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

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**Abstract:** *Objective*: To investigate the correlation between the expression of glucose-6-phosphate dehydrogenase (G6PD) and the clinicopathological characteristics, prognosis and immune cell infiltration of hepatocellular carcinoma (HCC). *Methods*: The expression of G6PD in liver cancer tissues and normal tissues is extracted from TCGA and GEO databases, validated by immunohistochemistry, and the correlation between G6PD expression and clinical features is analyzed. 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 for 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. *Results:* 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, dendritic cells, B cells, and follicular helper T cells in the tumor microenvironment. *Conclusion:* High expression of G6PD is a potential biomarker for poor prognosis of hepatocellular carcinoma, and G6PD may be a target for immunotherapy of HCC.

**Keywords:** Hepatocellular carcinoma; G6PD; Immunization; Prognosis; Bioinformatics analysis

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## 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 main risk factors for developing hepatocellular carcinoma (HCC).

Surgical resection, liver transplantation and percutaneous hepatic artery embolization are still the main treatment modalities for HCC, with a 5-year recurrence rate exceeding 70–80% [2]. HCC is prone to relapse, metastasis is the main cause of death, and to date, there is no effective treatment for metastatic HCC. Clinically proven poor response of hepatocellular carcinoma to molecularly targeted drugs (sorafenib, etc.) with 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 metabolic 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 important component of cellular energy metabolism. It is important for maintaining carbon homeostasis, providing precursors for nucleotide and amino acid biosynthesis, supplying reducing molecules for anabolism, and resisting oxidative stress [6]. *G6PD* is an prominent rate-limiting enzyme in PPP [8], Involves 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 is involved in various metabolic reactions as a hydrogen donor such as tetrahydrofolate, biotransformation of drugs and hormones, and maintenance of reduced glutathione(GSH) [6]. This study analyses the level of *G6PD* expression and prognostics of HCC patients in the Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/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 hepatocellular carcinoma.

## 2. Methods

### 2.1. Data collection

Gene expression profile tertiary data of hepatocellular carcinoma 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 due to the fact that 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 $\chi^2$ test. The diagnostic and prognostic value of *G6PD* in HCC patients was analyzed using the subject operating characteristic curve (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 clusterProfiler 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 liver cancer based on TCGA

| Characteristics | 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%) | |

**Table 1 (Continued)**

| Characteristics | Expression of G6PD | | *P*-value |
|---|---|---|---|
| | Low (*n* = 187) | High (*n* = 187) | |
| Child-Pugh grade, n (%) | | | |
| A | 123 (51%) | 96 (39.8%) | |
| B | 11 (4.6%) | 10 (4.1%) | 0.899 |
| C | 1 (0.4%) | 0 (0%) | |
| Fibrosis Ishak core, n (%) | | | |
| 0 | 52 (24.2%) | 23 (10.7%) | |
| 1/2 | 17 (7.9%) | 14 (6.5%) | 0.080 |
| 3/4 | 15 (7%) | 13 (6%) | |
| 5/6 | 40 (18.6%) | 41 (19.1%) | |
| T stage, n (%) | | | |
| T1 | 107 (28.8%) | 76 (20.5%) | |
| T2 | 37 (10%) | 58 (15.6%) | 0.007 |
| T3 | 37 (10%) | 43 (11.6%) | |
| T4 | 4 (1.1%) | 9 (2.4%) | |
| Histologic grade, n (%) | | | |
| G1 | 39 (10.6%) | 16 (4.3%) | |
| G2 | 98 (26.6%) | 80 (21.7%) | < 0.001 |
| 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%) | |
| Stage II | 36 (10.3%) | 51 (14.6%) | 0.017 |
| 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), 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), gastric 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), renal pheochromocytic carcinoma (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**).

