Glucose-6-Phosphate Dehydrogenase is a Prognostic Biomarker and Correlated with Immune Infiltrates in Hepatocellular Carcinoma

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) plays an important role in the metabolic and immunological aspects of tumors. In hepatocellular carcinoma (HCC), the alteration of tumor microenvironment influences recurrence and metastasis. We extract G6PD expression information from TCGA and GEO databases in liver cancer tissues and normal tissues, validated by immunohistochemistry, and the correlation between G6PD expression and clinical features is analyzed, and the clinical significance of G6PD in liver cancer is assessed by Kaplan-Meier, Cox regression and prognostic line graph models. Functional enrichment analysis is performed by protein-protein interaction (PPI) network, GO/KEGG, GSEA and G6PD-associated differentially expressed genes (DEGs). TIMER and ssGSEA packages are used to assess the correlation between expression and the level of immune cell infiltration. Analysis of TCGA and GEO datasets revealed that G6PD expression is significantly upregulated in hepatocellular carcinoma tissues ($P < 0.001$). G6PD expression is associated with histological grade, pathological stage, T-stage, vascular infiltration and AFP level ($P < 0.05$); HCC patients in the low G6PD expression group had longer overall survival and better prognosis compared with the high G6PD expression group ($P < 0.05$). The level of G6PD expression affects the levels of macrophages, unactivated dendritic cells, B cells, and follicular helper T cells in the tumor microenvironment. High expression of G6PD is a potential biomarker for poor prognosis of hepatocellular carcinoma, and G6PD may be a target for immunotherapy of HCC.

1. Introduction

Primary liver cancer is the sixth most common cancer in the world and the third most common cause of cancer mortality, with approximately 906,000 new cases and 830,000 deaths each year. Primary liver cancers include hepatocellular carcinoma (HCC) (75%-85% of cases) and intrahepatic cholangiocarcinoma (10%-15%) and mixed(1). Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin, alcohol consumption, obesity, type 2 diabetes, and smoking are the main risk factors for developing hepatocellular carcinoma (HCC). Surgical resection, liver transplantation and percutaneous hepatic artery embolization are still the primary treatment modalities for HCC, with a 5-year recurrence rate exceeding 70%-80%(2). HCC is easy to relapse, metastasis is the leading cause of death. At present, there is no effective treatment for metastatic HCC. There is evidence that hepatocellular carcinoma responds poorly to molecularly targeted drugs (sorafenib, etc.), with a limited survival benefit for patients. Immune checkpoint blockers are the most rapidly developing immunotherapy in recent years, mainly using PD1/PDL1 and CTLA4 as therapeutic targets to kill tumors by restoring the function of suppressed T cells(3),while the study found less than 20% anti-PD-1 response rate in liver cancer(4, 5).

Existing research shows that the energy metabolism requirements of tumor cells are exceptionally complex compared with normal cells, and abnormal metabolism of many pathways complements the energy requirements of tumor cells. Changes in abnormal glucose metabolism can be observed in many cancer cells, including HCC(5). Therefore, a better understanding of the metabolic abnormalities of HCC cells is crucial for improving the early diagnosis and treatment of HCC. The pentose phosphate pathway (PPP) is an essential component of cellular energy metabolism .It is crucial for maintaining carbon

homeostasis, providing precursors for nucleotide and amino acid biosynthesis, supplying reducing molecules for anabolism, and resisting oxidative stress(6). G6PD is a prominent rate-limiting enzyme in PPP(7), Involved in regulating the synthesis of ribulose-5-phosphate (R-5-P) and reduces coenzyme II (NADPH), of which R-5-P is a precursor for the synthesis of ATP, ADP, AMP, coenzyme A, NAD, etc(8). NADPH, a hydrogen donor, is involved in various metabolic reactions such as tetrahydrofolate, biotransformation of drugs and hormones, and maintenance of glutathione (GSH) in a reduced state. (6). This study analyses the level of G6PD expression and prognostics of HCC patients in the Cancer Genome Atlas (TCGA) and other public databases. It is aimed at assessing the correlation between G6PD-related genes and clinicopathological features, prognosis, and immune infiltration and providing possible theoretical support for exploring anti-G6PD therapy to improve the efficacy of HCC.

2. Methods

2.1. Data collection

Gene expression profile tertiary data of HCC were downloaded from the TCGA database (https: //portal.gdc.cancer.gov/) and normalized to extract G6PD gene expression information. Download clinical information on patients from the cBioportal database (https://www.cbioportal.org/). A total of 374 liver cancer samples and 50 paracancerous tissue samples were included in the study after excluding samples with incomplete clinical information and survival data. This study does not require the approval of the local ethics committee because the TCGA data are publicly available. In addition, gene expression profiles of the GSE39791 and GSE62232 datasets were collected from the GEO database to verify the expression levels of G6PD in normal and tumor tissues. The IHC data from THPA (https://www.proteinatlas.org/)is used to observe the distribution and subcellular localization of G6PD and expression in HCC.

2.2 Statistical analysis

SPSS 26.0 statistical software was applied for analysis, and the count data were analyzed by χ^2 test. The diagnostic and prognostic value of G6PD in HCC patients was analyzed using the Receiver operating characteristic (ROC) and Kaplan- Meier survival curve. Univariate and multifactorial COX regression analyses illustrated the relationship between G6PD expression and overall survival (OS) in HCC patients. P 0.05 was considered to be statistically significant.

2.3 Protein–protein interaction (PPI) and enrichment analysis

Based on the data of protein-protein interaction (PPI) in the online STRING database, we constructed the PPI network of differentially expressed proteins. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of *G6PD* and its related genes were performed using the cluster profiler package in R language to analyze the sites and pathways of action of G6PD and its related proteins on HCC. Enrichment analysis of G6PD-related biological functions and signaling

pathways was performed using the GSEA tool with the ggplot2 package in R language and gene sets with FDR (false discovery rate) < 0.25 and $P < 0.05$ were considered as significantly enriched gene sets.

2.4. Correlation analysis of immune cell infiltration

The relationship between G6PD and tumor purity and several immune cells, including B cells, neutrophils, macrophages, dendritic cells, CD4+ T cells and CD8+ T cells, was assessed by using the Tumor Immune Evaluation Resource (TIMER). Spearman correlation analysis was used to investigate the correlation between G6PD and immune cell infiltration.

3. Results

3.1. Clinical data collection

As shown in **Table 1**, the clinical data of 374 patients with HCC in TCGA were statistically analyzed. The results showed that the differences of G6PD expression levels in gender, age, BMI, Child stage, liver fibrosis score and tumor status were not statistically significant ($P>0.05$), and the differences of G6PD expression levels in AFP level, T stage, pathological stage, histological stage and vascular invasion were statistically significant ($P<0.05$).

