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Original Article

PYGO2 as a novel prognostic biomarker and its correlation with immune infiltrates in liver cancer

Jieyu Jin[1*], Yanqiu Zhang[2*], Jun Cao[1*], Junchao Feng[3], Yuting Liang[1], Longwei Qiao[4], Bin Feng[1], Qingqin Tang[1], Jun Qiu[1], Zhongping Qian[1]

[1]Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China; [2]Institute of Clinical Pharmacology, Anhui Medical University, Key Laboratory of Anti-inflammatory and Immune Medicine, Ministry of Education, Anhui Collaborative Innovation Center of Anti-inflammatory and Immune Medicine, Hefei 230000, Anhui, China; [3]Department of Nuclear Accident Medical Emergency, The Second Affiliated Hospital of Soochow University, Suzhou 215004, Jiangsu, China; [4]Center for Reproduction and Genetics, School of Gusu, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Nanjing Medical University, Suzhou 215008, Jiangsu, China. [*]Equal contributors.

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Abstract: Objective: The PYGO2 gene plays a significant role in various cancers. However, its prognostic significance and involvement in immune infiltration in liver cancer remain unclear. This study aimed to comprehensively evaluate PYGO2 expression and its associations with prognosis and clinicopathological features in liver cancer. Methods: Data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases were analyzed. Functional enrichment analysis and immune cell infiltration assessments were performed to explore potential pathogenic mechanisms. Results: PYGO2 was highly expressed in multiple cancer types, including bladder urothelial carcinoma, breast invasive carcinoma, cholangiocarcinoma, diffuse large B-cell lymphoma, and liver cancer. Analysis of 50 paired liver cancer tissues from TCGA revealed significant upregulation of PYGO2 expression. Moreover, high PYGO2 expression was significantly associated with pathological T stage, overall pathological stage, tumor status, and race. Kaplan-Meier survival analysis showed that low PYGO2 expression correlated with improved overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) in liver cancer patients. Functional enrichment analysis identified several enriched pathways, including the reactive oxygen species signaling pathway, MYC targets, interferon-alpha response, immune response regulation signaling pathway, and leukocyte migration. Additionally, PYGO2 overexpression was associated with lower proportions of cytotoxic cells, dendritic cells, immature dendritic cells, mast cells, neutrophils, plasmacytoid dendritic cell-like cells, Th17 cells, and regulatory T cells, but a higher proportion of Th2 cells. Furthermore, the high PYGO2 expression group exhibited increased immune checkpoint gene expression, particularly PDCD1. Conclusion: PYGO2 is a promising prognostic biomarker for liver cancer, given its strong associations with clinicopathological features, survival outcomes, and immune-related characteristics.

Keywords: PYGO2 gene, liver cancer, biomarker

Introduction

Liver hepatocellular carcinoma (LIHC) accounts for 80% to 90% of primary liver cancers and is one of the top three leading causes of cancer-related deaths worldwide [1]. The molecular mechanisms driving the occurrence and progression of liver cancer present significant challenges in its diagnosis, treatment, and prognosis evaluation. Although surgical interventions have shown some success, the overall prognosis for LIHC remains poor [2]. Currently, treatment decisions and prognosis predictions for hepatocellular carcinoma primarily rely on the tumor-node-metastasis (TNM) staging system and molecular typing. However, these methods alone are insufficient for accurately predicting prognosis or capturing the biological heterogeneity of liver cancer patients. Therefore, identifying novel prognostic biomarkers and therapeutic targets is essential to improving prognosis assessment, guiding more effective treatment strategies, and advancing liver cancer treatment options.

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PYGO2 (Pygopus homolog 2) is a member of the Pygopus protein family and plays a crucial role in embryonic development, cell proliferation, stem cell function, and tumorigenesis [3]. It is primarily involved in regulating the Wnt signaling pathway, which is essential for organismal development, tissue repair, and stem cell maintenance [4]. By interacting with the transcription factor β-catenin, PYGO2 facilitates the transcriptional activation of target genes in the nucleus [5]. Aberrant PYGO2 expression has been linked to tumor development and progression, making it a potential biomarker and therapeutic target in cancer [6]. However, its specific role in liver hepatocellular carcinoma (LIHC) remains poorly understood, and few studies have explored its prognostic significance. Further investigation into the molecular mechanisms of PYGO2 in liver cancer could provide valuable insights for prognosis assessment and the development of targeted therapies.

