

Original Research

Inhibition of G6PD-Mediated MAPK Signaling Triggers Mitochondrial Apoptosis and Cell Cycle Arrest in Hepatocellular Carcinoma

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) has been implicated in tumor progression. This study investigated the biological role of G6PD in the malignant progression of hepatocellular carcinoma (HCC) and explored the underlying molecular mechanisms. **Methods:** RNA-sequencing data and corresponding clinical data for patients with HCC were obtained from The Cancer Genome Atlas (TCGA). Independent validation datasets were obtained from Gene Expression Omnibus (GEO). We performed bioinformatics analyses to assess the expression of G6PD in HCC, as well as its relationship with clinical features and prognostic outcomes. Immunohistochemistry and western blotting were used to validate G6PD expression in human HCC tissues and cell lines. Stable G6PD-overexpressing and knockdown cell lines were established using lentiviral transduction. Functional assays were performed using G6PD-overexpressing and G6PD-knockdown cell lines to evaluate cell proliferation, cell cycle progression, apoptosis, mitochondrial function, and oxidative stress. Western blotting was conducted to assess the protein levels of key regulators of cell cycle progression, apoptosis, and the MAPK pathway. Finally, a BALB/c nude mouse xenograft model was used to evaluate the *in vivo* effects of G6PD on tumor growth. **Results:** G6PD was significantly overexpressed in HCC tissues and was significantly associated with histological grade ($p < 0.001$), T stage ($p = 0.039$), vascular invasion ($p = 0.002$), and pathological stage ($p = 0.031$). Patients with high G6PD expression exhibited significantly shorter overall, disease-specific, and progression-free survival. Multivariate analysis identified high G6PD expression as an independent predictor of mortality in patients with HCC. G6PD knockdown significantly inhibited HCCLM3 cell proliferation and induced G0/G1 cell-cycle arrest, accompanied by the downregulation of cyclin-dependent kinase 4 (CDK4), cyclin-dependent kinase 6 (CDK6), and cyclin D1 protein levels. In addition, G6PD silencing triggered mitochondrial apoptosis, including decreased mitochondrial membrane potential, BCL-2-associated X protein (BAX) and cysteine-aspartic acid protease-3 (caspase-3) upregulation, B-cell lymphoma 2 (BCL-2) downregulation, elevated reactive oxygen species (ROS) levels, and suppressed MEK1/2 and ERK1/2 phosphorylation. *In vivo*, G6PD knockdown significantly reduced xenograft tumor size and weight and decreased Ki67 and proliferating cell nuclear antigen (PCNA) expression. **Conclusions:** G6PD is an independent prognostic biomarker in HCC and promotes disease progression through pleiotropic mechanisms, including modulation of the MAPK/ERK pathway, promotion of cell proliferation, and suppression of mitochondrial apoptosis. These findings suggest that G6PD and its downstream signaling pathways may be promising therapeutic targets for HCC.

Keywords: hepatocellular carcinoma; glucose-6-phosphate dehydrogenase; apoptosis; cell cycle; MAPK

1. Introduction

Hepatocellular carcinoma, the most prevalent histological subtype of primary liver cancer, is one of the leading causes of cancer-related mortality worldwide and poses a major public health challenge [1]. The International Agency for Research on Cancer estimated approximately 865,000 new cases and 758,000 deaths from liver cancer in 2022, ranking hepatocellular carcinoma (HCC) third among all malignancies in terms of mortality [2,3]. Despite advances in surgical resection, liver transplantation, local ablation, and multitargeted systemic therapies, the 5-year overall survival rate for HCC remains <20%, since a considerable number of patients are diagnosed at intermediate or advanced stages [4]. Moreover, HCC is characterized by pronounced molecular heterogeneity and aggressive bi-

ological features. These traits continue to constrain long-term patient survival, and overall outcomes have seen little improvement. Meanwhile, emerging technologies such as single-cell sequencing, spatial transcriptomics, and integrated multi-omics approaches have deepened our insight into the molecular mechanisms that drive HCC pathogenesis [5,6]. Consequently, identifying reliable biomarkers and actionable therapeutic targets remains a critical priority for improving the diagnosis and treatment of HCC. Among the biological processes that contribute to HCC progression, dysregulated cell cycle control is a fundamental mechanism underlying uncontrolled tumor growth.

Cell cycle progression is tightly regulated by checkpoint mechanisms, including the G1/S, S, and G2/M checkpoints. Dysregulation of these pathways is a hallmark of



malignant transformation. Cell cycle progression is coordinated by core cell cycle proteins, cyclins, and cyclin-dependent kinases (CDKs), whose expression and activity vary throughout the cell cycle [7]. Cyclin D1 forms complexes with cyclin-dependent kinase 4 (CDK4) and CDK6 to regulate G1/S transition, and aberrant activation of the Cyclin D–CDK4/6–Rb pathway has been implicated in numerous malignancies, including breast, pancreatic, ovarian, and thyroid cancers [8,9,10]. Because cell cycle dysregulation is a key driver of malignant tumor cell proliferation, a deeper understanding of the underlying regulatory mechanisms may facilitate the identification of novel therapeutic targets to inhibit tumor growth. Among the metabolic pathways implicated in tumor growth, the pentose phosphate pathway (PPP) has emerged as an important regulator of cellular proliferation, redox homeostasis, and therapeutic resistance.

The PPP is an alternative route of glucose metabolism that provides essential substrates for biosynthesis and contributes to cellular antioxidant defenses [11]. Glucose-6-phosphate dehydrogenase (G6PD) is a major rate-controlling enzyme of the PPP. Located in the cytoplasm of blood cells, G6PD helps protect cells against oxidative damage. PPP provides NADPH to maintain glutathione in its reduced form, enhances antioxidant capacity, and promotes cell proliferation [12]. Increasing evidence indicates that G6PD is overexpressed in multiple malignancies, including melanoma, leukemia, colorectal cancer, and lung cancer, where it contributes to tumor progression [13]. Moreover, modulation of G6PD activity influences therapeutic resistance in several cancers, including ovarian, breast, and colorectal cancers, highlighting its potential as a therapeutic target [14,15,16,17]. Increased G6PD activity is associated with liver cancer metastasis, and elevated G6PD expression has been shown to promote HCC cell migration and invasion by inducing epithelial-mesenchymal transition (EMT) [18]. Furthermore, G6PD upregulation enhances PPP flux, resulting in substantial NADPH production, which reduces intracellular reactive oxygen species (ROS) levels and lipid peroxidation to suppress ferroptosis, ultimately leading to resistance to systemic treatments such as sorafenib [19]. However, the role of G6PD in regulating the biological functions of HCC is still not fully understood.

The present study evaluated the clinical significance of G6PD in HCC and investigated its regulatory role and underlying mechanisms in tumor cell proliferation, cell cycle progression, and apoptosis. In addition, this study explored the underlying molecular mechanisms, with particular emphasis on MAPK/ERK signaling, to assess the potential of G6PD as a prognostic biomarker in HCC.

2. Materials and Methods

2.1 Materials and Reagents

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Viva Cell (C3103, Shanghai, China). Fe-

tal bovine serum (FBS) was obtained from ExCell Bio (FSP500, Suzhou, Jiangsu, China). Trypsin-EDTA digestion solution (T1320) and puromycin (P8230) were purchased from Solarbio (Beijing, China). The Rabbit Two-Step Detection Kit (PV-9001) and DAB Chromogen Kit (ZLI9018) were obtained from ZSGB-BIO (Beijing, China).

Cell proliferation, cell cycle, apoptosis, mitochondrial membrane potential, and ROS analyses were performed using the Cell Counting Kit-8 (CCK-8) Kit (B34304, Biomake, Houston, TX, USA), BeyoClick™ EdU-555 Cell Proliferation Kit (C0075S, Beyotime, Shanghai, China), Cell Cycle Assay Kit (C1052, Beyotime), Apoptosis Assay Kit (C1062S, Beyotime), Mitochondrial Membrane Potential Assay Kit (C2006, Beyotime), and Reactive Oxygen Species Assay Kit (S0033M, Beyotime), respectively.

RNA extraction and quantitative PCR were performed using the MolarPure Cell/Tissue Total RNA Kit (19221ES, Yeasen Biotechnology, Shanghai, China), PrimeScript™ RT Master Mix (RR036A, Takara Bio, Kusatsu, Shiga, Japan), and TB Green® Premix Ex Taq™ (Tli RNaseH Plus) (RR820A, Takara Bio). Protein extraction and quantification were performed using the Total Protein Extraction Kit (KGB5303, KeyGEN BioTECH, Nanjing, Jiangsu, China) and BCA Protein Assay Kit (KGB2101, KeyGEN BioTECH). Protein electrophoresis was performed using the PAGE Gel Fast Preparation Kit (PG110, YaZen, Shanghai, China).

The primary antibodies used in this study were as follows: rabbit anti-G6PD (ab210702, Abcam, Cambridge, UK; 1:1000), rabbit anti-BCL-2 (ab182858, Abcam; 1:2000), rabbit anti-BAX (ab182733, Abcam; 1:2000), rabbit anti-Caspase-3 (ab32351, Abcam; 1:5000), rabbit anti-CDK4 (ab108357, Abcam; 1:5000), rabbit anti-CDK6 (ab222395, Abcam; 1:5000), rabbit anti-Cyclin D1 (ab16663, Abcam; 1:200), rabbit anti-p-MEK1/2 (9154, Cell Signaling Technology, Danvers, MA, USA; 1:1000), rabbit anti-MEK1/2 (9122, Cell Signaling Technology; 1:1000), rabbit anti-p-ERK1/2 (AF1015, Affinity Biosciences, Changzhou, Jiangsu, China; 1:1000), rabbit anti-ERK1/2 (11257-1-AP, Proteintech, Wuhan, Hubei, China; 1:2000), and rabbit anti-β-actin (ab213262, Abcam; 1:10,000).

2.2 The Cancer Genome Atlas (TCGA) Database

RNA-sequencing data and corresponding clinicopathological information for patients with HCC were obtained from the TCGA Liver Hepatocellular Carcinoma (TCGA-LIHC) database (<https://www.cancer.gov/tcga>). Expression data were log₂-transformed before analysis. We used R (version 4.2.1, The R Foundation for Statistical Computing, Vienna, Austria) with the stats and car packages to compare G6PD expression levels between HCC and normal liver samples, as well as between matched tumor and adjacent non-tumor tissues. The results were plotted

with the ggplot2 package. Receiver operating characteristic (ROC) analysis was performed using the pROC package. Survival analyses and Cox regression were performed using the survival and survminer packages, respectively. Prognostic nomograms were constructed and visualized using the rms package.