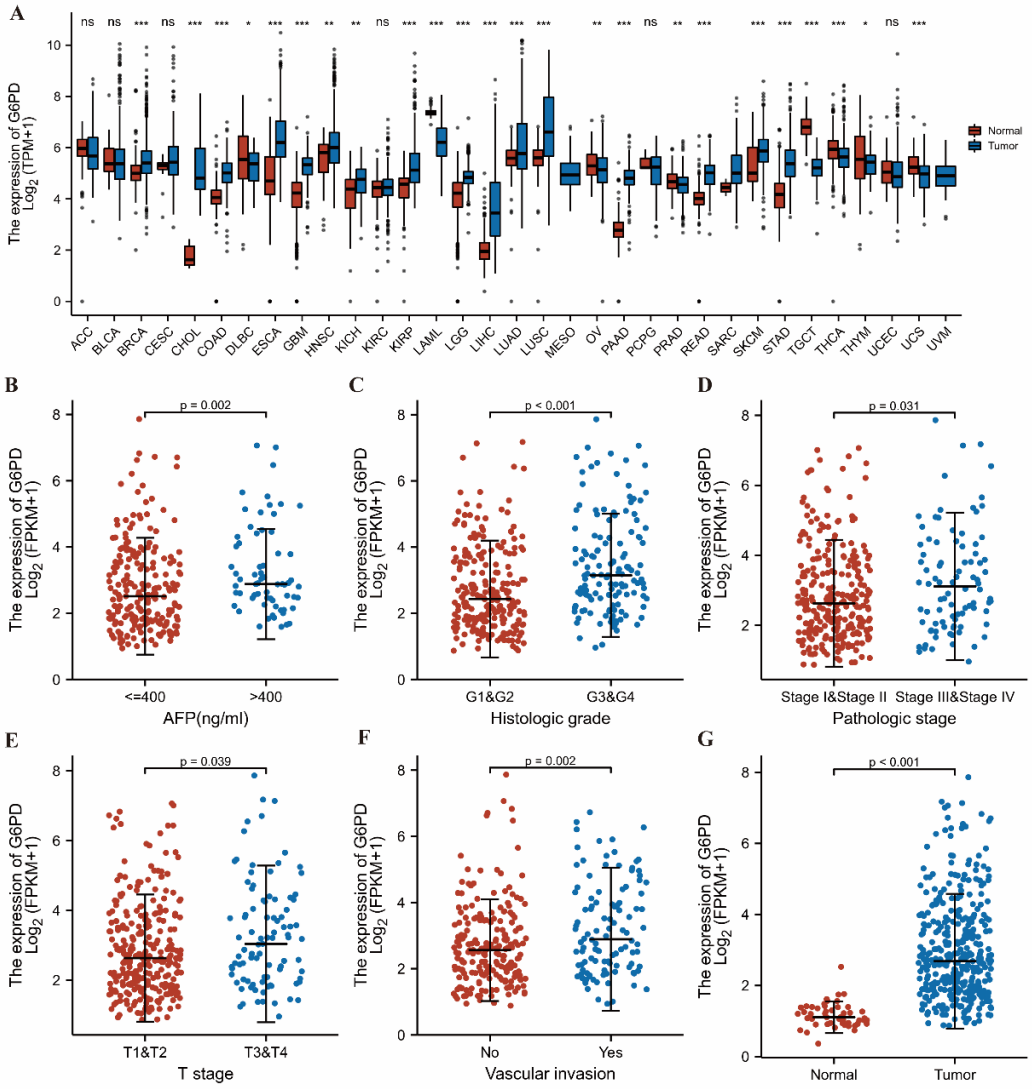



**Figure 1.** Expression levels of G6PD in cancer. (A) The expression levels of G6PD in different cancer tissues are different from the corresponding normal tissues. (B–F) Association between G6PD and clinical manifestations of HCC. The results showed that high expression of G6PD was associated with 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 ($p < 0.01$).