In this study, we analyzed the expression and prognostic significance of PYGO2 in liver hepatocellular carcinoma (LIHC) using data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. Additionally, we examined the co-expression of PYGO2 with other genes and explored its correlation with immune cell infiltration in the tumor microenvironment. These findings may provide valuable insights for clinical diagnosis and treatment decisions in LIHC.

Materials and methods

Analysis of PYGO2 expression in normal and cancerous tissues

RNA sequencing data and corresponding clinical information for 33 tumor and normal tissue types were obtained from the TCGA and GTEx databases using UCSC XENA (https://xenabrowser.net/), following previously reported methods [7-9]. Statistical analysis was conducted using R (version 4.3.2) to evaluate PYGO2 expression across various cancers. Additionally, PYGO2 expression was analyzed in both paired and unpaired liver cancer samples. Data visualization was performed using the ggplot2 package.

Analysis of differential PYGO2 expression across clinical variables of LIHC

PYGO2 expression data in normal tissues and liver hepatocellular carcinoma (LIHC) were obtained. Differential expression analysis was conducted based on key clinical variables, including T stage, pathological stage, tumor status, and ethnic background. This analysis aimed to investigate the association between PYGO2 expression and various clinicopathological characteristics in LIHC.

Analysis of the relationship between PYGO2 and LIHC prognosis

The receiver operating characteristic (ROC) curve for PYGO2 was analyzed using the ROC package in R to evaluate its clinical diagnostic value, with visualization performed using the ggplot2 package. Additionally, the survival package was used to generate Kaplan-Meier survival curves, assessing the association between PYGO2 expression and overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) in LIHC patients.

Screening for co-expressed genes and enrichment analysis

Co-expressed genes of PYGO2 were identified using R, applying the criteria of $P < 0.05$ and $|\log_2 \text{fold change}| > 1$. The top five upregulated and downregulated genes were selected for further analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using the ClusterProfiler package [10] to explore the functional roles of these co-expressed genes [11].

Analysis of the correlation between PYGO2 expression and immune cell infiltration in the tumor microenvironment

As previously described [8, 9], immune infiltration in liver cancer was assessed using the TCGA-LIHC dataset through single-sample gene set enrichment analysis (ssGSEA) with the GSVA package [12]. Enrichment scores for 24 immune cell types were calculated for each sample. The Wilcoxon rank sum test was applied to compare immune infiltration levels between high and low PYGO2 expression groups. Differences with $P < 0.05$ were considered statistically significant.

Statistical methods

Statistical analyses were performed using the default methods provided by the databases. The Wilcoxon test was used to compare PYGO2

expression between groups. Kaplan-Meier survival analysis was conducted to evaluate survival differences, while Cox regression analysis was used to assess the impact of PYGO2 expression on patient outcomes. A $P < 0.05$ was considered statistically significant.

Results

Elevated expression of PYGO2 in liver cancer

Pan-cancer analysis revealed that PYGO2 expression was significantly higher in liver cancer samples compared to normal liver tissues ($P < 0.001$) (**Figure 1B**). Analysis of the GTEx and TCGA databases further showed that PYGO2 was highly expressed in multiple cancer types, including bladder urothelial carcinoma, breast invasive carcinoma, cholangiocarcinoma, diffuse large B-cell lymphoma, and liver hepatocellular carcinoma (**Figure 1A**). Additionally, PYGO2 expression was significantly upregulated in 50 paired liver cancer tissues ($P < 0.001$) (**Figure 1C**). Receiver operating characteristic (ROC) curve analysis demonstrated that PYGO2 had strong predictive power for distinguishing liver cancer tissues from normal tissues, with an area under the curve (AUC) of 0.984 (95% confidence interval [CI] = 0.974-0.994) (**Figure 1D**).

Associations between PYGO2 expression and clinicopathologic variables

As shown in **Figure 2**, high PYGO2 expression was significantly associated with advanced tumor characteristics. Specifically, its expression was higher in patients with advanced pathologic T stage (T3 & T4 vs. T1, $P < 0.001$), advanced pathologic stage (Stage III & IV vs. Stage I, $P < 0.001$), and tumor status ($P < 0.05$). PYGO2 expression levels increased with tumor progression. Additionally, PYGO2 expression varied by race, with significantly higher expression in Asian patients compared to White patients ($P < 0.01$).