Table 1. Clinical characteristics of patients with HCC based on TCGA

Characteristic	Expression of G6PD		*P-value*
	Low (n=187)	High (n=187)	
Gender, n (%)			
Female	63 (16.8%)	58 (15.5%)	0.658
Male	124 (33.2%)	129 (34.5%)	
Age, n (%)			
<=60	89 (23.9%)	88 (23.6%)	1.00
>60	98 (26.3%)	98 (26.3%)	
BMI, n (%)			
<=25	84 (24.9%)	93 (27.6%)	0.246
>25	87 (25.8%)	73 (21.7%)	
AFP(ng/ml), n (%)			
<=400	120 (42.9%)	95 (33.9%)	**0.011**
>400	24 (8.6%)	41 (14.6%)	
Child-Pugh grade, n (%)			
A	123 (51%)	96 (39.8%)	0.899
B	11 (4.6%)	10 (4.1%)	
C	1 (0.4%)	0 (0%)	
Fibrosis ishak score, n (%)			
0	52 (24.2%)	23 (10.7%)	0.080
1/2	17 (7.9%)	14 (6.5%)	
3/4	15 (7%)	13 (6%)	
5/6	40 (18.6%)	41 (19.1%)	
T stage, n (%)			
T1	107 (28.8%)	76 (20.5%)	**0.007**
T2	37 (10%)	58 (15.6%)	
T3	37 (10%)	43 (11.6%)	
T4	4 (1.1%)	9 (2.4%)	
Histologic grade, n (%)			

G1	39 (10.6%)	16 (4.3%)	**< 0.001**
G2	98 (26.6%)	80 (21.7%)	
G3	44 (11.9%)	80 (21.7%)	
G4	4 (1.1%)	8 (2.2%)	
Pathologic stage, n (%)			
Stage I	101 (28.9%)	72 (20.6%)	**0.017**
Stage II	36 (10.3%)	51 (14.6%)	
Stage III	36 (10.3%)	49 (14%)	
Stage IV	2 (0.6%)	3 (0.9%)	
Vascular invasion, n (%)			
No	118 (37.1%)	90 (28.3%)	**0.035**
Yes	48 (15.1%)	62 (19.5%)	
Tumor status, n (%)			
Tumor free	112 (31.5%)	90 (25.4%)	0.052
With tumor	68 (19.2%)	85 (23.9%)	

3.2. G6PD expression in HCC

G6PD mRNA levels were expressed in breast cancer (BRCA), cholangiocarcinoma (CHOL), colon cancer (COAD), esophageal cancer (ESCA), glioblastoma multiforme (GBM), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), low-grade glioma of the brain (LGG), Hepatocellular carcinoma (HCC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian plasmacytoid cystic adenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), rectal adenocarcinoma (READ), sarcoma (SARC), cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumor (TGCT), thyroid carcinoma (THCA), uterine carcinosarcoma (UCS), lymphoid tumor diffuse large b-cell lymphoma (DLBC), head and neck squamous cell carcinoma (HNSC), kidney Chromophobe (KICH), prostate adenocarcinoma (PRAD) and other tumor tissues and normal tissues (**Figure 1A**). G6PD expression levels were higher in HCC tissues compared to normal tissues (P<0.05). Correlation analysis showed that G6PD was statistically different from tissue grade (P<0.05), vascular infiltration (P<0.05), AFP level (P<0.05), pathological stage (P<0.05), and T stage (**Figure 1B**). G6PD expression was verified in GSE60502 and GSE62232 (P<0.001) (**Figure 2A-B**). Further analysis showed agreement with IHC results (**Figure 2C-D**).

3.3. Prognostic correlation analysis of G6PD and HCC

Plotting the ROC curve yields an AUC value of 0.944, from which it can be concluded that G6PD can distinguish between normal and tumor tissues. Meanwhile, the Kaplan-Meier curve showed that high G6PD levels were associated with poor prognosis (**Figure 3**). The univariate Cox regression model showed that G6PD level, T-stage, tumor status, and pathological stage were associated with the prognosis of HCC patients (P<0.01). Multivariate Cox regression analysis showed that tumor status and G6PD level were independent risk factors for survival of HCC patients (P<0.01; **Figure 4**). Nomogram column line plots were constructed using the RMSR package based on the results of Cox regression analysis to further predict the 1-, 3-, and 5-year survival rates of HCC patients (**Figure 5**).

3.4.PPI Networks and Enrichment Analysis

In this study, we constructed a network of G6PD and its related genes using the STRING tool. The G6PD-related genes included GAPDH, TPI1, PGLS, TP53, PGD, PKM, TALDO1, LDHB, GPI. Their scores were greater than 0.9 (**Figure 6**). Based on the cluster profiler package and *ggplot2* package in R language, we enriched the differential genes in tumor tissues and normal tissues and the results of GO functional enrichment analysis showed that G6PD-related genes were mainly involved in the metabolism of pyridine compounds, nicotinamide nucleotides, pyridine nucleotides, carbohydrate binding, monosaccharide binding, and NADP binding in biological processes. and NADP binding. KEGG results suggest that G6PD-related genes are enriched in the biological processes of carbon metabolism, gluconeogenesis and pentose phosphate pathway, among which G6PD, NAPDH, GPI, HK2, PGD, PKM, TALDO1, TPL1 and PGLS are enriched in the carbon metabolism pathway (**Figure 7**). GSEA analysis results suggested a high G6PD gene expression phenotype significantly enriched in the REACTOME_INNATE_IMMUNE_SYSTEM gene set (NES = 1.997; *P*.adjust = 0.017; FDR = 0.012); in the EACTOME_INFECTIOUS_DISEASE gene set (NES = 2.124; *P*.adjust = 0.017; FDR = 0.012) (NES = 2.124; *P*.adjust = 0.017; FDR = 0.012).

3.5. Correlation Analysis of Immune Cell Infiltration

Hepatocellular carcinoma is considered to be an immunogenic tumor, which is closely associated with a viral infection and inflammatory environment. TIMER data suggested statistically significant differences between G6PD and B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (all P< 0.05, **Figure 8A**). Compared with the G6PD low expression group, B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cells in G6PD high expression group were significantly increased (all P< 0.05). Meanwhile, ssGESA analysis showed: Helper T cells, TFH cells, Th1 cells, and Th2 cells were elevated in the *G6PD* high expression group compared to the low expression group (all P< 0.05, **Figure 8B**). In contrast, Th17 cells and TReg cells (P< 0.01) were decreased in the high expression group compared to the low expression group.

4. Discussion

Recurrence and metastasis of HCC are still primary causes of patients' death and these factors are closely related to the proliferation and invasion capacities of tumor cells. Recently, although a lot of progress has been made in the pathogenesis of HCC. Therefore, exploring new therapeutic targets for

HCC is of great significance to improve postoperative survival rates and facilitate the survival prognosis of patients. As a key enzyme in the PPP process, the main role of G6PD is to provide sufficient reducing capacity to support cell growth and keep cells in redox homeostasis. Its product, NADPH, acts as a pro-oxidant, generates reactive oxygen species (ROS) and reactive nitrogen species (RNS), which ameliorate tumor cell proliferation and metastasis, cell cycle and apoptosis. Severe deficiency of G6PD can impair embryonic development and retard the organism's growth. Hence, altering the activity of G6PD is associated with pathophysiology such as autophagy, insulin resistance, infection, inflammation, diabetes, hypertension, etc. Besides, abnormal activation of G6PD can also lead to cell proliferation in many cancers(9). In the current study, G6PD activity is significantly increased in tumor tissues of gastric cancer, breast cancer, bladder cancer, cervical cancer, and colorectal cancer(10–14). This is consistent with the result that G6PD is highly expressed in pan-cancer in the TCGA data. Therefore, G6PD is also considered as a potentially effective target for tumor treatment(15–17).