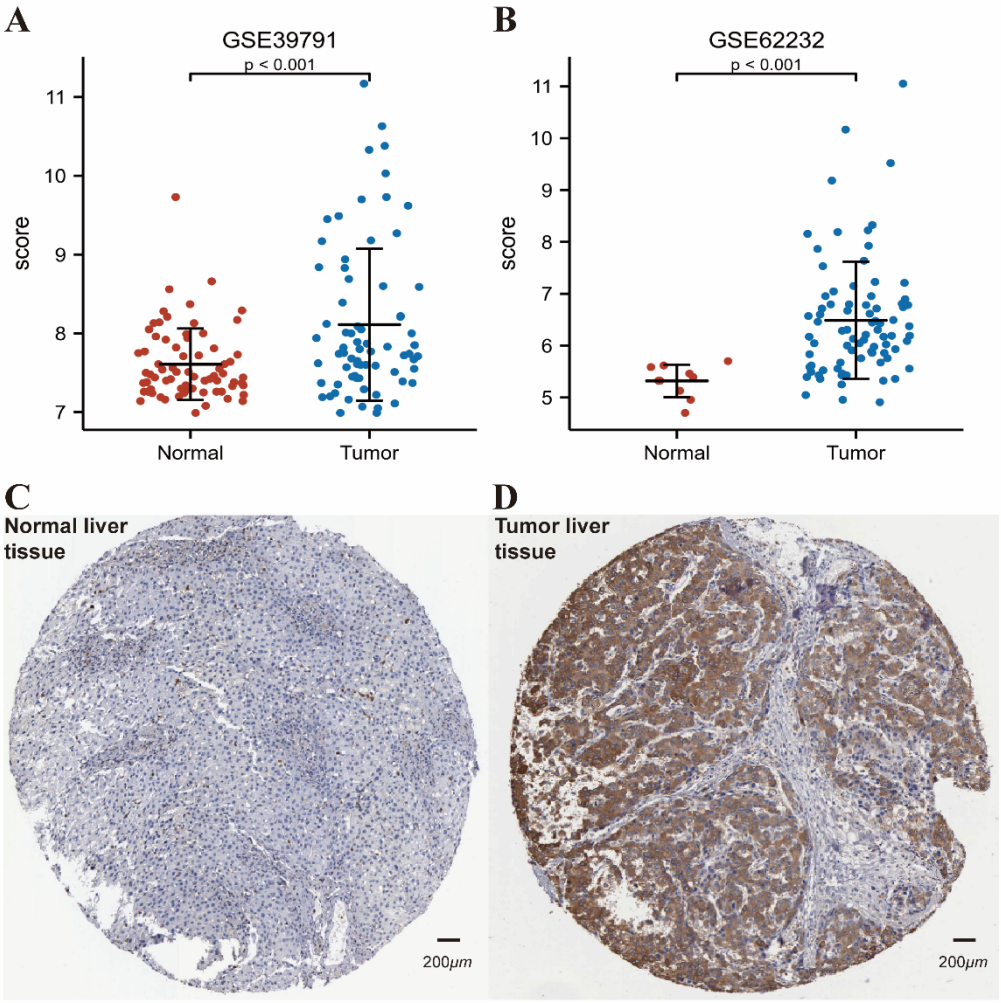



**Figure 2.** (A–B) *G6PD* is overexpressed in HCC. *G6PD* expression was higher in GSE 39791 and GSE 62232 than in normal tissues ($p < 0.001$). (C–D) The level of *G6PD* protein was higher in HCC than in normal tissues.