Prognostic value of PYGO2 in liver cancer

As shown in **Figure 3A**, PYGO2 was identified as a high-risk prognostic factor for liver cancer. Kaplan-Meier survival analysis was used to evaluate the correlation between PYGO2 expression and patient prognosis. Based on the optimal cut-off score, patients were divided into high and low PYGO2 expression groups. Patients in the high PYGO2 expression group had significantly worse overall survival (OS) and disease-specific survival (DSS) compared to those in the low expression group (OS: hazard ratio [HR] = 2.05, 95% CI = 1.45-2.91, $P < 0.001$; DSS: HR = 2.52, 95% CI = 1.61-3.94, $P < 0.001$) (**Figure 3B**, **3C**). Furthermore, in the TCGA-LIHC cohort, high PYGO2 expression was associated with a lower progression-free interval (PFI) (PFI: HR = 1.54, 95% CI = 1.13-2.09, $P = 0.006$) (**Figure 3D**).

Identification of differentially expressed genes (DEGs) in liver cancer

A total of 1,674 differentially expressed genes (DEGs) were identified between the high and low PYGO2 expression groups, including 1,221 upregulated DEGs (72.9%) and 453 downregulated DEGs (27.1%) (*adjusted* $P < 0.05$, $|Log_2FC| > 1$) (**Figure 4A**). Additionally, the relationships between PYGO2 and the top 10 DEGs - LGALS14, CEACAM7, SMR3A, SAA2, ARHGAP36, HMGA2, WIF1, SAA1, CIDEA, and OR12D2 - are illustrated in **Figure 4B**.

Functional enrichment analyses: GO, KEGG, and GSEA

GO enrichment analysis categorized DEGs into three major aspects: Molecular Functions (MF): Enriched GO terms included hormone activity (GO:0005179), serine-type peptidase activity (GO:0017171), channel activity (GO:0015267), serine-type endopeptidase activity (GO:0008236), and receptor-ligand activity (GO:0048018). Cellular Components (CC): Significant terms included blood microparticles (GO:0072562), apical plasma membrane (GO:0016324), plasma lipoprotein particles (GO:0034358), lipoprotein particles (GO:1990777), and high-density lipoprotein particles (GO:0034364). Biological Processes (BP): DEGs were enriched in regulation of hormone levels (GO:0010817), amino acid catabolic processes (GO:0009063), small molecule catabolic processes (GO:0044282), pattern specification processes (GO:0007389), and responses to metal ions (GO:0010038).

KEGG pathway analysis identified significant enrichment in the retinol metabolism pathway (hsa00830), bile acid metabolism pathway (hsa04976), tryptophan metabolism pathway