As an independent predictor of prognosis for gastric cancer patients,G6PD expression was higher in gastric cancer tissues than in paired normal gastric mucosa groups, and high G6PD expression was closely associated with tumor size, depth of infiltration and tumor size, lymph node metastasis, distant metastasis, TNM stage and survival rate in previous studies(10). Zhang et al.(18)found that intracellular high expression of G6PD decreased matrix metalloproteinase expression through the G6PD /HIF-1α/ Notch1 axis and promoted the migration of tumor cells. Chen J et al.(19)showed that G6PD-based metabolic markers can be used as predictors of prostate cancer metastasis. G6PD was expressed at high levels in melanoma. In animal models, wild-type nude mice models had faster tumor growth, larger tumor size, and higher malignancy than G6PD-deficient nude mice(20). Hong et al. (21)found that high G6PD expression was significantly associated with poor prognosis in HCC, especially in HCC patients who are in the advanced stages of HCC treated with sorafenib after hepatocellular carcinoma surgery, high G6PD expression was significantly associated with worse PFS and OS. This may also have a link with PPP and tumor cell resistance. Yin X et al.(22) found that Inhibitor of Differentiation-1(ID-1) in HCC cells leads to reduce G6PD and NADPH activity and increase ROS production, and transfection of G6PD into ID-1 knockdown HCC cells reversed these changes and induced oxaliplatin resistance, and the above evidence also provides new ideas for the study of hepatocellular carcinoma in terms of resistance to sorafenib and oxaliplatin. In our research, G6PD expression is associated with histological grading, pathological stage, T-stage, vascular infiltration and AFP levels (P<0.05). HCC patients in the G6PD low expression group have more prolonged overall survival and better prognosis than the G6PD high expression group (P<0.05), and high G6PD expression is a potential biomarker for poor prognosis of HCC. In addition, we combined G6PD with age, AFP, and Child classification to construct columnar line graph prognostic plots to obtain a more accurate prognostic prediction model, and the C-index G6PD-related Cox model predicted OS of 0.686. The calibration plots show that the best agreement between the predictions of the column line graphs is associated with G6PD and the actual observations of the 1-year, 3-year and 5-year OS probabilities. Thus, our model can provide personalized scoring for HCC patients.

In this study, the PPI network was used to identify co-expressed proteins of G6PD, and we identified G6PD-related genes, including GAPDH, TPI1, PGLS, TP53, PGD, PKM, TALDO1, LDHB, and GPI. Among

them, TP53 has become a key antitumor factor since its discovery, and TP53 can be activated under conditions of genotoxic stress, oncogene activation, ribosomal stress, hypoxic state, and abnormal energy metabolism(23). Furthermore, the MDM2-TP53 axis may be manifested in hepatocyte glycolipid metabolism by enhancing glycolipid catabolism, but can promote hepatocyte injury in the early and late stages of glycolipid metabolism disorders (24). Oxidative stress, steatosis and abnormal cell growth can be detected in hepatocytes with disorders of glucolipid metabolism, all of which may contribute to the development of HCC(24). GAPDH and LDHB are all glycolysis-related genes, and it has been shown that TFB2M activates aerobic glycolysis in hepatocellular carcinoma cells through NAD /SIRT3/HIF-1α Signaling pathway(25). Among them, TFB2M (mitochondrial transcription factor B2) is a core mitochondrial transcription factor, and its overexpression is significantly associated with the malignancy and prognosis of hepatocellular carcinoma(26). Enrichment analysis of differential genes in tumor tissues and normal tissues based on the DAVID database was performed, and the results of GO functional enrichment analysis showed that G6PD-related genes were enriched in the metabolism of pyridine compounds, metabolism of nicotinamide nucleotides, metabolism of pyridine nucleotides, carbohydrate-binding, monosaccharide binding, and NADP binding. Among them, nicotinamide ribonucleotide (NMN) is a precursor of coenzyme 1NAD+ (nicotinamide adenine dinucleotide), which is not only a coenzyme involved in intracellular redox reactions, but also involved as a substrate in regulating apoptosis, DNA repair, immune response, and many other physiological roles (27).Moreover, due to the high rate of tumor cell appreciation and DNA repair, the demand for NAD is increased, and some studies have shown an important role of nicotinamide phosphoribosyl transferase (NAMPT)-mediated NAD remediation pathway in the energy homeostasis of hepatocellular carcinoma cells and suggested that NAMPT inhibition is a potential therapeutic option for hepatocellular carcinoma (28). KEGG results suggest that G6PD-related genes are enriched in carbon metabolism, gluconeogenesis, and the pentose phosphate pathway (PPP), which is consistent with the function of G6PD. The pentose phosphate pathway (PPP) is the first step reaction of glycolysis, and its product NADPH has an important role in biosynthesis as well as in maintaining cellular redox homeostasis. ROS is a collective term for a variety of oxygen radicals in intracellular metabolic processes, which can promote normal cell proliferation when ROS levels are low, and trigger apoptosis when ROS levels are too high(29). The significance of PPP at this time lies largely in reducing excess ROS and maintains cellular energy metabolism in a redox homeostasis. In tumor cells, making PPP at high levels is able to resist ROS-induced apoptosis (30). In hepatocellular carcinoma cells, high expression of the G6PDH gene is closely related to the occurrence, development, metastasis and prognosis of hepatocellular carcinoma(31).

In addition, tumor cell metabolic reprogramming is involved in tumor immune regulation, and metabolic reprogramming plays a crucial role in antitumor immunotherapy. Tumor cells require large amounts of energy during proliferation, while body has to compete with tumor cells for these nutrients by tumor-infiltrating effector cells CD8+T lymphocytes (CD8TILs) in order to fight tumors. Studies have shown that sustained antitumor immunity can be triggered by upregulating glycolysis and oxidative phosphorylation in CD8TILs(32).

T cells also play an important role in the immune microenvironment against infections and tumors (33), Naïve T cells have low metabolic demand and rely mainly on oxidative phosphorylation for energy production, but with the onset of infections and tumors, Naïve T cells will be activated, and activated T cell energy metabolism will shift to aerobic glycolysis and increase oxidative phosphorylation, which is essential for effector T cell production and function(34, 35). Gu M et al.(36) found that the G6PD-NADPH redox system plays an important role in the stability and metabolism of hexokinase 2 (HK2) in activated T cells. However, there are few studies on the relationship between G6PD and immune cells in HCC. In the present study, the correlation between G6PD and immune cell infiltration was assessed using TIMER and ssGSEA. Our results showed that significant differences between G6PD and $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, B cells, neutrophils, macrophages, dendritic cells, helper T cells, follicular helper T cells, Th1, Th2, Th17, regulatory T cells (Treg). This also provides new ideas for immunotherapy in HCC patients with elevated G6PD. However, there are some limitations in this study, such as the relatively small sample size in the TCGA database and the hypothesis of this study was not validated using animal models. Therefore, we will conduct further cellular assays to further prove in the following studies.

5. Conclusion

In conclusion, our study confirmed that G6PD expression levels were significantly associated with the prognosis of HCC patients and that G6PD expression levels were significantly associated with immune cell infiltration. Thus, our findings suggest that G6PD expression may have a unique prognostic value in HCC patients and may be a potential target for HCC immunotherapy.