2.3 Gene Expression Omnibus (GEO) Database

We retrieved the GSE39791 and GSE62232 datasets from the GEO repository (<https://www.ncbi.nlm.nih.gov/gco/>) via the GEOquery package. GSE39791 includes 72 tumor and 72 normal samples, whereas GSE62232 contains 81 tumor and 11 normal samples. Differential G6PD expression between tumor and normal tissues was analyzed using the limma package with unpaired two-tailed Student's *t*-tests. Data are presented as mean $\pm$ standard error of the mean (SEM), and the threshold for statistical significance was set at $p < 0.05$. Data were visualized with the aid of ggplot2.

2.4 TIMER2.0 Database

G6PD expression levels in multiple tumor types were analyzed using TIMER2.0 (<http://timer.cistrome.org/>).

2.5 Immunohistochemistry

Paraffin-embedded sections were prepared from mouse xenograft tumors, human HCC, and paired adjacent noncancerous tissues. Sections were deparaffinized, rehydrated sequentially with xylene and ethanol, and blocked with goat serum for 1 hour at room temperature. The sections were then incubated overnight at 4 °C with primary antibodies against G6PD, proliferation Marker Ki67 (Ki67), and proliferating cell nuclear antigen (PCNA). After washing, sections were incubated with enhanced enzyme-labeled goat anti-rabbit immunoglobulin G (IgG) polymer for 1 hour at room temperature. The sections were then washed three times with phosphate-buffered saline (PBS), followed by incubation with 3,3'-diaminobenzidine (DAB) chromogen solution for 3–5 minutes. Finally, sections were counterstained with hematoxylin, dehydrated, mounted, and photographed. The collection and use of human tissues were approved by the Ethics Committee of the General Hospital of Ningxia Medical University (Approval No. KYLL-2022-0412), and written informed consent was obtained from all participating patients.

2.6 Cell Culture

Human HCC lines HCCLM3, MHCC97L, MHCC97H, Hep3B, and Huh-7, as well as LO-2 cells, were obtained from the Liver Disease Research Institute, Affiliated Hospital of Fudan University, Shanghai. All cells were cultured in high-glucose DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, and maintained at 37 °C with 5% CO₂. All cell lines used in the experiments were vali-

dated by STR profiling, and mycoplasma contamination test results were negative. Cells were passaged when they reached 80–90% confluence. All experiments were performed using cells at passages 5–15 to ensure stable phenotypes and consistent experimental outcomes.

2.7 Lentiviral Transduction

HCCLM3 cells were inoculated into six-well plates. They were then incubated at 37 °C in an atmosphere of 5% CO₂. We performed lentiviral transduction once the cells attained 30–50% confluence. The lentiviral solution was thawed on ice before being gently pipetted into the mix. An appropriate volume of viral stock was added to six-well plates according to the multiplicity of infection (MOI) value. We aspirated the virus-laden supernatant at 12 hours post-transduction and supplied fresh medium to the cells. To obtain stable transductants from each group, we applied puromycin selection at a concentration of 2 µg/mL. All lentiviruses used in this study were designed and constructed by Beijing Tsingke Biotechnology Co., Ltd.

2.8 5-Ethynyl-2'-Deoxyuridine (EdU) Assay

Cell proliferation was assessed using a BeyoClick EdU-555 Cell Proliferation Kit. HCCLM3 cells were cultured in 24-well plates for 24 hours. Cells were then incubated with the prepared EdU working solution at 37 °C for 2 hours. We then treated the cells with 4% paraformaldehyde for 15 minutes to achieve fixation, followed by permeabilization using 0.3% Triton X-100 for 10 minutes. Click reaction solution was then added, and the cells were incubated in the dark for 30 minutes, followed by Hoechst 33342 staining in the dark for 5 minutes. Finally, images were captured using an inverted fluorescence microscope.

2.9 Cell Counting Kit-8 (CCK-8) Cell Proliferation Assay

Stably transfected HCCLM3 cells in log-growth phase were digested with trypsin and seeded into 96-well plates at a density of 2000 cells/well. After allowing 12 hours for cell attachment, 110 µL of CCK-8 detection solution (prepared by mixing 10 µL of CCK-8 reagent with 100 µL of complete culture medium) was added to each well. After incubation at 37 °C for 1 hour, the plates were read at 450 nm on a microplate reader to collect the measurement. Subsequent measurements were performed at 24, 48, 72, and 96 hours.

2.10 Colony Formation Assay

Stably transfected HCCLM3 cells in log-growth phase were digested with trypsin. We stopped the digestion by adding complete culture medium, then gently pipetted the cells to create a uniform cell suspension. We then seeded the cells into 6-well plates (1000 cells/well) and maintained them at 37 °C in a 5% CO₂ incubator for a period of two weeks. The cells were rinsed with PBS in three cycles and subsequently incubated with 4% paraformaldehyde for 20 minutes for fixation. After three additional PBS rinses, the

colonies were incubated with crystal violet for 15 minutes. The surplus stain was then eliminated by washing with water. Finally, images were captured.

2.11 Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from cells using the Mol-
larPure Cell/Tissue Total RNA Kit, and the concentration
was measured. Reverse transcription was performed us-
ing Takara PrimeScript™ RT Master Mix, followed by RT-
qPCR on a Thermo Fisher Scientific PCR system. All
primers were designed and synthesized by Beijing Tsingke
Biotechnology Co., Ltd. (Beijing, China).

Listed below are the primer sequences (Table 1):

Table 1. Primer sequences for G6PD and GAPDH.

Gene		Primer Sequence (5'-3')
G6PD	Forward	CGAGGCCGTCACCAAGAAC
	Reverse	GTAAGTGGTCGATGCGGTAGA
GAPDH	Forward	GGCAAATTCATGGCACCGTCAAGG
	Reverse	GCCAGCATCGCCCCACTTGATTTTG
G6PD, Glucose-6-phosphate dehydrogenase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.		

The reaction conditions are as follows (Table 2):

Table 2. Thermal cycling conditions for RT-qPCR.

Steps	Temperature	Time	Cycle
Initial denaturation	95 °C	30sec	1
PCR reaction	95 °C	5 sec	40
	60 °C	34 sec	
Dissociation	95 °C	15 sec	1
	65 °C	1 min	

2.12 Western Blotting

Culture dishes with >80% cell confluence were lysed
in Lysis Buffer supplemented with protease and phos-
phatase inhibitors to extract the total protein. The protein
concentration was determined using a BCA assay kit, and
a standard curve was generated. Samples containing 15
µg of protein per well were prepared for loading. Pro-
tein samples were run on 10% sodium dodecyl sulfate-
polyacrylamide gel electrophoresis (SDS-PAGE) and then
electroblotted onto polyvinylidene fluoride (PVDF) mem-
branes (pre-activated in methanol). Blocking was done with
5% non-fat milk for 2 hours. The membranes were subse-
quently probed with primary antibodies (target proteins and
β-actin) overnight (14 hours) at 4 °C under gentle shaking.
Following this, HRP-linked secondary antibodies were in-
cubated with the membranes for 2 hours at RT. Finally, im-
munoreactive bands were exposed using a chemilumines-
cent detection kit.

2.13 Gene Set Enrichment Analysis (GSEA)

We stratified patients from the TCGA-LIHC cohort
into high and low G6PD expressers according to the me-
dian value. For enrichment evaluation, GSEA was then em-
ployed to evaluate enrichment.

2.14 Flow cytometry

Cells in good growth conditions from each group were
selected and analyzed using the Beyotime Apoptosis Assay
Kit. Following the wash step, we added 100 µL of bind-
ing buffer (1×) to each sample, followed by gentle pipet-
ting to obtain a homogeneous single-cell suspension. Sub-
sequently, 5 µL of Annexin V-FITC and 5 µL of propidium
iodide (PI) staining solution were added to each sample, and
the mixtures were gently pipetted to ensure thorough mix-
ing. The samples were then incubated in the dark for 10
minutes to allow sufficient staining. After the incubation
period, 400 µL of binding buffer (1×) was applied to each
specimen and mixed well. All specimens were examined
on the flow cytometer within 1 hour. For flow cytometric
analysis, debris and cell aggregates were excluded based on
forward scatter (FSC) and side scatter (SSC) properties. A
minimum of 10,000 events per sample were acquired. Cells
were gated on the FSC-A/SSC-A population, and Annexin
V-FITC and PI fluorescence were measured using appropri-
ate fluorescence detectors.

Cell cycle distribution was analyzed using the Bey-
otime Cell Cycle Assay Kit. Cells were harvested, fixed
in ice-cold 70% ethanol at 4 °C for at least 2 hours, and
then stained with propidium iodide (PI) staining solution
(containing PI and RNase A) at 37 °C for 30 minutes in
the dark. For flow cytometric analysis, debris and cell ag-
gregates were excluded based on forward scatter (FSC) and
side scatter (SSC) properties. Doublets were discriminated
using PI-A versus PI-W gating. A minimum of 10,000
single-cell events per sample were acquired. DNA content
was measured using a flow cytometer at an excitation wave-
length of 488 nm.

2.15 Mitochondrial Membrane Potential Assay

Cells were treated according to the experimental
grouping and seeded in six-well plates (5 × 10⁵ cells/well).
Following the addition of 0.5 mL 5,5',6,6'-tetrachloro-
1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide
(JC-1) working solution per well, the cells were incubated
for 30 minutes at 37 °C. The plate was then centrifuged
at 2000 rpm for 3 minutes, after which the supernatant
was discarded and the cell pellets were re-suspended in
JC-1 buffer. Fluorescent images were taken under a
fluorescence microscope.

2.16 In Vivo Animal Model

A total of 20 male BALB/c nude mice (7 weeks old)
were acquired from Jiangsu Huachuang Sino Pharmaceu-
tical Technology Co., Ltd. The animals were kept in SPF

conditions with a 12-hour light/dark cycle at 25–27 °C and had free access to chow and water in the Laboratory Animal Center of Ningxia Medical University. For the experiment, they were randomized into four groups (5 mice/group). The sample size was selected to account for potential tumor formation failure or unexpected death during the experiment.

Four groups of HCCLM3 cells (control, G6PD-overexpression, shControl, and shG6PD) were cultured separately, and each mouse was subcutaneously inoculated with the corresponding HCCLM3 cells (5×10^6 cells per mouse) into the right forelimb. After four weeks, all mice developed measurable tumors. Subsequently, the animals were euthanized by 100% CO₂ inhalation, followed by cervical dislocation, and tumors were excised for photographic documentation, weighing, and immunohistochemical analysis.