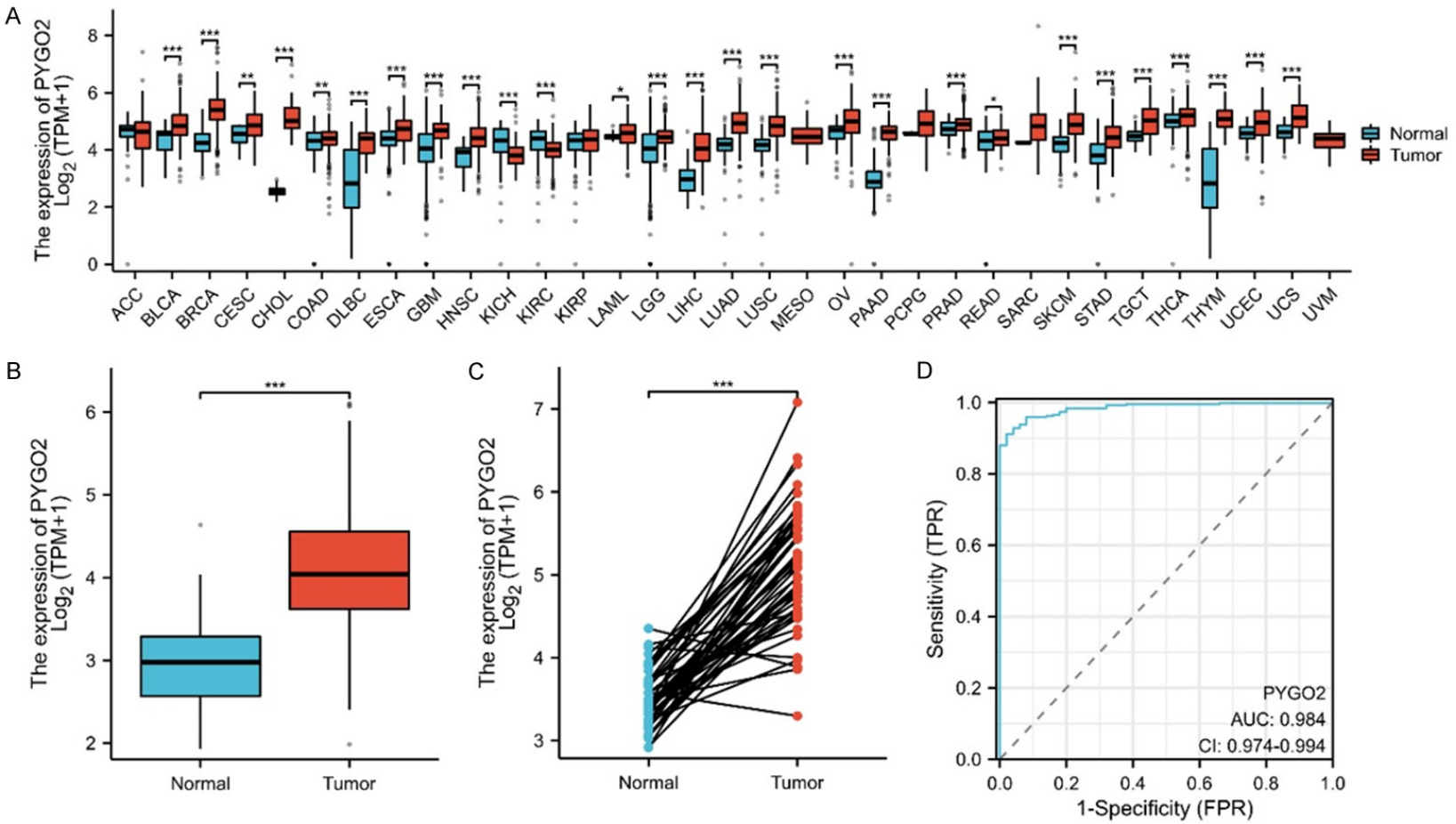

Figure 1. Expression levels of PYGO2 in different types of tumors and liver cancer. A. Expression of PYGO2 in various tumor types compared with normal tissues in the TCGA and GTEx databases. B. Expression of PYGO2 in liver cancer and non-matched normal tissues from the TCGA and GTEx databases. C. Expression of PYGO2 in liver cancer and matched normal tissues from the TCGA database. D. Diagnostic ROC curve of PYGO2 in liver cancer. TCGA: The Cancer Genome Atlas; GTEx: Genotype-Tissue Expression Project; ROC: Receiver Operating Characteristic. *P < 0.05, **P < 0.01, and ***P < 0.001.