## 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**).

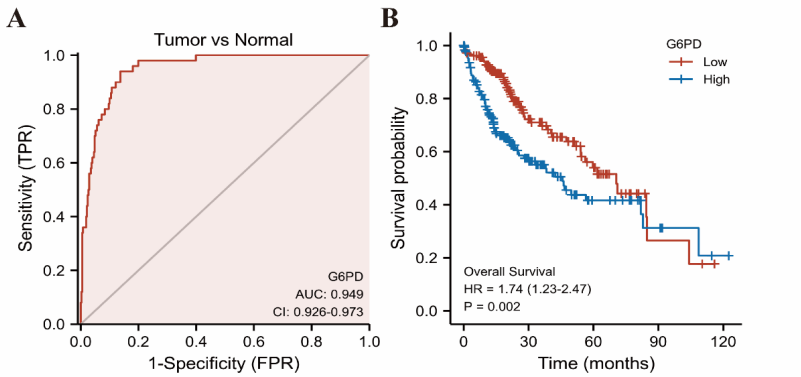



**Figure 3.** (A) ROC curve and Kaplan-Meier curve of G6PD. ROC curve analysis showed that G6PD was able to accurately identify tumor and normal tissue with an AUC of 0.949; (B) Kaplan-Meier survival curve showed poor prognosis of HCC patients with higher G6PD levels.

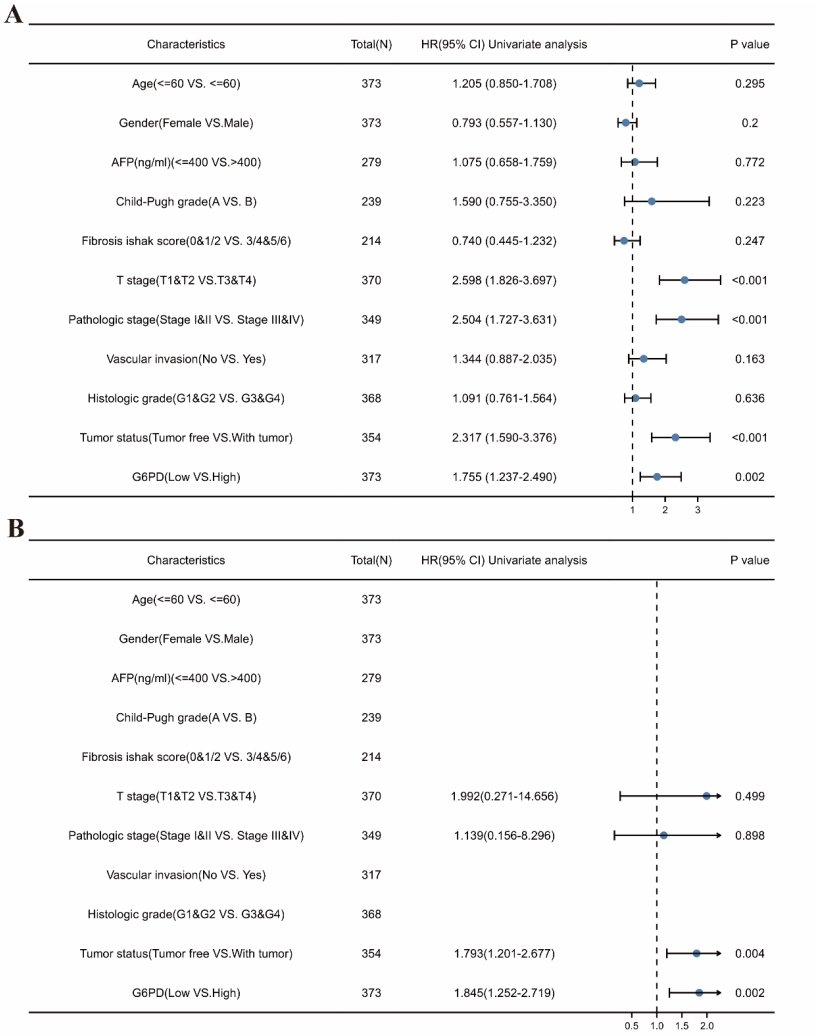



**Figure 4.** Univariate and multifactorial regression analyses were calculated for *G6PD* expression and other OS clinicopathological factors in HCC. (A) The univariate Cox regression model. Among these factors, T-stage, tumor status, and pathological stage were determined to be statistically significantly 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.