The animal studies were conducted in accordance with Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. Cell inoculation, tumor measurement, and euthanasia were performed within the same time window (9:00–11:00). To reduce measurement errors, the measurements of tumor size and weight were conducted by a researcher who was unaware of the grouping situation. The inclusion criterion was survival after inoculation, without severe infection or ulcers. Exclusion criteria included failure of tumor formation, animal death, or severe cachexia.

2.17 Statistical Analysis

All statistical analyses were performed using R version 4.2.1. For normally distributed quantitative data, comparisons between two groups were performed using unpaired or paired *t*-tests. Chi-square and Fisher's exact tests were used to analyze correlations between G6PD expression and clinicopathological characteristics. Survival analysis was conducted using the Kaplan–Meier method with the log-rank test. Receiver operating characteristic (ROC) curves were employed to evaluate the clinical utility of G6PD as a diagnostic marker. Survival predictors were identified through Cox proportional hazards regression. A nomogram for predicting patient prognosis was constructed based on G6PD expression levels, and its calibration and predictive performance were evaluated using calibration curves. $p < 0.05$ was considered statistically significant.

3. Results

3.1 G6PD is Upregulated in HCC

Pan-cancer analysis using the TIMER2.0 database revealed that G6PD was significantly upregulated in multiple tumor types, including liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), breast invasive carcinoma (BRCA), pancreatic adenocarcinoma (PAAD), colorectal adenocarcinoma (COAD and READ), and stomach adenocarcinoma (STAD), whereas lower expression was observed in selected malignancies, including acute myeloid leukemia

(LAML), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) (Fig. 1A).

Analyses of the TCGA and GEO databases revealed elevated G6PD expression in HCC tissues compared with normal liver tissues, a difference that was statistically significant (Fig. 1B–E). ROC curve analysis indicated good diagnostic efficacy of G6PD in HCC, with an AUC of 0.949 (Fig. 1F). Validation of the findings from public database analyses and immunohistochemistry showed significantly higher G6PD expression in HCC tissues than in the adjacent noncancerous tissues (Fig. 1G). Additionally, Western blot analysis further confirmed higher G6PD expression levels in HCC tissues than in normal liver tissues (Fig. 1H), consistent with the results of the database analyses.

3.2 Abnormal Upregulation of G6PD is Associated With Poor Prognosis in HCC

Analysis of the correlations between G6PD expression and clinical features of HCC from TCGA datasets revealed significant correlations between G6PD expression levels and histologic grade ($p < 0.001$), T stage ($p = 0.039$), vascular invasion ($p = 0.002$), alpha-fetoprotein (AFP) levels ($p = 0.002$), and pathologic stage ($p = 0.031$) (Fig. 2A).

Kaplan–Meier survival curve analysis of the relationship between G6PD and the prognosis of patients with HCC in the TCGA database showed shorter overall, disease-specific, and progression-free survival in patients with high G6PD expression than those with low expression (Fig. 2B).

Univariate analysis preliminarily identified four significant risk factors—T stage, pathologic stage, tumor status, and high G6PD expression—all of which were associated with poor prognosis (Fig. 2C). In multivariate analysis, after controlling for confounders, both tumor status and elevated G6PD levels emerged as independent predictors of poor outcome (hazard ratio [HR] > 1 , $p \leq 0.004$). Notably, high G6PD expression itself was significantly associated with increased mortality risk in the HCC cohort (Fig. 2D).

Analysis of the goodness-of-fit and predictive performance of a nomogram incorporating G6PD expression together with multiple clinical variables to estimate 1-, 3-, and 5-year survival prognosis risk scores in HCC cases demonstrated good predictive capability (Fig. 2E,F).

3.3 G6PD Promotes HCC Cell Proliferation

G6PD expression was significantly higher in MHCC97L, MHCC97H, and HCCLM3 HCC cell lines compared with LO-2 cells (Fig. 3A,B). Because HCCLM3 cells exhibited the highest endogenous expression, they were selected for subsequent gain- and loss-of-function studies. Successful overexpression and knockdown of G6PD were confirmed before functional analyses (Fig. 3C,D).

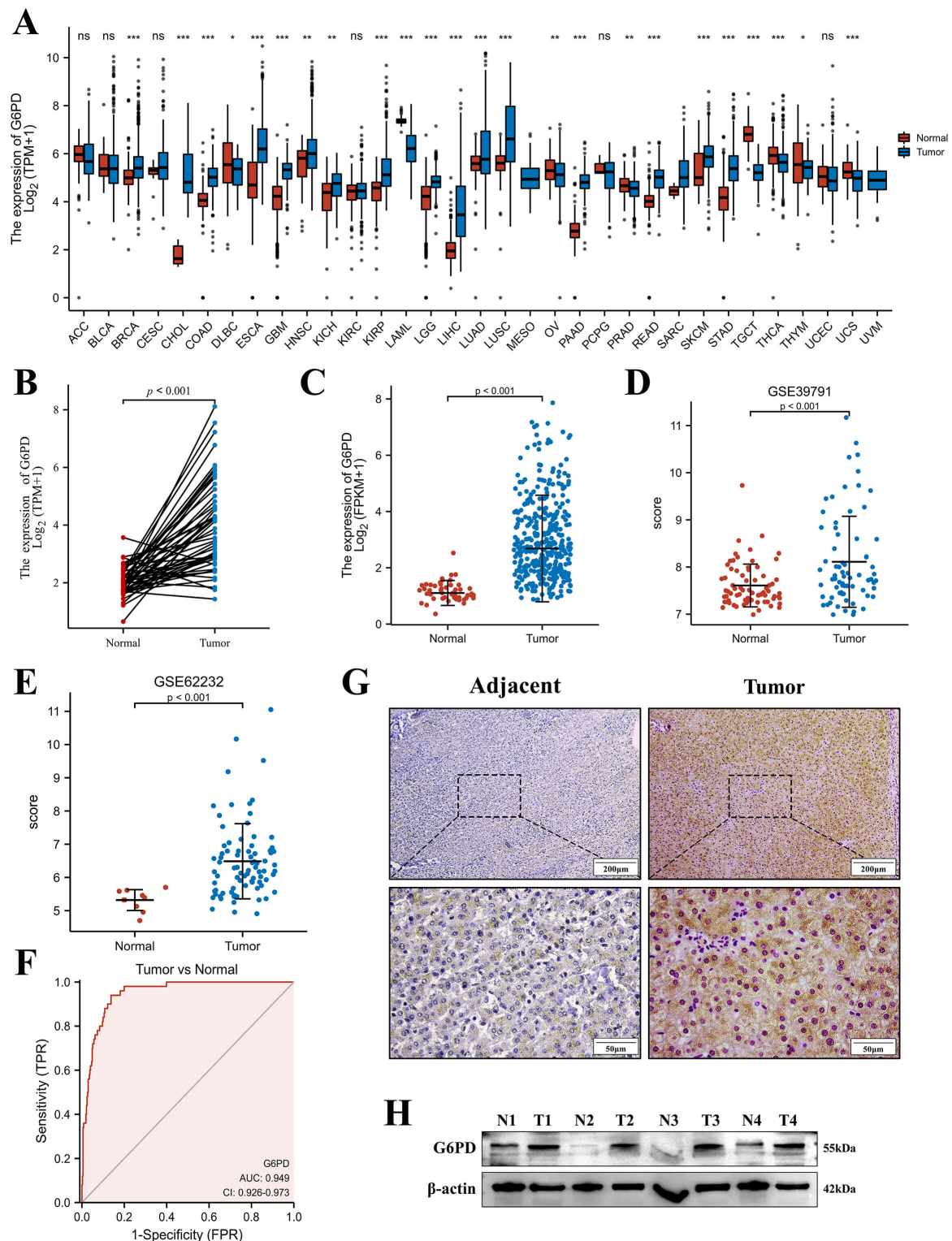


Fig. 1. Expression of G6PD in hepatocellular carcinoma. (A) Expression levels of G6PD across various TCGA cancer types analyzed using the TIMER 2.0 database. (B–E) Differential expression of G6PD between HCC tissues and normal liver tissues based on TCGA and GEO datasets. (F) ROC curve of G6PD expression for distinguishing HCC from non-cancerous tissues in TCGA. (G) Immunohistochemical detection of G6PD protein levels in paired adjacent non-cancerous and HCC tissues. Scale bar: 200 μm (main images) and 50 μm (insets). (H) Western blot analysis of G6PD expression in adjacent non-cancerous liver tissues and HCC tissues (n = 4 per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant. G6PD, glucose-6-phosphate dehydrogenase; TCGA, the Cancer Genome Atlas program; GEO, the Gene Expression Omnibus; HCC, hepatocellular carcinoma; ROC, receiver operating characteristic.