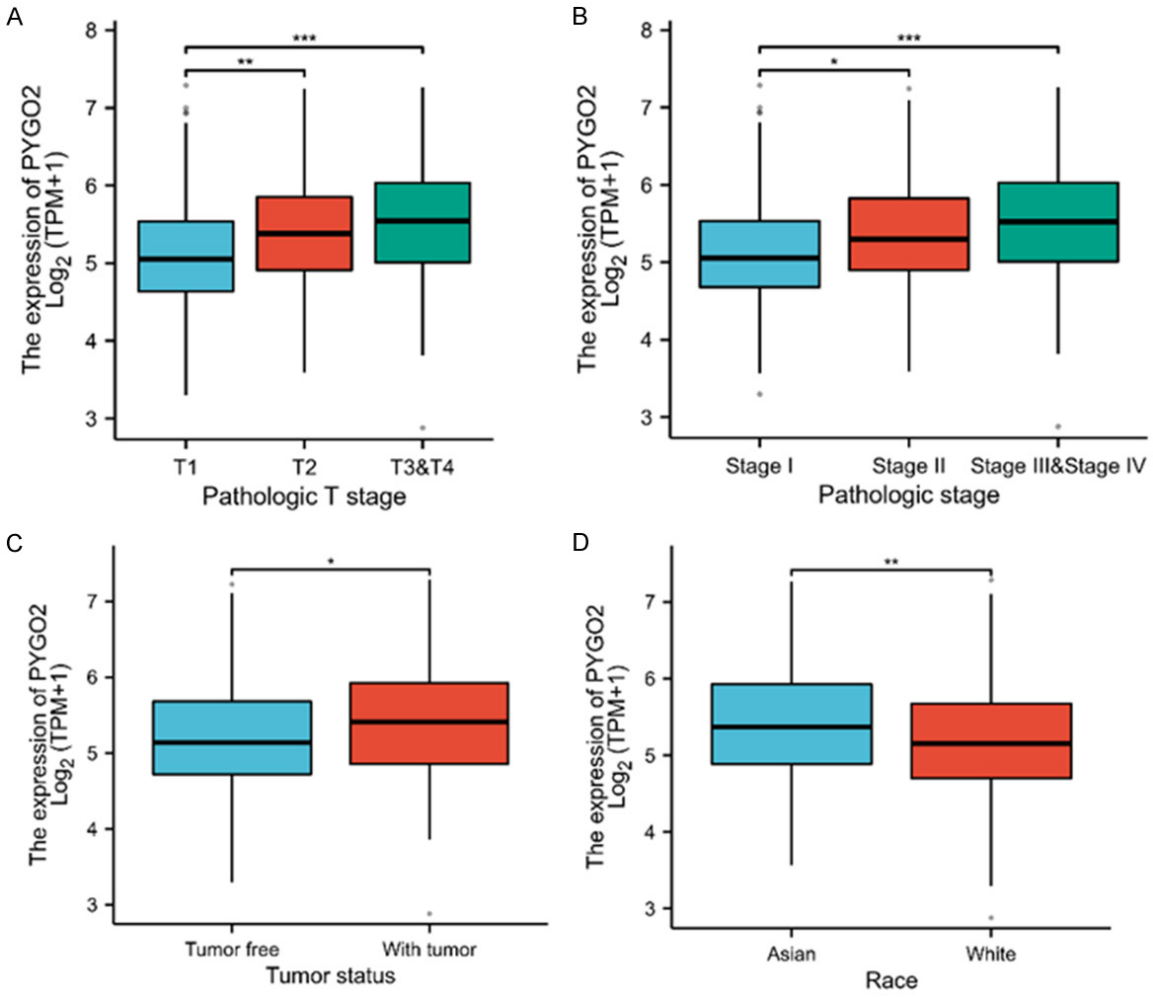

Figure 2. Associations between PYGO2 expression and clinicopathological characteristics. A. Pathological T stage. B. Pathological stage. C. Tumor type. D. Race. *P < 0.05, **P < 0.01, and ***P < 0.001.

(hsa00380), and alanine, aspartate, and glutamate metabolism pathway (hsa00250) (**Figure 4C**).

Additionally, GSEA analysis comparing high and low PYGO2 expression groups revealed a significant enrichment of immune-related biological processes in the low PYGO2 expression group, suggesting that high PYGO2 expression is associated with a decreased immune phenotype in liver cancer (**Figure 5A**, **5B**).

Correlation between PYGO2 expression and immune infiltration

In addition, the proportions of 24 immune cell types in liver cancer were analyzed using the ssGSEA algorithm. Compared to the low-expression group, the high-expression group had lower proportions of cytotoxic cells, dendritic cells, immature dendritic cells, mast cells, neutrophils, plasmacytoid dendritic cell-like cells, Th17 cells, and regulatory T cells. In contrast, the proportion of Th2 cells was higher in the high expression group (**Figure 6A**). To assess the potential effect of immunotherapy, we analyzed the expression levels of immune checkpoint genes in the high- and low-PYGO2 expression groups. It was found that CTLA4, HAVCR2, LAG3, PDCD1, and TIGIT were more highly expressed in the high-expression group (**Figure 6B**).

Discussion

Liver hepatocellular carcinoma (LIHC) progresses rapidly, leading to a poor prognosis, with a five-year survival rate of less than 10% [13].