Abbreviations

G6PD, Glucose-6-phosphate dehydrogenase; HCC, Hepatocellular carcinoma; PPI, Protein-protein interaction; DEGs, Differentially expressed genes; HBV, Hepatitis B virus; HCV, Hepatitis C virus; PPP, Pentose phosphate pathway; R-5-P, Ribulose-5-phosphate; TCGA , The Cancer Genome Atlas; BRCA ,Breast cancer; CHOL, Cholangiocarcinoma；COAD：Colon cancer；ESCA, Esophageal cancer; GBM, Glioblastoma multiforme; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Low-grade glioma of the brain; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; OV, Ovarian plasmacytoid cystic adenocarcinoma; PAAD, Pancreatic adenocarcinoma; READ, Rectal adenocarcinoma; SARC, Sarcoma; SKCM, Cutaneous melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular germ cell tumor; THCA, Thyroid carcinoma; UCS, Uterine carcinosarcoma; DLBC, Lymphoid tumor diffuse large b-cell lymphoma; HNSC, Head and neck squamous cell carcinoma; KICH, Kidney Chromophobe; PRAD, Prostate adenocarcinoma; ROS, Reactive oxygen species; RNS, Reactive nitrogen species; ID-1, Inhibitor of Differentiation-1; HK2, Hexokinase 2; ROC, Receiver operating characteristic; OS, Overall survival; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TIMER, Tumor Immune Evaluation Resource.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets TCGA for this study can be found in the https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga. And the datasets GEO can be found in the https://www.ncbi.nlm.nih.gov/geo/.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Authors' contributions

KJL and YMN were responsible for the design of the study, data acquisition, and analysis, as well as drafting the manuscript. LC, CQL and JH participated in data acquisition, analysis, and interpretation. YB and BDC participated in drafting the manuscript and troubleshooting. KJL, YMN, LC, CQL, JH, BDC and YB participated in its design and coordination. All authors read and approved the final manuscript.

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References

1. Sung H, Ferlay J, Siegel R, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. 2021;71(3):209-49.
2. Llovet J, Kelley R, Villanueva A, et al. Hepatocellular carcinoma. 2021;7(1):6.
3. Chu P, Chan SJC. Cure the Incurable? Recent Breakthroughs in Immune Checkpoint Blockade for Hepatocellular Carcinoma. 2021;13(21).
4. Marrero J, Kulik L, Sirlin C, et al. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. 2018;68(2):723-50.

5. Boroughs L, DeBerardinis RJNcb. Metabolic pathways promoting cancer cell survival and growth. 2015;17(4):351-9.
6. Wood TJCb, function. Physiological functions of the pentose phosphate pathway. 1986;4(4):241-7.
7. Wang A, Chen B, Jian S, Cai W, Xiao M, Du GJA-cd. miR-206-G6PD axis regulates lipogenesis and cell growth in hepatocellular carcinoma cell. 2021;32(5):508-16.
8. Yan Y, Wang J, Dong X, et al. Quantitative proteomic analysis of hepatic tissue in allotetraploid hybridized from Red Crucian Carp and Common Carp identified crucial proteins and pathways associated with metabolism and growth rate. 2021:e2100115.
9. Yang H, Wu Y, Yen W, et al. The Redox Role of G6PD in Cell Growth, Cell Death, and Cancer. 2019;8(9).
10. Wang J, Yuan W, Chen Z, et al. Overexpression of G6PD is associated with poor clinical outcome in gastric cancer. 2012;33(1):95-101.
11. Dong T, Kang X, Liu Z, et al. Altered glycometabolism affects both clinical features and prognosis of triple-negative and neoadjuvant chemotherapy-treated breast cancer. 2016;37(6):8159-68.
12. Ohl F, Jung M, Radonić A, ,et al. Identification and validation of suitable endogenous reference genes for gene expression studies of human bladder cancer. 2006;175(5):1915-20.
13. Van Driel B, Valet G, Lyon H, , et al. Prognostic estimation of survival of colorectal cancer patients with the quantitative histochemical assay of G6PDH activity and the multiparameter classification program CLASSIF1. 1999;38(4):176-83.
14. Fang Z, Jiang C, Feng Y, et al. Effects of G6PD activity inhibition on the viability, ROS generation and mechanical properties of cervical cancer cells. 2016;1863(9):2245-54.
15. Budihardjo I, Walker D, Svingen P, et al. 6-Aminonicotinamide sensitizes human tumor cell lines to cisplatin. 1998;4(1):117-30.
16. Polimeni M, Voena C, Kopecka J, et al. Modulation of doxorubicin resistance by the glucose-6-phosphate dehydrogenase activity. 2011;439(1):141-9.
17. Mele L, Paino F, Papaccio F, et al. A new inhibitor of glucose-6-phosphate dehydrogenase blocks pentose phosphate pathway and suppresses malignant proliferation and metastasis in vivo. 2018;9(5):572.
18. Zhang H, Zhang Z, Du G, et al. Nrf2 promotes breast cancer cell migration via up-regulation of G6PD/HIF-1α/Notch1 axis. 2019;23(5):3451-63.
19. Chen J, Cao S, Situ B, et al. Metabolic reprogramming-based characterization of circulating tumor cells in prostate cancer. 2018;37(1):127.
20. Hu T, Zhang C, Tang Q, et al. Variant G6PD levels promote tumor cell proliferation or apoptosis via the STAT3/5 pathway in the human melanoma xenograft mouse model. 2013;13:251.
21. Hong X, Song R, Song H, et al. PTEN antagonises Tcl1/hnRNPK-mediated G6PD pre-mRNA splicing which contributes to hepatocarcinogenesis. 2014;63(10):1635-47.
22. Yin X, Tang B, Li J, et al. ID1 promotes hepatocellular carcinoma proliferation and confers chemoresistance to oxaliplatin by activating pentose phosphate pathway. 2017;36(1):166.

23. Levine AJCd, differentiation. Reviewing the future of the P53 field. 2018;25(1):1-2.
24. Cao H, Chen X, Wang Z, et al. The role of MDM2-p53 axis dysfunction in the hepatocellular carcinoma transformation. 2020;6:53.
25. Chang H, Li J, Luo Y, et al. TFB2M activates aerobic glycolysis in hepatocellular carcinoma cells through the NAD /SIRT3/HIF-1α signaling. 2021.
26. Geng X, Geng Z, Li H, , et al. Over-expression of TFB2M facilitates cell growth and metastasis via activating ROS-Akt-NF-κB signalling in hepatocellular carcinoma. 2020;40(7):1756-69.
27. Xu L, Yang C, Ma J, et al. NAMPT-mediated NAD biosynthesis suppresses activation of hepatic stellate cells and protects against CCl-induced liver fibrosis in mice. 2021:9603271211052991.
28. Schuster S, Penke M, Gorski T, et al. FK866-induced NAMPT inhibition activates AMPK and downregulates mTOR signaling in hepatocarcinoma cells. 2015;458(2):334-40.
29. Lennicke C, Cochemé HJMc. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. 2021;81(18):3691-707.
30. Simon-Molas H, Vallvé-Martínez X, Caldera-Quevedo I, et al. TP53-Induced Glycolysis and Apoptosis Regulator (TIGAR) Is Upregulated in Lymphocytes Stimulated with Concanavalin A. 2021;22(14).
31. Kowalik M, Guzzo G, Morandi A, et al. Metabolic reprogramming identifies the most aggressive lesions at early phases of hepatic carcinogenesis. 2016;7(22):32375-93.
32. Udono H, Nishida MJIi. Pharmacological effects on anaplerotic pathways alters the metabolic landscape in the tumor microenvironment, causing unpredictable, sustained antitumor immunity. 2021.
33. Durgeau A, Virk Y, Corgnac S, , et al. Recent Advances in Targeting CD8 T-Cell Immunity for More Effective Cancer Immunotherapy. 2018;9:14.
34. Lim A, Rathmell W, Rathmell JJe. The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. 2020;9.
35. Odegaard J, Chawla AJI. The immune system as a sensor of the metabolic state. 2013;38(4):644-54.
36. Gu M, Zhou X, Sohn J, et al. NF-κB-inducing kinase maintains T cell metabolic fitness in antitumor immunity. 2021;22(2):193-204.