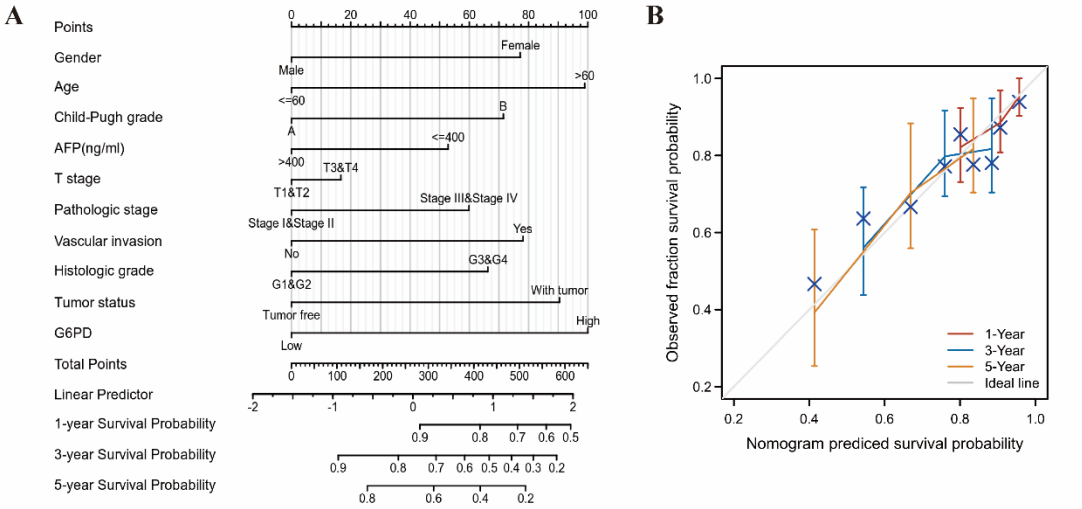
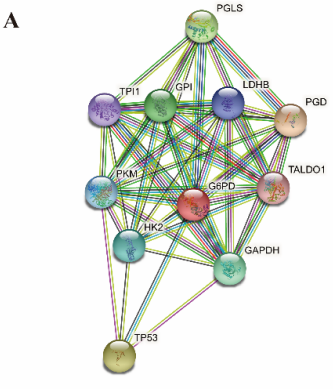



**Figure 5.** Nomogram plot: 1, 3, and 5-year survival rates of HCC patients can be predicted based on the column line plot.

## 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 clusterProfiler 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).




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.** (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.