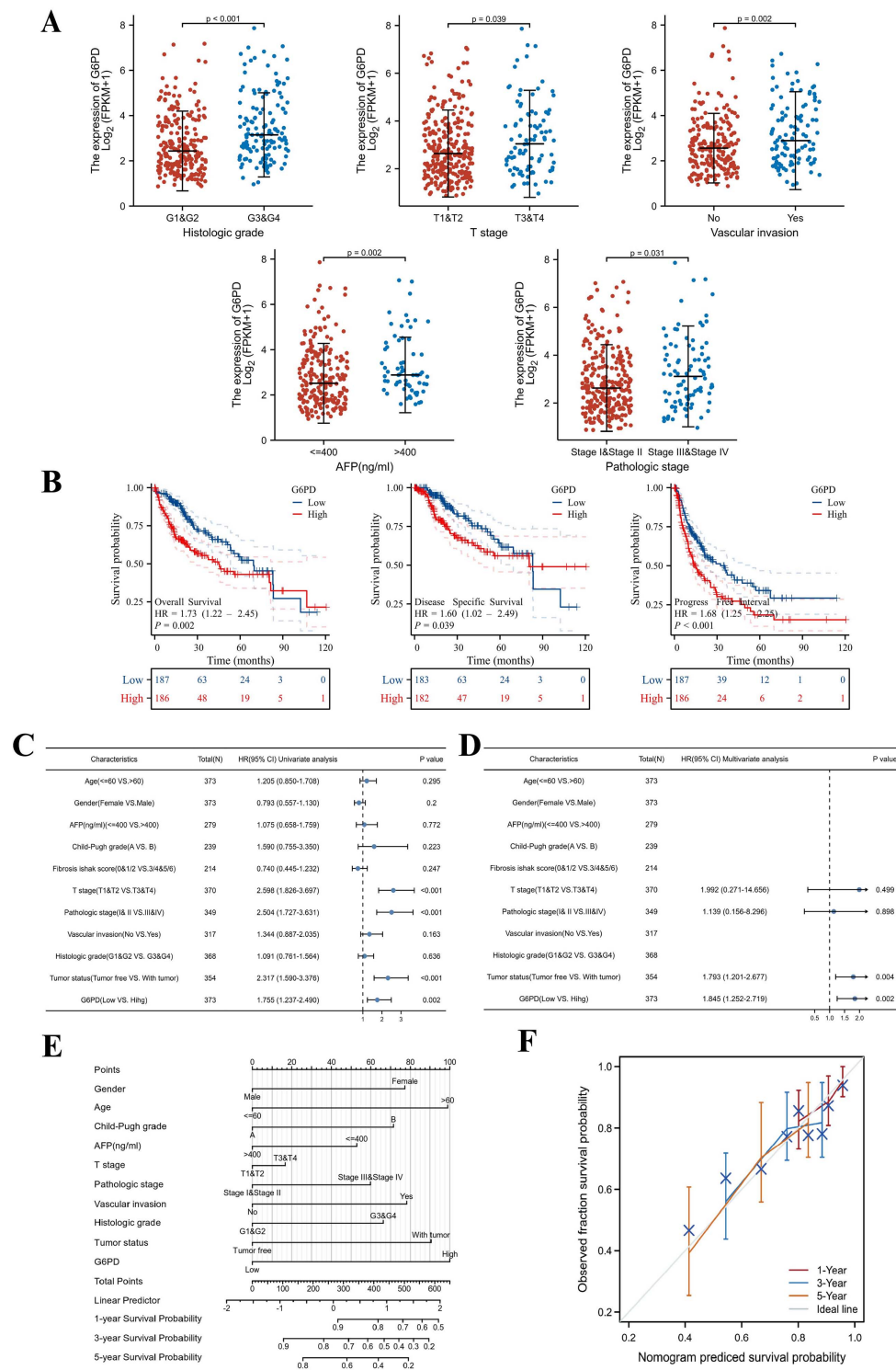


Fig. 2. Correlation between G6PD expression and clinicopathological characteristics in hepatocellular carcinoma. (A) Differential expression of G6PD analyzed using the TCGA database across different histologic grades, T stages, vascular invasion status, AFP levels, and pathologic stages. (B) Kaplan-Meier curves for OS, DSS, and PFI stratified by G6PD expression. Group sizes were $n = 187$ (low) and $n = 186$ (high) for OS and PFI, and $n = 183$ (low) and $n = 182$ (high) for DSS. p values were calculated using the two-sided log-rank test. (C,D) Univariate and multivariate Cox regression analyses of factors influencing survival prognosis in hepatocellular carcinoma patients. (E) Nomogram predicting 1-, 3-, and 5-year survival prognosis risk scores for hepatocellular carcinoma patients. (F) Calibration curves of the nomogram for 1-, 3-, and 5-year survival, demonstrating good agreement between predicted and observed outcomes. AFP, alpha fetoprotein; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

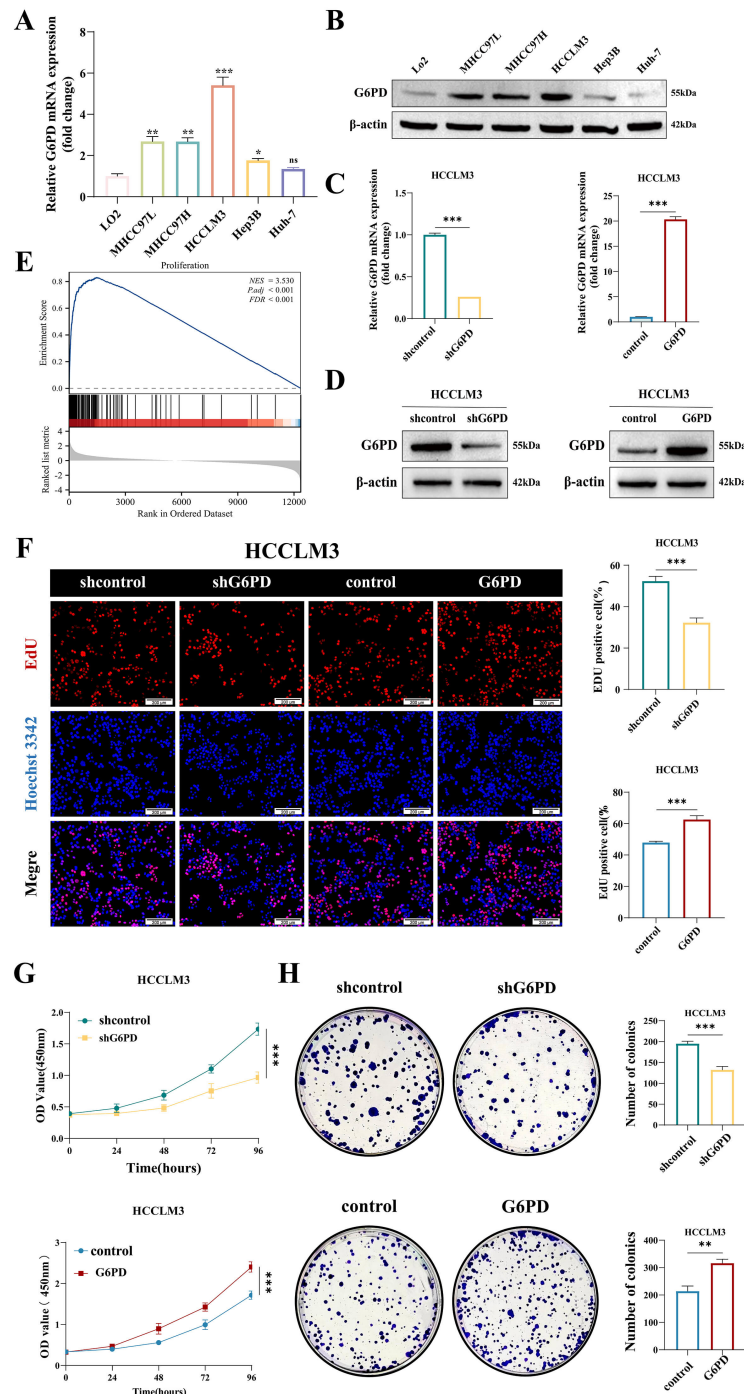


Fig. 3. Effects of G6PD on the biological behavior of hepatocellular carcinoma cells. (A,B) mRNA and protein expression levels of G6PD in various HCC cell lines (MHCC97L, MHCC97H, HCCLM3, Hep3B, Huh-7) and LO-2 cells detected by RT-qPCR and Western blot, respectively. Data are shown as mean $\pm$ SD from three independent experiments. (C,D) Verification of G6PD knockdown (shG6PD) and overexpression (G6PD) efficiency in HCCLM3 stable cell lines by RT-qPCR and Western blot, compared with corresponding controls. (E) GSEA showing that low G6PD expression is associated with suppression of proliferation-related pathways in HCC. Normalized enrichment score (NES) and nominal p value are indicated. (F) EdU assay showing proliferating cells (red) and nuclei (blue) under G6PD overexpression or knockdown. Scale bar = 200 μ m. (G) CCK-8 assay measuring cell viability at 0, 24, 48, 72, and 96 h. (H) Colony formation assay assessing long-term proliferative capacity, the comparison between the two groups was conducted using Student's t -test. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant. GSEA, Gene Set Enrichment Analysis; EdU, 5-ethynyl-2'-deoxyuridine; CCK-8, Cell Counting Kit-8.

GSEA to explore the relationship between G6PD and HCC cell proliferation demonstrated suppression of proliferation-related pathways in tumors with low G6PD expression (Fig. 3E). Consistent with this finding, G6PD overexpression significantly enhanced colony formation and proliferative activity, whereas G6PD knockdown markedly inhibited both processes (Fig. 3F–H), further confirming that G6PD is a key hub in malignant proliferation.

3.4 G6PD Inhibition Induces Mitochondrial Apoptosis in HCC Cells

GSEA to investigate the relationship between G6PD and apoptosis revealed an association between high G6PD expression and enrichment of apoptosis-related pathways, whereas low G6PD expression was associated with suppression of these pathways (Fig. 4A). Because mitochondrial apoptosis is a major mechanism regulating tumor cell survival and progression [20], we next examined the effects of G6PD on mitochondrial apoptotic signaling.

Mitochondrial membrane potential analysis demonstrated that G6PD overexpression maintained mitochondrial membrane integrity, whereas G6PD knockdown significantly increased mitochondrial depolarization, as indicated by a shift from JC-1 aggregates to JC-1 monomers (Fig. 4B). Consistent with these findings, flow cytometric analysis revealed a significantly higher apoptotic rate in G6PD-knockdown cells and a lower apoptotic rate in G6PD-overexpressing cells compared with their respective controls (Fig. 4C).

Western blot assays confirmed that silencing G6PD elevated pro-apoptotic BAX and Caspase-3, but G6PD knockdown reduced anti-apoptotic BCL-2, when compared with controls. G6PD overexpression produced the opposite effects (Fig. 4D). Together, these findings suggest that G6PD promotes HCC cell survival, at least in part, through suppression of mitochondria-mediated apoptosis.

3.5 G6PD Inhibition Induces Cell Cycle Arrest

GSEA to further elucidate the mechanism by which G6PD affects HCC cell proliferation revealed significant enrichment of high G6PD expression with cell cycle-related pathways, including mitotic G1 phase, G1/S transition activation, and regulation of G1-to-S phase progression (Fig. 5A).

Analysis of cell cycle distribution by flow cytometry to validate these findings showed a significantly increased proportion of HCC cells in the G0/G1 phase and a significantly decreased proportion of cells in the S phase following G6PD knockdown. No significant difference was observed in the proportion of cells in G2/M phase. In contrast, G6PD overexpression reduced the proportion of cells in the G0/G1 phase and increased the proportion of cells in the S phase (Fig. 5B). These data pointed to a key function of G6PD in governing the G1/S transition.

Cyclin D1 and CDK4/6 are key regulators of G1/S phase transition, and dysregulation of this pathway has been implicated in multiple malignancies [21,22]. Western blotting of cell cycle regulatory proteins to further examine the molecular basis of the observed cell cycle changes revealed that G6PD knockdown decreased CDK4, CDK6, and Cyclin D1 expression, whereas G6PD overexpression promoted CDK4, CDK6, and Cyclin D1 expression (Fig. 5C).

Collectively, these results indicate that G6PD promotes HCC cell proliferation, at least in part, by facilitating G1/S cell cycle progression.

3.6 G6PD Inhibition Promotes Oxidative Stress and Suppresses MAPK Signaling

Evaluation of intracellular ROS levels in HCC cells following G6PD modulation revealed significantly increased ROS signal in the shG6PD group and significantly decreased levels in the G6PD-overexpressing group compared with their respective controls (Fig. 6A), indicating that G6PD negatively regulates intracellular ROS levels in HCC cells.