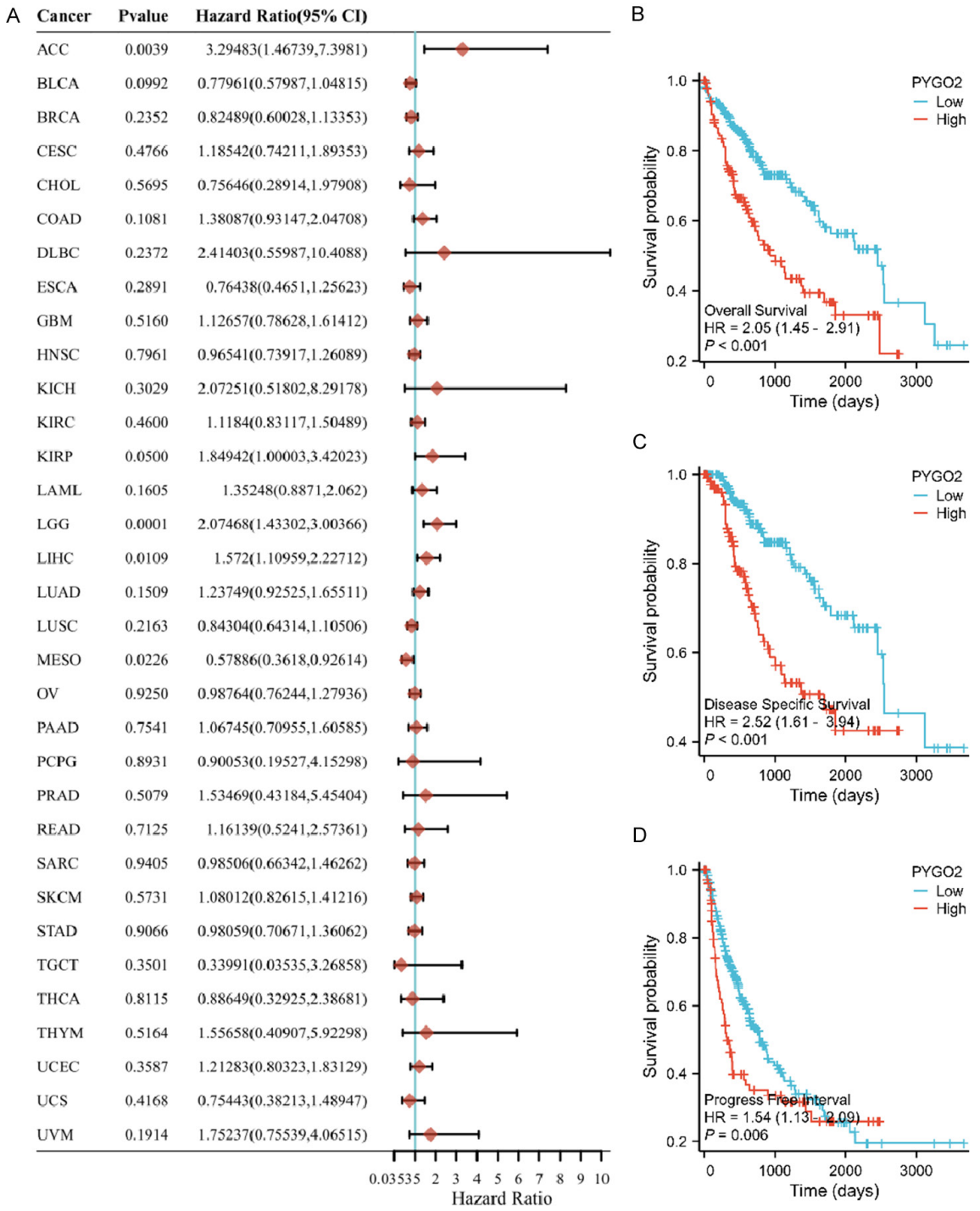

Figure 3. Associations between PYGO2 expression and prognosis. A. Forest plot showing the association between PYGO2 expression and pan-cancer prognosis. B. Kaplan-Meier survival curve for overall survival (OS) in liver cancer. C. Kaplan-Meier survival curve for disease-specific survival (DSS) in liver cancer. D. Kaplan-Meier survival curve for progression-free interval (PFI) in liver cancer. OS: Overall Survival; DSS: Disease-Specific Survival; PFI: Progression-Free Interval.