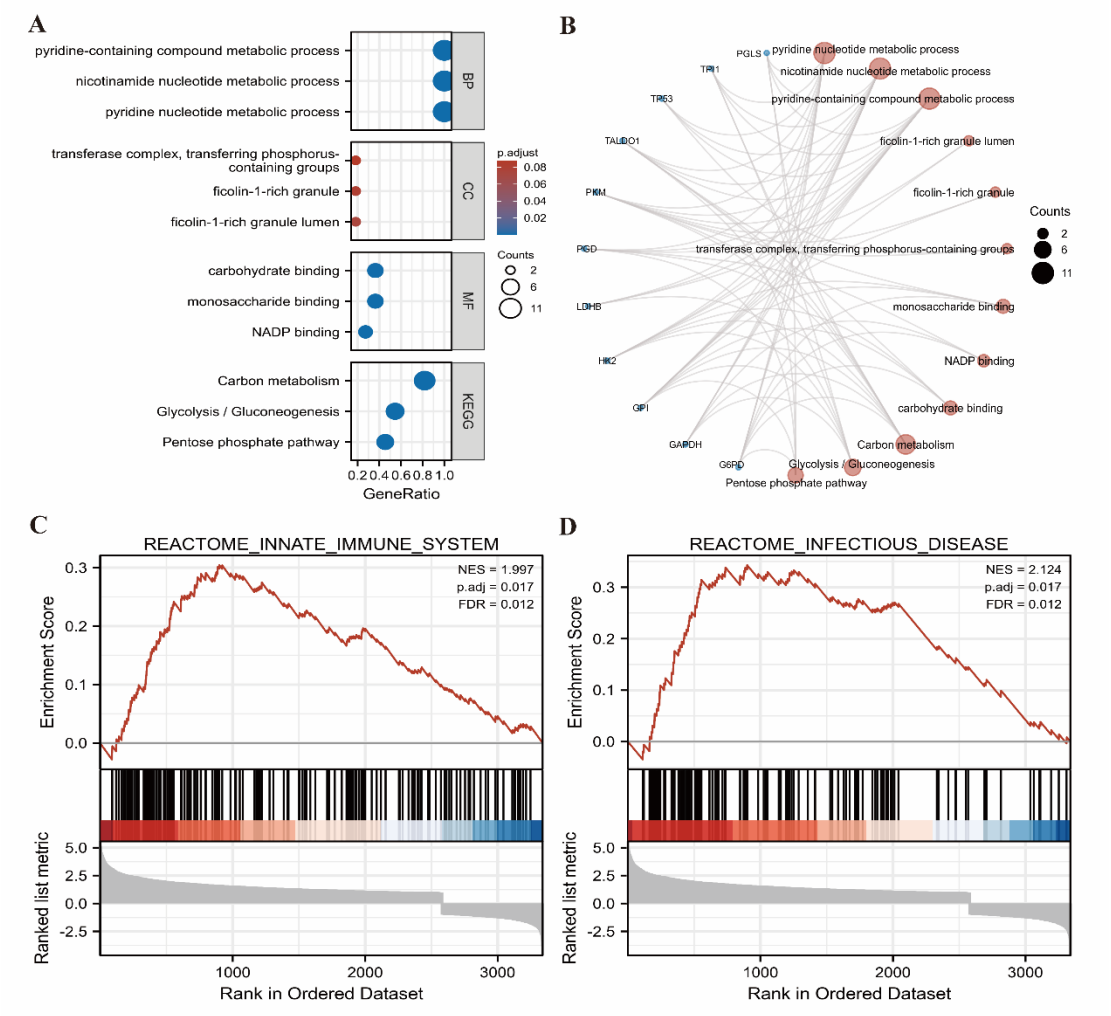



**Figure 7.** Enrichment analysis of *G6PD*.

## 3.5. Correlation analysis of immune cell infiltration

Hepatocellular carcinoma is considered to be an immunogenic tumor, which is closely associated with viral infection and inflammatory environment. TIMER data suggested statistically significant differences between *G6PD* and B cells, CD4+ T cells, CD4+ T cells, neutrophils, macrophages, and dendritic cells (all $p < 0.05$). Compared with *G6PD* low expression group, B cells, CD4+ T cells, CD4 + 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 ($p < 0.05$. In contrast, Th17 cells and TReg cells ($p < 0.01$) were decreased in the high expression group compared to the low expression group.