Figures

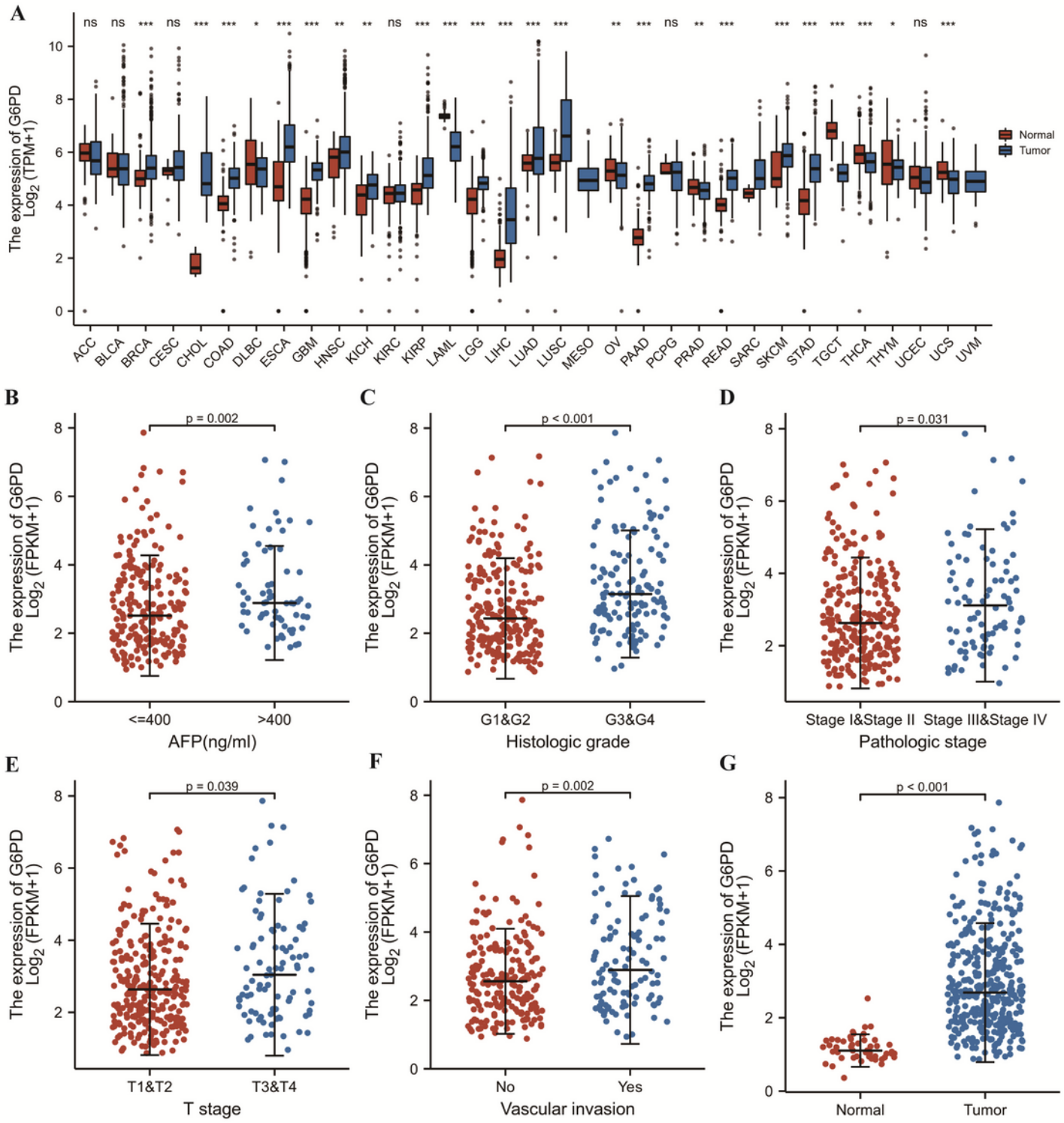


Figure 1

Expression levels of G6PD in cancer. (A) The expression levels of G6PD in different cancer tissues are different from the corresponding normal tissues. (B-F) Association between G6PD and clinical manifestations of HCC. The results showed that high expression of G6PD was associated with higher histological grade, higher pathological stage, T-stage, vascular infiltration and AFP level (P<0.05). (G) The expression level of G6PD was significantly higher in HCC tissues compared to normal tissues (P<0.01).