Because G6PD contributes to cellular redox homeostasis through NADPH and ROS can regulate the MAPK pathway [23,24,25], we examined the expression of key molecules in the MAPK/ERK signaling pathway. Western blot analysis showed that G6PD knockdown inhibited MEK1/2 and ERK1/2 phosphorylation, whereas the opposite was observed for G6PD overexpression (Fig. 6B).

Collectively, these findings suggest that G6PD modulates ROS levels inside cells and correlates with MAPK pathway activation.

3.7 G6PD Promotes Tumor Growth In Vivo

Xenograft tumor experiments to investigate the effect of G6PD on HCC *in vivo* showed significantly reduced tumor size and weight in the shG6PD group compared with the control group, whereas the opposite results were observed in the overexpression group (Fig. 7A). Moreover, tumor sections from the shG6PD group demonstrated decreased Ki67 and PCNA expression (Fig. 7B).

These results suggest that G6PD plays an important and multifaceted role in HCC development.

4. Discussion

HCC remains a major global health challenge, with rising incidence despite advances in diagnosis and treatment. Increasing evidence indicates that metabolic reprogramming is a hallmark of HCC progression, yet the contribution of specific metabolic enzymes to tumor growth and survival remains incompletely understood [26,27,28]. G6PD, the rate-limiting enzyme of the PPP, has emerged as an important regulator of cellular metabolism, redox homeostasis, and stress responses [13,29]. Analysis of the TCGA and GEO databases, together with immunohisto-

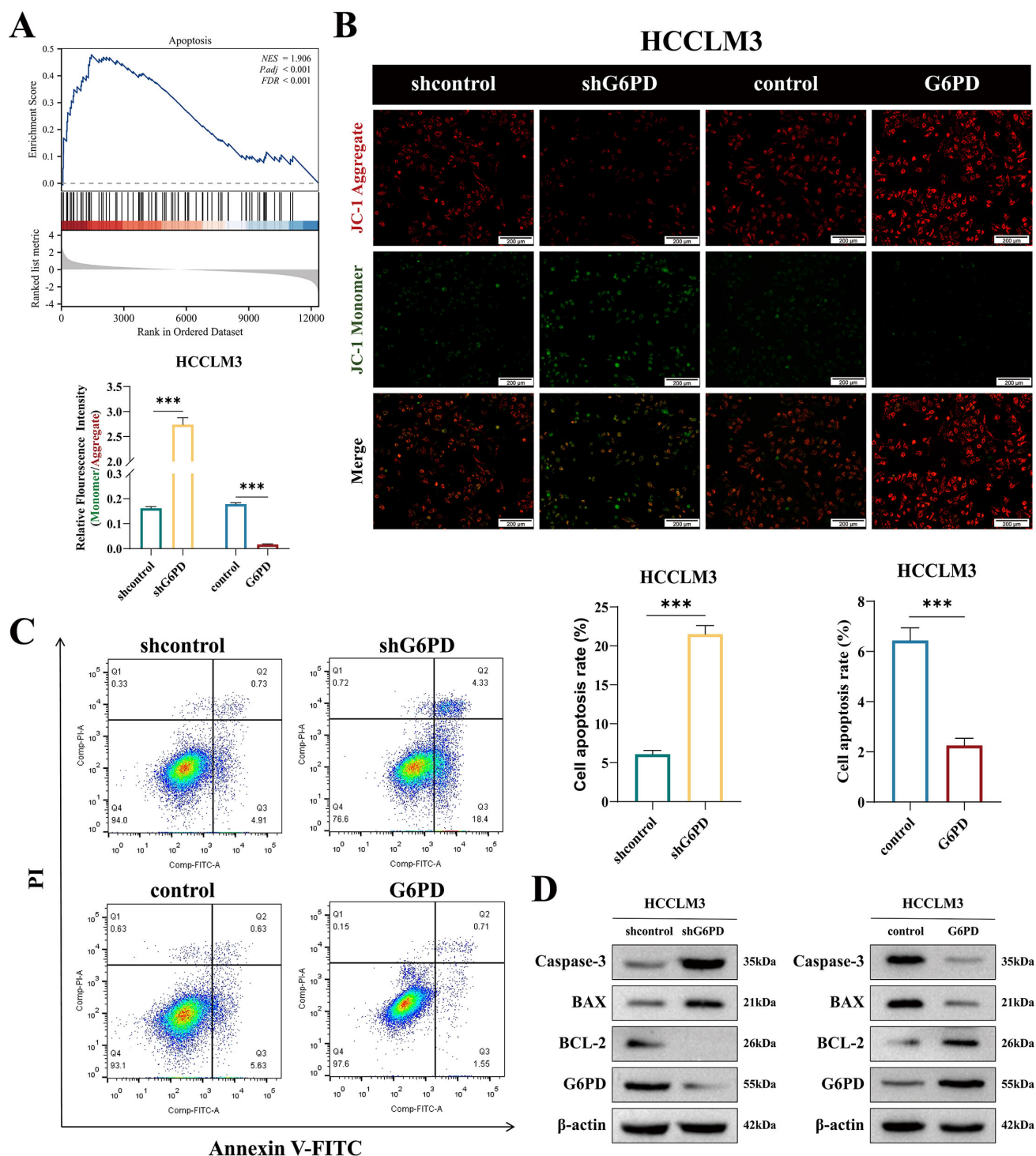


Fig. 4. G6PD regulates mitochondrial apoptosis in hepatocellular carcinoma cells. (A) GSEA enrichment analysis showing enrichment of apoptosis-related pathways in the G6PD-high expression group. NES and nominal p value are indicated. (B) JC-1 staining for mitochondrial membrane potential. Representative fluorescence images show JC-1 aggregates (red, healthy mitochondria) and monomers (green, depolarized mitochondria). Scale bar = 200 μ m. Quantitative analysis of green/red fluorescence intensity ratio (JC-1 monomer/aggregate) is shown. (C) Flow cytometry analysis of apoptosis using Annexin V-FITC/PI double staining. Representative dot plots show early (Annexin V⁺/PI⁻) and late (Annexin V⁺/PI⁺) apoptotic cells. Bar graph indicates apoptotic rate. (D) Western blot detection of BAX, Caspase-3, and BCL-2 protein levels. β -actin served as a loading control. The comparison between the two groups was conducted using Student's t -test. *** p < 0.001. GSEA, Gene Set Enrichment Analysis; JC-1, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; BAX, BCL-2-associated X protein; Caspase-3, cysteine-aspartic acid protease-3; BCL-2, B-cell lymphoma 2.

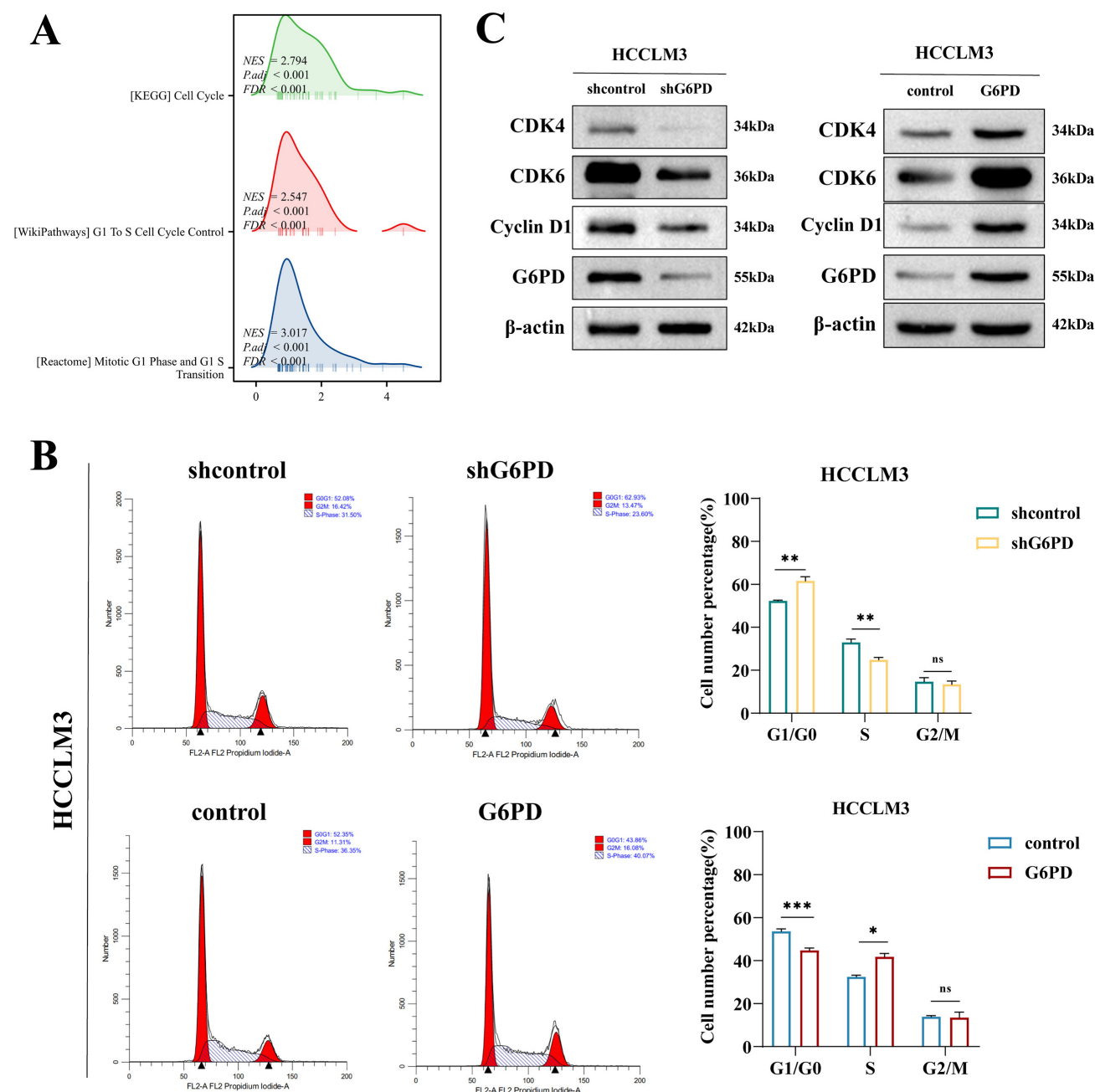


Fig. 5. Effect of G6PD on cell cycle in hepatocellular carcinoma cells. (A) GSEA enrichment analysis showing that high G6PD expression is associated with activation of cell cycle, G1/S transition, and mitotic progression pathways. NES and nominal p value are indicated. (B) Cell cycle distribution analyzed by flow cytometry after G6PD overexpression or knockdown, compared with respective controls. Representative DNA content histograms and quantitative bar graphs of cell percentages in G0/G1, S, and G2/M phases are shown. Statistical comparisons were performed using Student's t -test. (C) Western blot analysis of CDK4, CDK6, and Cyclin D1 protein levels following G6PD overexpression or knockdown. β -actin served as a loading control. ns indicates $p \geq 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6.

chemical validation, demonstrated significantly increased G6PD expression in HCC tissue. ROC analysis further indicated good diagnostic performance (AUC = 0.949). Previous studies have shown that G6PD promotes EMT to enhance tumor cell migration and invasion and accelerate disease progression [18,30]. In addition, G6PD contributes to

HCC development through ferroptosis inhibition and regulation of lipid metabolism [31]. Our findings further expand the evidence supporting a multifaceted oncogenic role for G6PD in HCC.