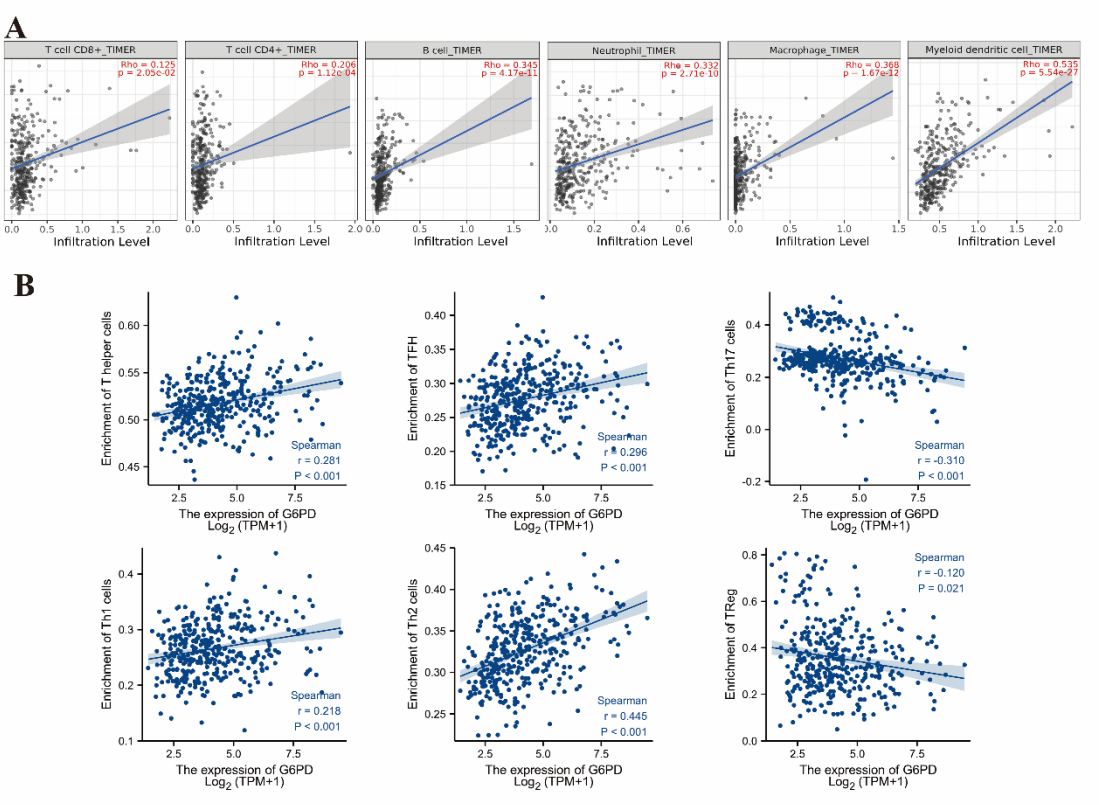



**Figure 8.** (A) Correlation of *G6PD* expression with immune cell infiltration in HCC. Correlation of *G6PD* expression with tumor purity and six immune cells was analyzed by TIMER database. (B) Correlation between *G6PD* and other immune cells was calculated by ssGSEA.

## 4. Discussion

Recurrence and metastasis of HCC are still the primary causes of patients' death, and these factors are closely related to the proliferation and invasion capacities of tumor cells. Recently, a lot of progress has been made in the pathogenesis of HCC. Therefore, exploring new therapeutic targets for hepatocellular carcinoma is of great significance to improve postoperative survival rates and to ameliorate 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, generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), which help ameliorate tumor cell proliferation and metastasis, as well as regulate the cell cycle and apoptosis. Severe deficiency of *G6PD* can impair embryonic development and retard the growth of the organism. 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 a potential 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. 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 [18]. Chen J et al. showed that *G6PD*-based metabolic markers can be used as predictors of prostate cancer metastasis [19]. *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 compared with G6PD-deficient nude mice [20]. Hong et al. 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 [21]. Yin X et al. found that Inhibitor of Differentiation 1(ID1) in HCC cells leads to reduce *G6PD* and NADPH activity and increase ROS production, and transfection of *G6PD* into ID1 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 [22]. 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 longer 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 hepatocellular carcinoma. 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 (0.645–0.728). 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, P53 has become a key antitumor factor since its discovery, and P53 can be activated under conditions of genotoxic stress, oncogene activation, ribosomal stress, hypoxic state, and abnormal energy metabolism. Furthermore, the MDM2-p53 axis may play a dual role in hepatocyte glycolipid metabolism, which is manifested by enhanced glycolipid catabolism, but in early and late stages of the disorder, promoting hepatocyte injury in a study by Cao H et al. [23] The MDM2-p53 axis may play a dual role in hepatocyte glycolipid metabolism, which is manifested by enhanced glycolipid catabolism, but in early and late stages of the disorder, promoting hepatocyte injury [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 [23]. 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 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 proliferation 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 maintaining cellular energy metabolism in a redox homeostasis. In tumor cells, making PPP at high levels is able to induce 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, but the body has to compete with tumor cells for these nutrients through 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 a 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. found that the G6PD-NADPH redox system plays an important role in the stability and metabolism of hexokinase 2 (HK2) in activated T cells [36]. 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 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, and 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 this 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.

## Disclosure statement

The authors declare no conflict of interest.

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