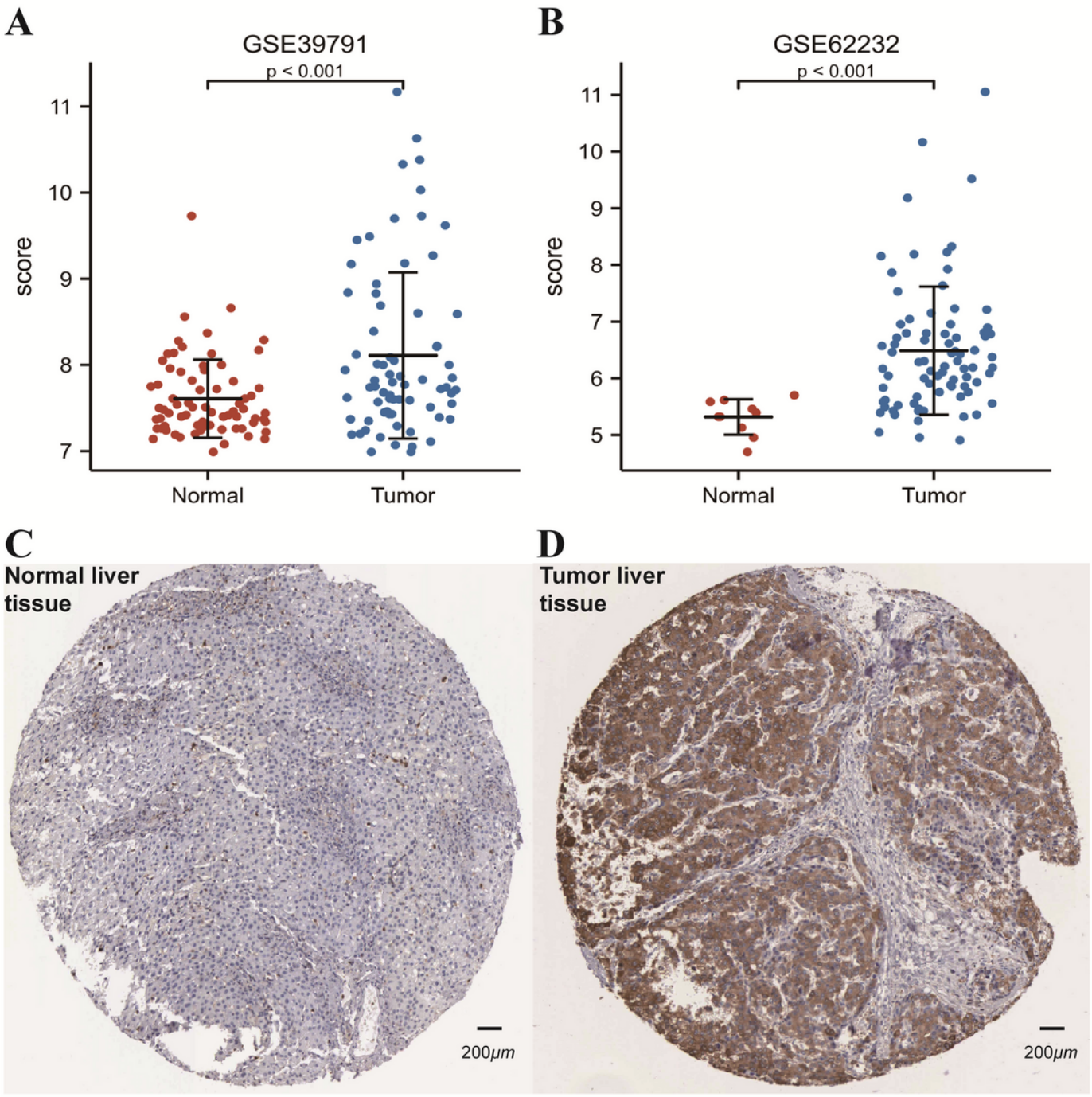


Figure 2

Overexpression of G6PD was in HCC. (A-B) G6PD is overexpressed in HCC. G6PD expression was higher in GSE 39791 and GSE 62232 than in normal tissues ($p<0.001$). (C-D) The level of G6PD protein was higher in HCC than in normal tissues(Anti-HPA000834).

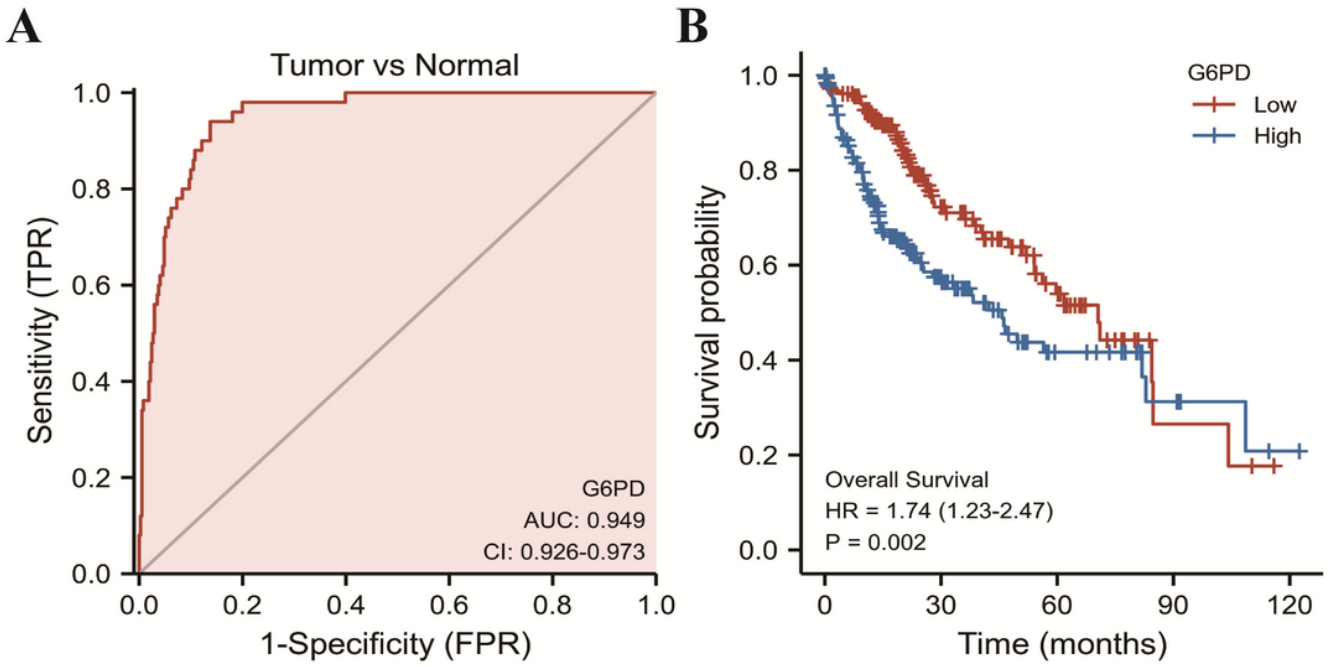


Figure 3

ROC and Kaplan-Meier survival curve of G6PD. (A) ROC curve and Kaplan-Meier curve of G6PD. ROC curve analysis showed that G6PD was able to accurately identify tumor and normal tissue with an AUC of 0.949; (B) Kaplan-Meier survival curve showed poor prognosis of HCC patients with higher G6PD levels.