Analysis of clinicopathological characteristics demonstrated that high G6PD expression was significantly

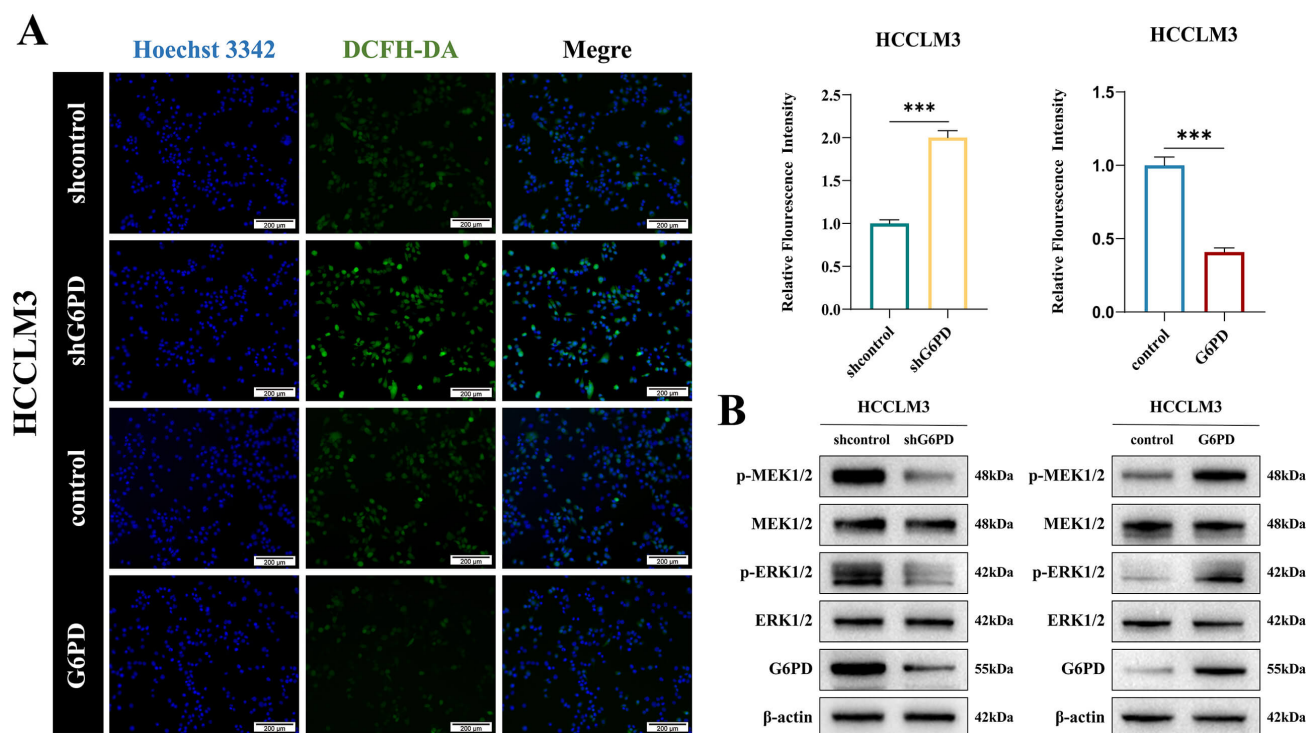


Fig. 6. G6PD regulates oxidative stress and the MAPK signaling pathway in hepatocellular carcinoma cells. (A) Detection of ROS levels using DCFH-DA assay following G6PD knockdown (shG6PD) or overexpression (OE-G6PD), compared with respective controls. Representative fluorescence images and quantitative analysis of mean fluorescence intensity are shown. Scale bar = 200 μ m. (B) Western blot analysis of key proteins in the MAPK/ERK signaling pathway, including total and phosphorylated MEK1/2 and ERK1/2, following G6PD knockdown or overexpression, β -actin served as a loading control. *** $p < 0.001$; ROS, reactive oxygen species; DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate.

associated with histological grade, T stage, vascular invasion, AFP level, and pathological stage. Furthermore, G6PD remained an independent prognostic risk factor for HCC, consistent with previous studies that incorporated HCC into prognostic nomograms [32,33]. The G6PD-based nomogram developed in the present study showed good calibration and predictive performance, suggesting potential utility as a quantitative tool for individualized risk stratification. Previous studies have attempted to build models using G6PD with machine learning algorithms and have achieved good performance [34,35]. However, this model requires external validation in independent cohorts to help fully evaluate its generalizability and clinical reliability.

To better understand the biological basis underlying these clinical associations, we investigated the functional role of G6PD in HCC progression using *in vitro* and *in vivo* experimental models. G6PD knockdown significantly suppressed HCC cell colony formation and proliferation, whereas G6PD overexpression enhanced these phenotypes. GSEA identified significant enrichment of cell cycle-related pathways in tumors with high G6PD expression, indicating that G6PD is involved in regulating the cell cycle. Consistent with this observation, G6PD knockdown

induced G0/G1 arrest and reduced the expression of CDK4, CDK6, and Cyclin D1, whereas G6PD overexpression promoted S phase entry. These findings suggest that G6PD facilitates HCC cell proliferation, at least in part, through promotion of G1/S cell cycle progression. Similar observations have been reported with herbal compounds such as EZW, which suppress HCC cell cycle progression by targeting genes, including CDK1, CDK4, and G6PD [36,37].

In addition to its effects on proliferation, G6PD influenced apoptosis-related pathways. G6PD inhibition led to mitochondrial depolarization, upregulation of pro-apoptotic BAX and Caspase-3, downregulation of anti-apoptotic BCL-2, and a consequent rise in apoptosis. This indicates that G6PD inhibition triggers mitochondria-dependent apoptosis. Similar mechanisms have been reported in other studies that induce apoptosis in HCC [38,39,40,41]. Collectively, these findings support the role of G6PD as a key driver of cell proliferation and cell cycle progression.

The tumor-promoting effects of G6PD were further supported by xenograft experiments. G6PD knockdown significantly reduced tumor volume and weight, and suppressed Ki-67 and proliferating cell nuclear antigen (PCNA) expression. These results are consistent with pre-

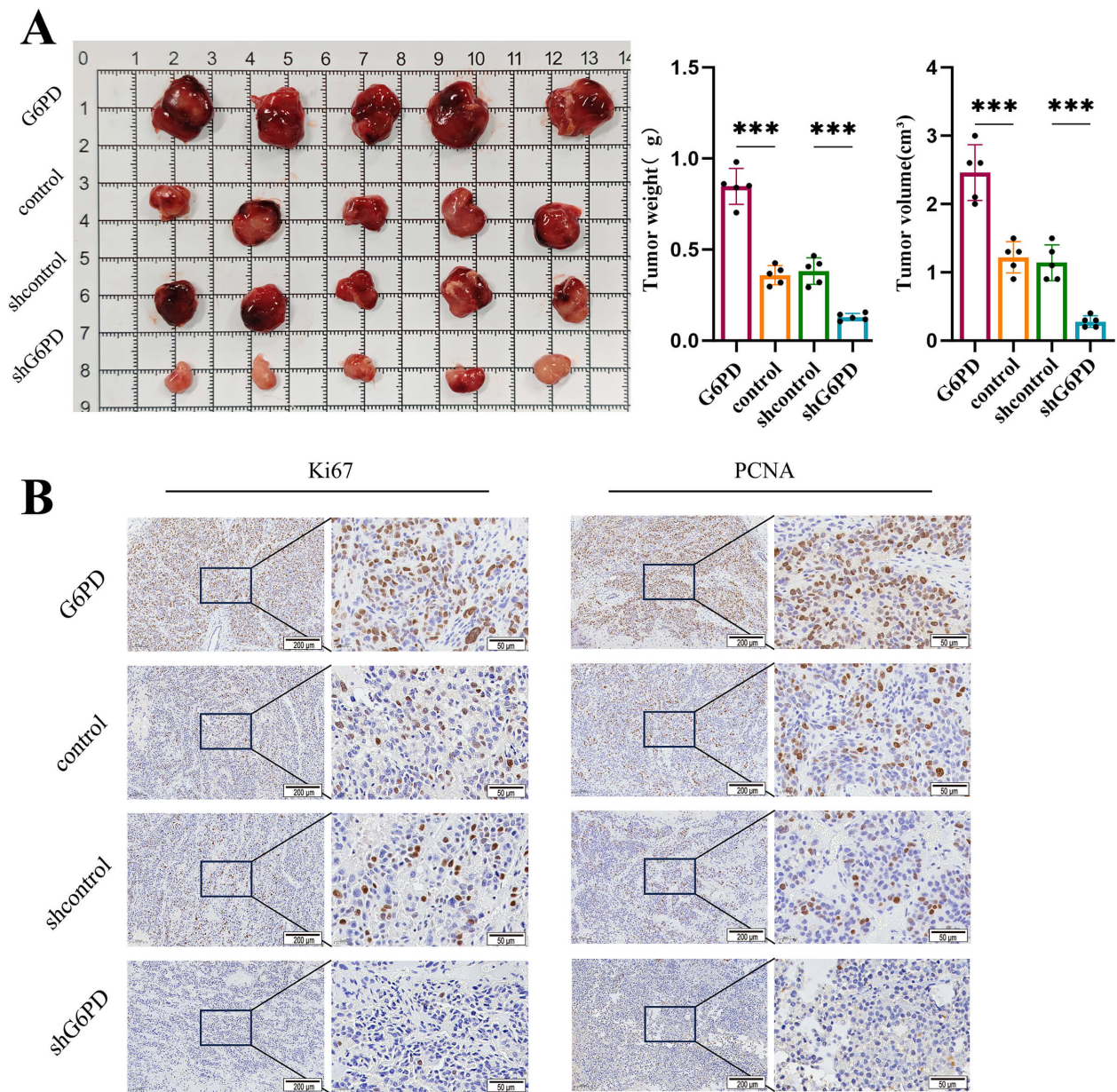


Fig. 7. The effect of G6PD on the proliferation of liver cancer *in vivo*. (A) Representative images of xenografted tumors and quantitative analysis of tumor volume and tumor weight from nude mice. Statistical significance was determined by one-way ANOVA. Data are presented as mean $\pm$ SD ($n = 5$ per group). (B) Immunohistochemical staining of Ki67 and PCNA in tumor sections from each group, scale bar: 200 μ m (main images) and 50 μ m (insets). *** $p < 0.001$; Ki67, proliferation Marker Ki67; PCNA, proliferating cell nuclear antigen.

vious reports describing a pro-tumorigenic role for G6PD in multiple cancer types [31,42,43]. The agreement between cell-based and animal experiment findings strengthens the evidence that G6PD contributes to HCC growth and supports its potential value as a therapeutic target.

