Pelletier J, Bellot G, Gounon P, Lacas-Gervais S, Pouyssegur J, Mazure NM. Glycogen Synthesis is Induced in Hypoxia by the Hypoxia-Inducible Factor and Promotes Cancer Cell Survival. *Frontiers in oncology*. 2012;2:18. PubMed PMID: 22649778. Pubmed Central PMCID: 3355943.
37. Iida Y, Aoki K, Asakura T, Ueda K, Yanaihara N, Takakura S, et al. Hypoxia promotes glycogen synthesis and accumulation in human ovarian clear cell carcinoma. *International journal of oncology*. 2012 Jun;40(6):2122-30. PubMed PMID: 22447231.
38. Lee WN, Guo P, Lim S, Bassilian S, Lee ST, Boren J, et al. Metabolic sensitivity of pancreatic tumour cell apoptosis to glycogen phosphorylase inhibitor treatment. *British journal of cancer*. 2004 Dec 13;91(12):2094-100. PubMed PMID: 15599384. Pubmed Central PMCID: 2409791.
39. Govender D, Ramdial PK, Clarke B, Chetty R. Clear cell (glycogen-rich) gastric adenocarcinoma. *Annals of diagnostic pathology*. 2004 Apr;8(2):69-73. PubMed PMID: 15060883.
40. Rousset M, Chevalier G, Rousset JP, Dussaulx E, Zweibaum A. Presence and cell growth-related variations of glycogen in human colorectal adenocarcinoma cell lines in culture. *Cancer research*. 1979 Feb;39(2 Pt 1):531-4. PubMed PMID: 761227.
41. Rousset M, Chevalier G, Rousset JP, Robine-Leon S, Dussaulx E, Zweibaum A. Kinetics of glycogen levels in asynchronous and synchronous cultures of a human colon carcinoma cell line, HT 29. *Frontiers of gastrointestinal*

research. 1979;4:73-9. PubMed PMID: 428897.

42. Rousset M, Zweibaum A, Fogh J. Presence of glycogen and growth-related variations in 58 cultured human tumor cell lines of various tissue origins. *Cancer research*. 1981 Mar;41(3):1165-70. PubMed PMID: 7459858.
43. Takahashi S, Satomi A, Yano K, Kawase H, Tanimizu T, Tuji Y, et al. Estimation of glycogen levels in human colorectal cancer tissue: relationship with cell cycle and tumor outgrowth. *Journal of gastroenterology*. 1999 Aug;34(4):474-80. PubMed PMID: 10452680.
44. McBride A, Ghilagaber S, Nikolaev A, Hardie DG. The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. *Cell metabolism*. 2009 Jan 7;9(1):23-34. PubMed PMID: 19117544. Pubmed Central PMCID: 2642990.
45. Skwarski L, Namiot Z, Stasiewicz J, Kemon A, Kralisz M, Gorski J. Glycogen content in the gastric mucosa of partially resected stomach; a possible relationship with the development of cancer. *Cancer letters*. 1998 May 15;127(1-2):123-8. PubMed PMID: 9619867.
46. Fang CL, Hseu YC, Lin YF, Hung ST, Tai C, Uen YH, et al. Clinical and prognostic association of transcription factor SOX4 in gastric cancer. *PloS one*. 2012;7(12):e52804. PubMed PMID: 23285187. Pubmed Central PMCID: 3527618.
47. Kamphorst AO, Ahmed R. CD4 T-cell immunotherapy for chronic viral infections and cancer. *Immunotherapy*. 2013 Sep;5(9):975-87. PubMed PMID: 23998732. Pubmed Central PMCID: 3864579.
48. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2008 Sep 20;26(27):4410-7. PubMed PMID: 18802153.
49. Simpson JA, Al-Attar A, Watson NF, Scholefield JH, Ilyas M, Durrant LG. Intratumoral T cell infiltration, MHC class I and STAT1 as biomarkers of good prognosis in colorectal cancer. *Gut*. 2010 Jul;59(7):926-33. PubMed PMID: 20581241.
50. Hu ZI, Ho AY, McArthur HL. Combined Radiation Therapy and Immune Checkpoint Blockade Therapy for Breast Cancer. *International journal of radiation oncology, biology, physics*. 2017 Sep 1;99(1):153-64. PubMed PMID: 28816141.
51. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England journal of medicine*. 2003 Jan 16;348(3):203-13. PubMed PMID: 12529460.
52. Ahearne MJ, Allchin RL, Fox CP, Wagner SD. Follicular helper T-cells: expanding roles in T-cell lymphoma and targets for treatment. *British journal of haematology*. 2014 Aug;166(3):326-35. PubMed PMID: 24815671.
53. Lu X, Yang L, Yao D, Wu X, Li J, Liu X, et al. Tumor antigen-specific CD8(+) T cells are negatively regulated by PD-1 and Tim-3 in human gastric cancer. *Cellular immunology*. 2017 Mar;313:43-51. PubMed PMID: 28110884.
54. Aoki K, Aoki M, Sugai M, Harada N, Miyoshi H, Tsukamoto T, et al. Chromosomal instability by beta-catenin/TCF transcription in APC or beta-catenin mutant cells. *Oncogene*. 2007 May 24;26(24):3511-20. PubMed PMID: 17160019.
55. Huang Q. Single disease entity for both chromosomal instable subtype gastric adenocarcinoma and esophageal adenocarcinoma. *Journal of digestive diseases*. 2017 Jun;18(6):319-22. PubMed PMID: 28509354.

Tables

Table 1. Correlation between OS and clinicopathologic features of GC patients.

Variables	Events/total (n=351)	MST (months)	HR (95% CI)	Log-rank P-value
<i>PPP1R3B</i>	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			
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.

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.

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: ^a adjusted 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.

Table 4 Joint effects survival analysis of clinical factors and the *PPP1R3B* expression with OS in GC patients.

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

Notes: ^a adjusted 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 GC patients 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.

Figures

Figure 1

Expression of PPP1R3B in gastric adenocarcinoma in terms of clinical variables. *P<0.05, ** P <0.01, ***P < 0.001.

Figure 2

Kaplan–Meier survival curves for PPP1R3B gene in GC of TCGA cohort.

Figure 3

Joint effects analysis of overall survival stratified by PPP1R3B and GC 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).

Figure 4

Prognostic value evaluation of PPP1R3B in patients with GC. Notes: A. PPP1R3B gene distribution; B. patient overall survival status and survival time; C. Gene expression heatmap of the PPP1R3B expression profiles; D. Receiver operating characteristic curve for predicting overall survival in GC patients by the PPP1R3B.

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). PPP1R3B, protein phosphatase 1 regulatory subunit 3.

Figure 6

Prognostic value of PPP1R3B (1552670_a_at) expression determined by Kaplan–Meier plotter database. Notes: Overall survival curves for intestinal-type (A), diffuse-type (B), mixed-type (C), poor differentiation (D), moderate differentiation (E), well differentiation (F). PPP1R3B, protein phosphatase 1 regulatory subunit 3.

A Go term enrichment

B KEGG enrichment

Figure 7

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

Figure 8

GSEA analysis of PPP1R3B in gastric cancer patients using the TCGA database. Notes: (A–C) GSEA results of C2 reference gene sets for high PPP1R3B expression groups; (D–F) GSEA results of C5 reference gene sets for low PPP1R3B expression groups. NES, normalized enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; PPP1R3B, protein phosphatase 1 regulatory subunit 3.