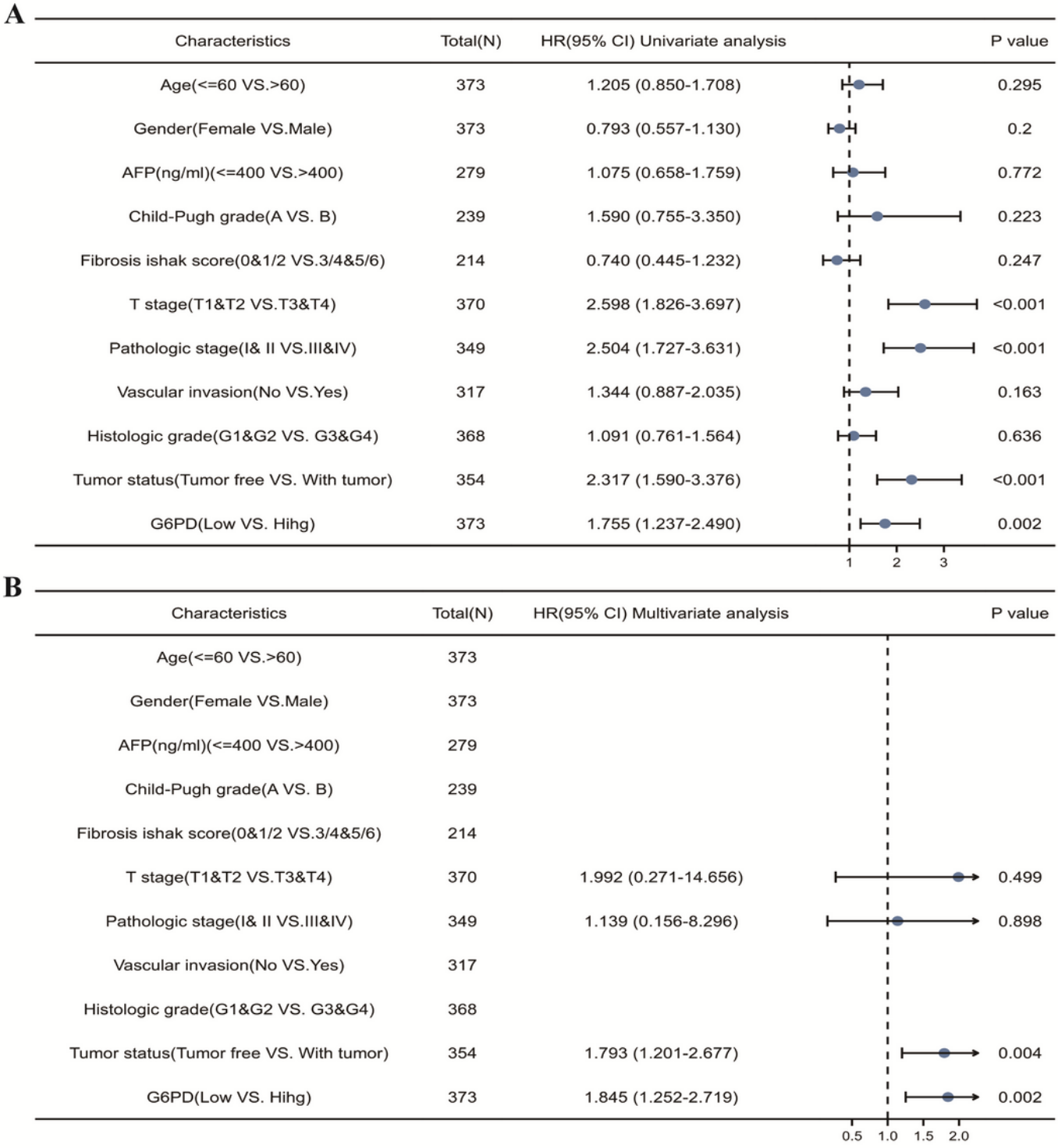


Figure 4

Univariate and multifactorial regression analyses were calculated for G6PD expression and other OS clinicopathological factors in HCC. (A) The univariate Cox regression model. Among these factors, T-stage, tumor status, and pathological stage were determined to be statistically significantly associated with the likelihood of OS. (B) Multiple Cox regression analysis in which tumor status and G6PD level were independent risk factors for survival in HCC patients.

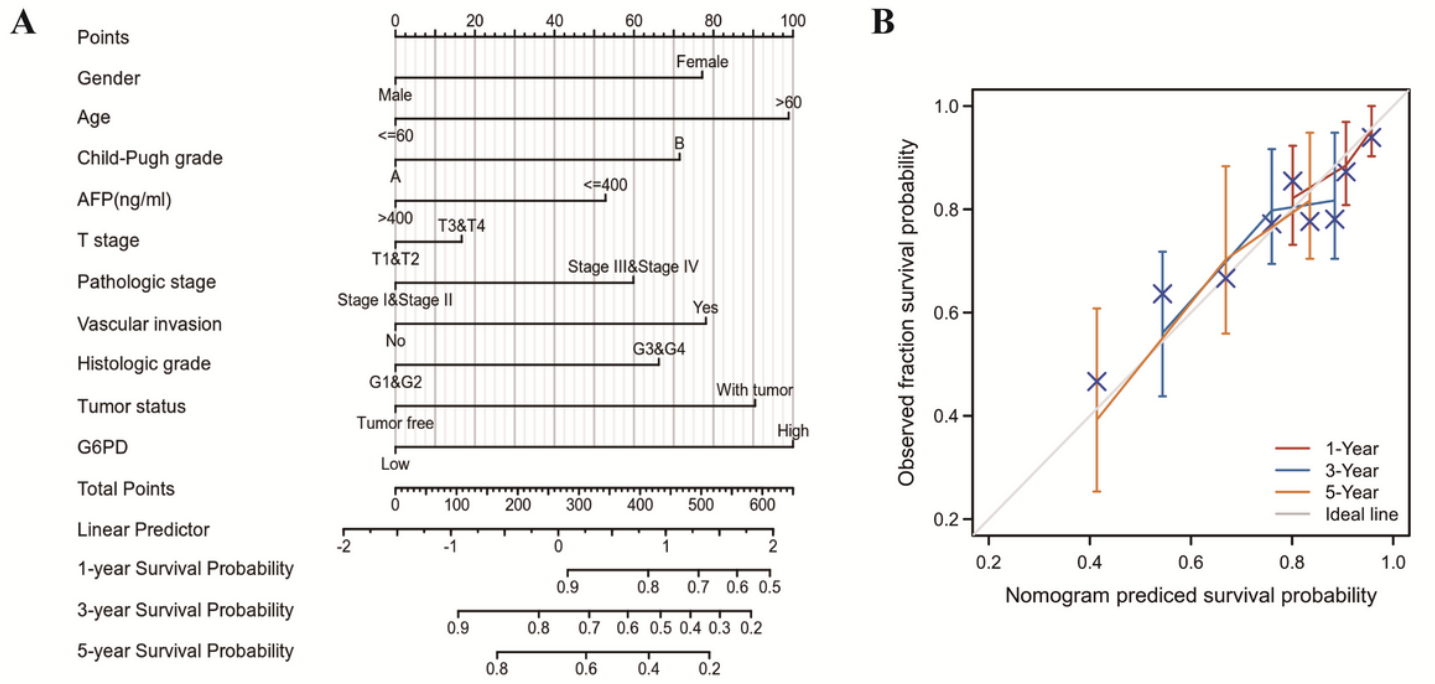
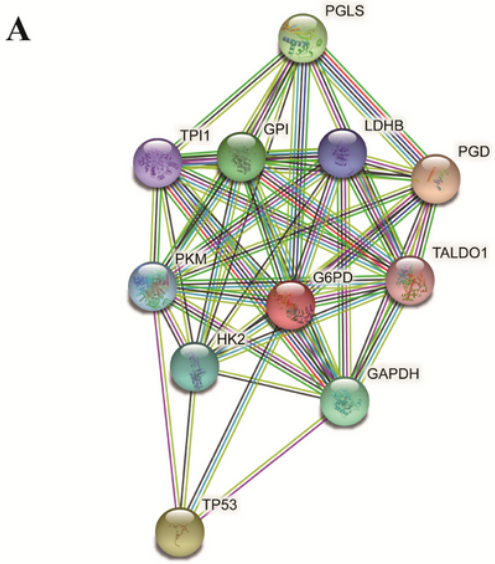


Figure 5

Nomogram plot: 1, 3, and 5-year survival rates of HCC patients can be predicted based on the column line plot



B

Gene symbol	Annotation	Score
GAPDH	glyceraldehyde phosphate dehydrogenase	0.975
TPI1	Triosephosphate isomerase 1	0.957
PGLS	6-phosphogluconolactonase	0.995
TP53	Tumor Protein53	0.996
PGD	phosphogluconate dehydrogenase	0.998
HK2	hexokinase 2	0.965
PKM	Pyruvate kinase PKM	0.962
TALDO1	transaldolase 1	0.954
LDHB	lactate dehydrogenase B	0.961
GPI	glucose-6-phosphate isomerase	0.992

Figure 6

Protein-protein interaction comprehensive analysis of G6PD. (A) Comprehensive analysis of G6PD-related protein interactions. The network of G6PD and its potential co-expressed genes was analyzed using the

STRING tool. The results are shown in the bubble diagram. (B) Details of G6PD-related genes are listed.