A central function of G6PD is the maintenance of cellular redox homeostasis through the generation of NADPH via the PPP. This may be the basis for its protective effects on mitochondrial function and inhibition of intrinsic apoptosis and ferroptosis triggered by excessive ROS accumulation [19]. Our results confirmed that G6PD knockdown

in HCC cells significantly increased intracellular ROS levels. In contrast, G6PD overexpression reduced ROS accumulation. These findings are consistent with the well-established antioxidant role of G6PD as a major source of NADPH. NADPH is essential for glutathione regeneration and ROS elimination [44,45]. When G6PD function is impaired, NADPH supply becomes insufficient, leading to a redox imbalance [30,46]. Therefore, the regulation of ROS levels by G6PD further indicates that G6PD promotes the malignant phenotype of HCC by maintaining redox homeostasis. Abnormal MAPK pathway activation has

been linked to worse outcomes, higher proliferative activity, and greater metastatic capacity across different cancers, as reported in numerous studies [47,48]. Thus, the ability of G6PD to maintain redox homeostasis may represent an important mechanism through which it promotes the malignant phenotype of HCC.

G6PD regulates the phosphorylation of MEK1/2 and ERK1/2 by modulating ROS levels, thereby modulating MAPK/ERK signaling activity. ROS can act as secondary messengers that influence signaling cascades, and G6PD-mediated redox homeostasis may contribute to this regulation [40]. This signaling pathway serves as a major regulator of cell growth and survival. The regulation of this pathway by G6PD is consistent with the observation that metabolic enzymes promote tumor growth by affecting signal transduction. For example, G6PD inhibitors suppress cancer cell proliferation by inducing ROS accumulation and cell cycle arrest [30,49].

In summary, our findings identify G6PD as a clinically relevant biomarker and functional driver of HCC progression. By linking metabolic reprogramming, redox homeostasis, cell cycle regulation, apoptosis, and MAPK/ERK signaling, this study offers further understanding of the molecular events driving HCC and highlights G6PD as a potential therapeutic target.

5. Limitations

This study has certain limitations that deserve recognition. First, the mechanistic investigations focused primarily on a single candidate gene, G6PD. Integrating transcriptomic, proteomic, and metabolomic data in subsequent research could yield a more complete picture of the metabolic rewiring driven by G6PD and the associated signaling cascades, as well as identify key upstream and downstream regulators and potential feedback mechanisms.

Second, the functional studies *in vitro* were performed using a single cell line, HCCLM3. This line originates from a highly metastatic HCC model and exhibits robust proliferation and metabolism, supporting its use for preliminary mechanistic exploration [50]. Nevertheless, we recognize that results from one cell line may not capture the full molecular diversity of HCC. Future work should verify our observations across other cell lines and in patient-derived tumor models.

Finally, the prognostic model developed in this study was based primarily on publicly available datasets and requires external validation in independent patient cohorts. We need multicenter investigations that include larger, more varied cohorts to assess the general applicability and clinical dependability of our findings. In addition, its diagnostic and prognostic utility in clinical settings warrants further evaluation through prospective studies.

6. Conclusion

In summary, our findings indicate that G6PD is markedly upregulated in HCC and correlates with adverse clinicopathological features and worse survival outcomes. Mechanistically, G6PD facilitates proliferation and antagonizes mitochondrial apoptosis via ROS-mediated MAPK/ERK signaling. G6PD knockdown inhibited tumor growth *in vivo*. Collectively, these results point to G6PD as a promising candidate for targeted HCC therapy. However, further validation in clinical settings is required.

Availability of Data and Materials

All data are available from the corresponding authors upon reasonable request.

Author Contributions

XM and JHL designed the research study, wrote the manuscript and collected clinical samples. JX performed part of the experiments and analyzed the data; KJL and YB participated in the experimental design, contributed to data interpretation, and critically revised the manuscript for important intellectual content. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of General Hospital of Ningxia Medical University (Approval No. KYLL-2022-0412). The collection and use of human tissues were conducted in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from all participating patients or legal guardians. All animal procedures were carried out in compliance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and the NIH Guide for the Care and Use of Laboratory Animals. The approval covered both human and animal components of this study.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used DeepSeek in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL53651>.

References

- [1] Donne R, Lujambio A. The liver cancer immune microenvironment: Therapeutic implications for hepatocellular carcinoma. *Hepatology* (Baltimore, Md.). 2023; 77: 1773–1796. <https://doi.org/10.1002/hep.32740>
- [2] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*. 2024; 74: 229–263. <https://doi.org/10.3322/caac.21834>
- [3] Cui L, Liu J, Lv Y, Chen B, Liu K, Bu Y. Study on the Mechanism of CEN2 Promoting Sorafenib Resistance in HCC and Its Combined Intervention Strategy. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 45454. <https://doi.org/10.31083/FBL45454>
- [4] Xue Y, Ruan Y, Wang Y, Xiao P, Xu J. Signaling pathways in liver cancer: pathogenesis and targeted therapy. *Molecular Biomedicine*. 2024; 5: 20. <https://doi.org/10.1186/s43556-024-00184-0>
- [5] Ye J, Lin Y, Liao Z, Gao X, Lu C, Lu L, et al. Single cell-spatial transcriptomics and bulk multi-omics analysis of heterogeneity and ecosystems in hepatocellular carcinoma. *NPJ Precision Oncology*. 2024; 8: 262. <https://doi.org/10.1038/s41698-024-00752-1>
- [6] Hong Y, Wang D, Liu Z, Chen Y, Wang Y, Li J. Decoding per- and polyfluoroalkyl substances (PFAS) in hepatocellular carcinoma: a multi-omics and computational toxicology approach. *Journal of Translational Medicine*. 2025; 23: 504. <https://doi.org/10.1186/s12967-025-06517-z>
- [7] Glaviano A, Singh SK, Lee EHC, Okina E, Lam HY, Carbone D, et al. Cell cycle dysregulation in cancer. *Pharmacological Reviews*. 2025; 77: 100030. <https://doi.org/10.1016/j.pharmr.2024.100030>
- [8] Costa-Guda J, Corrado K, Bellizzi J, Romano R, Saria E, Saucier K, et al. CDK4/6 Dependence of Cyclin D1-Driven Parathyroid Neoplasia in Transgenic Mice. *Endocrinology*. 2020; 161: bqaa159. <https://doi.org/10.1210/endo/bqaa159>
- [9] Xue Y, Meehan B, Macdonald E, Venneti S, Wang XQD, Witkowski L, et al. CDK4/6 inhibitors target SMARCA4-determined cyclin D1 deficiency in hypercalcemic small cell carcinoma of the ovary. *Nature Communications*. 2019; 10: 558. <https://doi.org/10.1038/s41467-018-06958-9>
- [10] Cheng X, Sun Y, Highkin M, Vemalapally N, Jin X, Zhou B, et al. Breast Cancer Mutations HER2V777L and PIK3CAH1047R Activate the p21-CDK4/6-Cyclin D1 Axis to Drive Tumorigenesis and Drug Resistance. *Cancer Research*. 2023; 83: 2839–2857. <https://doi.org/10.1158/0008-5472.CAN-22-3558>
- [11] Chen X, Xu Z, Zhu Z, Chen A, Fu G, Wang Y, et al. Modulation of G6PD affects bladder cancer via ROS accumulation and the AKT pathway in vitro. *International Journal of Oncology*. 2018; 53: 1703–1712. <https://doi.org/10.3892/ijo.2018.4501>
- [12] Zheng P, Pan HH, Zhou XH, Qiu YY, Hu J, Qin ZS, et al. Glucose 6 phosphatase dehydrogenase (G6PD): a novel diagnosis marker related to gastrointestinal cancers. *American Journal of Translational Research*. 2023; 15: 2304–2328.
- [13] Yang HC, Wu YH, Yen WC, Liu HY, Hwang TL, Stern A, et al. The Redox Role of G6PD in Cell Growth, Cell Death, and Cancer. *Cells*. 2019; 8: 1055. <https://doi.org/10.3390/cells8091055>
- [14] Luo M, Fu A, Wu R, Wei N, Song K, Lim S, et al. High Expression of G6PD Increases Doxorubicin Resistance in Triple Negative Breast Cancer Cells by Maintaining GSH Level. *International Journal of Biological Sciences*. 2022; 18: 1120–1133. <https://doi.org/10.7150/ijbs.65555>
- [15] Wang C, Yu C, Chang H, Song J, Zhang S, Zhao J, et al. Glucose-6-phosphate dehydrogenase: a therapeutic target for ovarian cancer. *Expert Opinion on Therapeutic Targets*. 2023; 27: 733–743. <https://doi.org/10.1080/14728222.2023.2247558>
- [16] Wang J, Qiao Y, Sun M, Sun H, Xie F, Chang H, et al. FTO promotes colorectal cancer progression and chemotherapy resistance via demethylating G6PD/PARP1. *Clinical and Translational Medicine*. 2022; 12: e772. <https://doi.org/10.1002/ctm2.772>
- [17] Feng Q, Li X, Sun W, Sun M, Li Z, Sheng H, et al. Targeting G6PD reverses paclitaxel resistance in ovarian cancer by suppressing GSTP1. *Biochemical Pharmacology*. 2020; 178: 114092. <https://doi.org/10.1016/j.bcp.2020.114092>
- [18] Lu M, Lu L, Dong Q, Yu G, Chen J, Qin L, et al. Elevated G6PD expression contributes to migration and invasion of hepatocellular carcinoma cells by inducing epithelial-mesenchymal transition. *Acta Biochimica et Biophysica Sinica*. 2018; 50: 370–380. <https://doi.org/10.1093/abbs/gmy009>
- [19] Li S, Guo W, Qian Y, Li L, Li Q, Zhu Z, et al. Targeting KDM1A suppresses G6PD expression to induce oxidative stress and ferroptosis in TP53-mutated liver cancer. *View*. 2025; 6: 20250036. <https://doi.org/10.1002/VIW.20250036>
- [20] Lopez J, Tait SWG. Mitochondrial apoptosis: killing cancer using the enemy within. *British Journal of Cancer*. 2015; 112: 957–962. <https://doi.org/10.1038/bjc.2015.85>
- [21] Fang M, Wu HK, Pei Y, Zhang Y, Gao X, He Y, et al. E3 ligase MG53 suppresses tumor growth by degrading cyclin D1. *Signal Transduction and Targeted Therapy*. 2023; 8: 263. <https://doi.org/10.1038/s41392-023-01458-9>
- [22] Piezzo M, Cocco S, Caputo R, Cianniello D, Gioia GD, Lauro VD, et al. Targeting Cell Cycle in Breast Cancer: CDK4/6 Inhibitors. *International Journal of Molecular Sciences*. 2020; 21: 6479. <https://doi.org/10.3390/ijms21186479>
- [23] Lin CJ, Ho HY, Cheng ML, You TH, Yu JS, Chiu DTY. Impaired dephosphorylation renders G6PD-knockdown HepG2 cells more susceptible to H(2)O(2)-induced apoptosis. *Free Radical Biology & Medicine*. 2010; 49: 361–373. <https://doi.org/10.1016/j.freeradbiomed.2010.04.019>
- [24] Zhang Q, Han Q, Yang Z, Ni Y, Agbana YL, Bai H, et al. G6PD facilitates clear cell renal cell carcinoma invasion by enhancing MMP2 expression through ROS-MAPK axis pathway. *International Journal of Oncology*. 2020; 57: 197–212. <https://doi.org/10.3892/ijo.2020.5041>
- [25] Ham M, Lee JW, Choi AH, Jang H, Choi G, Park J, et al. Macrophage glucose-6-phosphate dehydrogenase stimulates