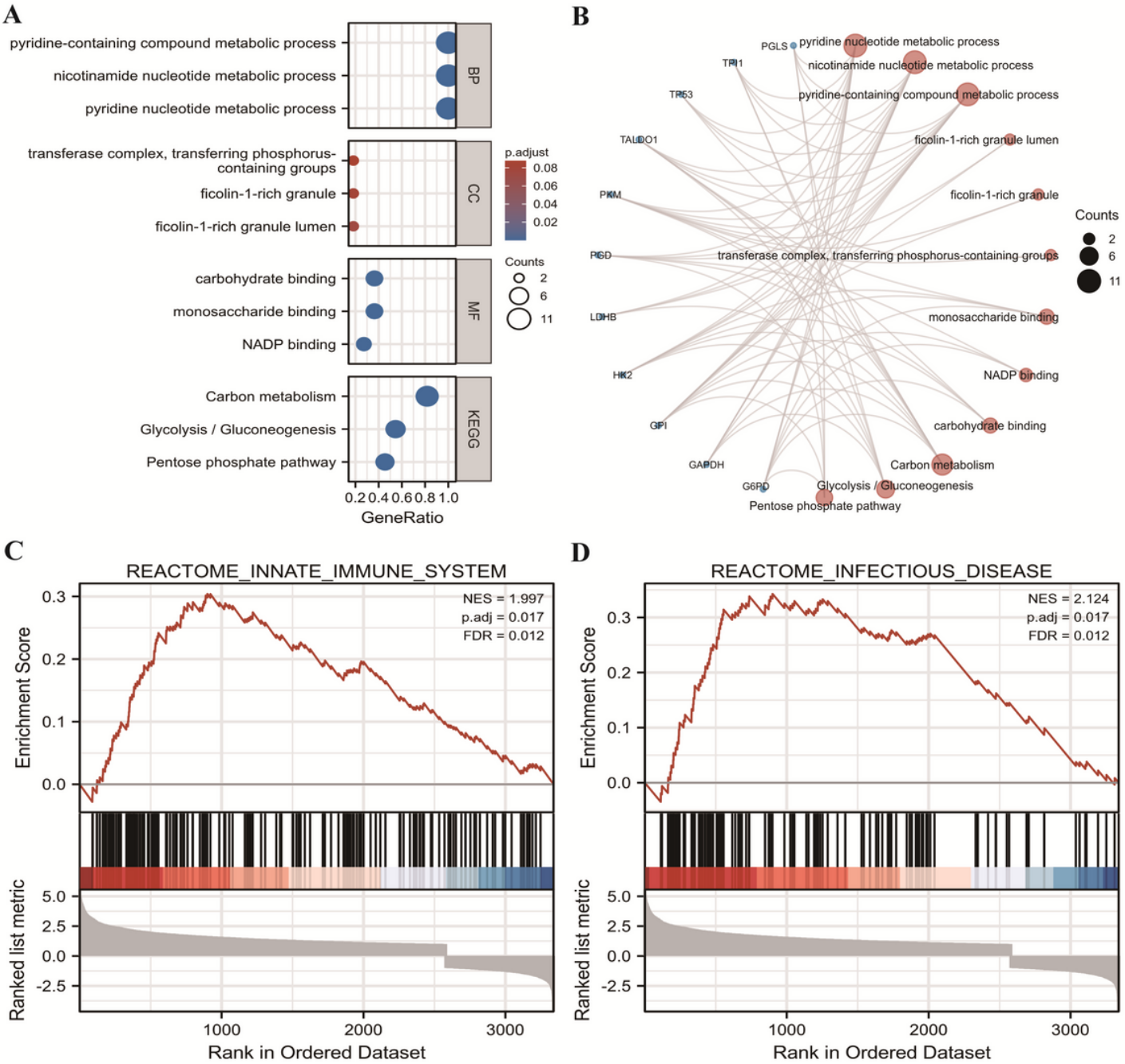


Figure 7

Enrichment analysis of G6PD.

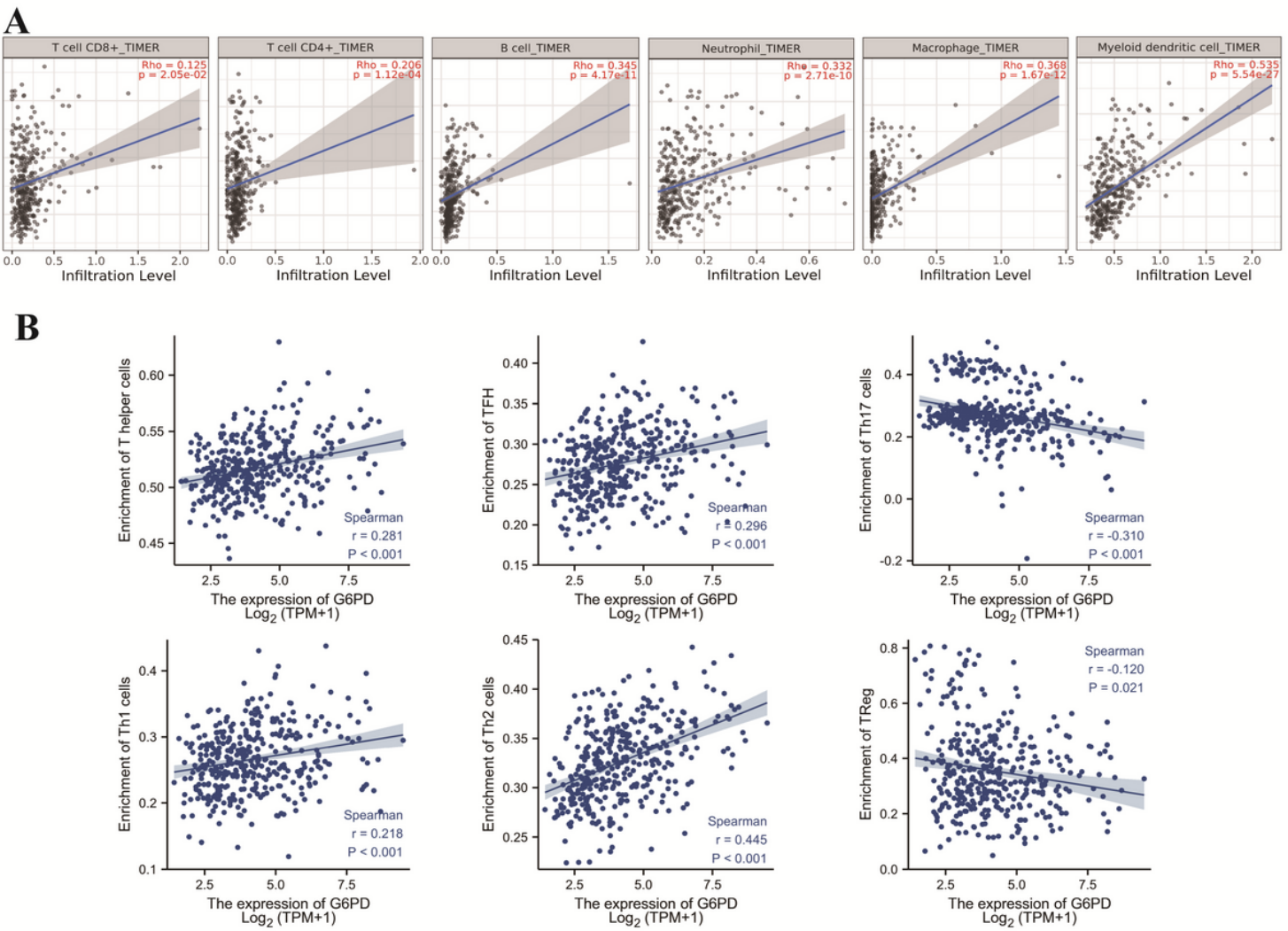


Figure 8

Correlation of G6PD expression with immune cell infiltration in HCC. (A)Correlation of G6PD expression with tumor purity and six immune cells was analyzed by TIMER database. (B) Correlation between G6PD and other immune cells was calculated by ssGSEA.