- proinflammatory responses with oxidative stress. *Molecular and Cellular Biology*. 2013; 33: 2425–2435. <https://doi.org/10.1128/MCB.01260-12>
- [26] Kowalik MA, Columbano A, Perra A. Emerging Role of the Pentose Phosphate Pathway in Hepatocellular Carcinoma. *Frontiers in Oncology*. 2017; 7: 87. <https://doi.org/10.3389/fonc.2017.00087>
- [27] Liu J, Zhu Y, Lv J, Hu X, Zhong Y. Farnesyl Diphosphate Synthase Promotes Proliferation of Hepatocellular Carcinoma Cells by Interacting With Glucose-6-Phosphate Dehydrogenase. *Cancer Medicine*. 2026; 15: e71620. <https://doi.org/10.1002/cam4.71620>
- [28] Cheng M, Wang B, Duan L, Jin Y, Zhang W, Li N. HOTAIR Knockdown Increases the Sensitivity of Hepatocellular Carcinoma Cells to Sorafenib by Disrupting miR-145-5p/HK2 Axis-Mediated Mitochondrial Function and Glycolysis. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 37368. <https://doi.org/10.31083/FBL37368>
- [29] Yang HC, Stern A, Chiu DTY. G6PD: A hub for metabolic reprogramming and redox signaling in cancer. *Biomedical Journal*. 2021; 44: 285–292. <https://doi.org/10.1016/j.bj.2020.08.001>
- [30] Lin X, Xu Y, Bai E, Deng Y, Zhang W, Xue R, et al. 6-aminonicotinamide, a G6PD inhibitor, mitigates CAPS1 reduction mediated HCC metastasis via ERK and GSK3 β signals. *Neoplasia (New York, N.Y.)*. 2025; 70: 101239. <https://doi.org/10.1016/j.neo.2025.101239>
- [31] Cao F, Luo A, Yang C. G6PD inhibits ferroptosis in hepatocellular carcinoma by targeting cytochrome P450 oxidoreductase. *Cellular Signalling*. 2021; 87: 110098. <https://doi.org/10.1016/j.cellsig.2021.110098>
- [32] Wang X, Mao M, He Z, Zhang L, Li H, Lin J, et al. Development and Validation of a Prognostic Nomogram in AFP-negative hepatocellular carcinoma. *International Journal of Biological Sciences*. 2019; 15: 221–228. <https://doi.org/10.7150/ijbs.28720>
- [33] Wang X, Liao X, Yang C, Huang K, Yu T, Yu L, et al. Identification of prognostic biomarkers for patients with hepatocellular carcinoma after hepatectomy. *Oncology Reports*. 2019; 41: 1586–1602. <https://doi.org/10.3892/or.2019.6953>
- [34] Li F, Wang B, Li H, Kong L, Zhu B. G6PD and machine learning algorithms as prognostic and diagnostic indicators of liver hepatocellular carcinoma. *BMC Cancer*. 2024; 24: 157. <https://doi.org/10.1186/s12885-024-11887-6>
- [35] Xiao J, Wang J, Zhou C, Luo J. Development and Validation of a Propionate Metabolism-Related Gene Signature for Prognostic Prediction of Hepatocellular Carcinoma. *Journal of Hepatocellular Carcinoma*. 2023; 10: 1673–1687. <https://doi.org/10.2147/JHC.S420614>
- [36] Zheng S, Pan B. Multilevel data integration and molecular docking approach to systematically elucidate the underlying pharmacological mechanisms of Er-Zhi-Wan against hepatocellular carcinoma. *Aging*. 2022; 14: 8783–8804. <https://doi.org/10.18632/aging.204369>
- [37] Gupta P, Bhatia N, Bansal MP, Koul A. Lycopene modulates cellular proliferation, glycolysis and hepatic ultrastructure during hepatocellular carcinoma. *World Journal of Hepatology*. 2016; 8: 1222–1233. <https://doi.org/10.4254/wjh.v8.i29.1222>
- [38] Zhang X, Wang M, Teng S, Wang D, Li X, Wang X, et al. Indolyl-chalcone derivatives induce hepatocellular carcinoma cells apoptosis through oxidative stress related mitochondrial pathway in vitro and in vivo. *Chemico-biological Interactions*. 2018; 293: 61–69. <https://doi.org/10.1016/j.cbi.2018.07.025>
- [39] Song J, Wang Y, Teng M, Zhang S, Yin M, Lu J, et al. Cordyceps militaris induces tumor cell death via the caspase-dependent mitochondrial pathway in HepG2 and MCF-7 cells. *Molecular Medicine Reports*. 2016; 13: 5132–5140. <https://doi.org/10.3892/mmr.2016.5175>
- [40] Zhao Q, Yu M, Li J, Guo Y, Wang Z, Hu K, et al. GLUD1 inhibits hepatocellular carcinoma progression via ROS-mediated p38/JNK MAPK pathway activation and mitochondrial apoptosis. *Discover Oncology*. 2024; 15: 8. <https://doi.org/10.1007/s12672-024-00860-1>
- [41] Luo Y, Liu L, Zhao J, Jiao Y, Zhang M, Xu G, et al. PI3K/AKT1 Signaling Pathway Mediates Sinomenine-Induced Hepatocellular Carcinoma Cells Apoptosis: An In Vitro and in Vivo Study. *Biological & Pharmaceutical Bulletin*. 2022; 45: 614–624. <https://doi.org/10.1248/bpb.b21-01063>
- [42] Zhang X, Gao F, Ai H, Wang S, Song Z, Zheng L, et al. TSP50 promotes hepatocyte proliferation and tumour formation by activating glucose-6-phosphate dehydrogenase (G6PD). *Cell Proliferation*. 2021; 54: e13015. <https://doi.org/10.1111/cpr.13015>
- [43] Zhu X, Ma W, Ji X, He J, Luo J, Kong X, et al. Loss of TFAM accelerates pentose phosphate pathway by unleashing G6PD oligomerization to drive hepatocarcinogenesis. *Cancer Letters*. 2026; 641: 218275. <https://doi.org/10.1016/j.canlet.2026.218275>
- [44] Lee D, Zhang MS, Tsang FHC, Bao MHR, Xu IMJ, Lai RKH, et al. Adaptive and Constitutive Activations of Malic Enzymes Confer Liver Cancer Multilayered Protection Against Reactive Oxygen Species. *Hepatology (Baltimore, Md.)*. 2021; 74: 776–796. <https://doi.org/10.1002/hep.31761>
- [45] Guo H, Xu J, Zheng Q, He J, Zhou W, Wang K, et al. NRF2 SUMOylation promotes de novo serine synthesis and maintains HCC tumorigenesis. *Cancer Letters*. 2019; 466: 39–48. <https://doi.org/10.1016/j.canlet.2019.09.010>
- [46] Gao J, Shi X, Sun Y, Liu X, Zhang F, Shi C, et al. Deficiency of betaine-homocysteine methyltransferase activates glucose-6-phosphate dehydrogenase (G6PD) by decreasing arginine methylation of G6PD in hepatocellular carcinogenesis. *Science China. Life Sciences*. 2024; 67: 1648–1665. <https://doi.org/10.1007/s11427-023-2481-3>
- [47] Ganesan K, Xu C, Wu S, Sui Y, Du B, Zhang J, et al. Ononin Inhibits Tumor Bone Metastasis and Osteoclastogenesis By Targeting Mitogen-Activated Protein Kinase Pathway in Breast Cancer. *Research (Washington, D.C.)*. 2024; 7: 0553. <https://doi.org/10.34133/research.0553>
- [48] Wu L, Huang S, Tian W, Liu P, Xie Y, Qiu Y, et al. PIWI-interacting RNA-YBX1 inhibits proliferation and metastasis by the MAPK signaling pathway via YBX1 in triple-negative breast cancer. *Cell Death Discovery*. 2024; 10: 7. <https://doi.org/10.1038/s41420-023-01771-w>
- [49] Yin X, Tang B, Li JH, Wang Y, Zhang L, Xie XY, et al. ID1 promotes hepatocellular carcinoma proliferation and confers chemoresistance to oxaliplatin by activating pentose phosphate pathway. *Journal of Experimental & Clinical Cancer Research*. 2017; 36: 166. <https://doi.org/10.1186/s13046-017-0637-7>
- [50] Guo Z, Jiang JH, Zhang J, Yang HJ, Zhong YP, Su J, et al. Side population in hepatocellular carcinoma HCCLM3 cells is enriched with stem-like cancer cells. *Oncology Letters*. 2016; 11: 3145–3151. <https://doi.org/10.3892/ol.2016.4343>