Due to the heterogeneity of LIHC, current pathological indicators used to predict prognosis - such as tumor stage, grade, and serum alpha-fetoprotein (AFP) levels - have certain limitations

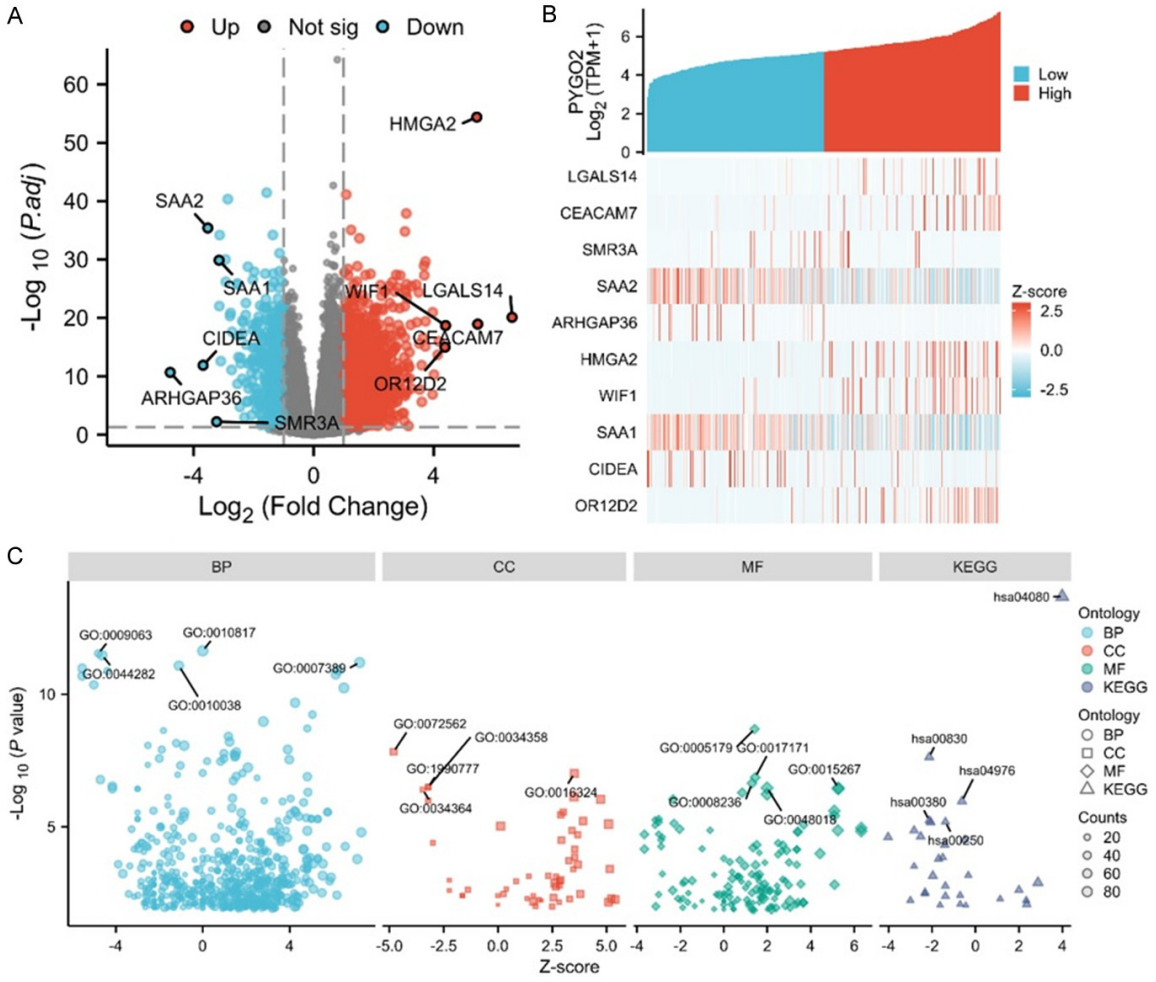

Figure 4. Identification of DEGs and functional enrichment analysis. A. Volcano plot of differentially expressed genes (DEGs). Blue dots represent significantly downregulated DEGs, and red dots represent significantly upregulated DEGs. B. Heatmap of the correlation between PYGO2 expression and the top 10 DEGs. C. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs. DEGs: Differentially Expressed Genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

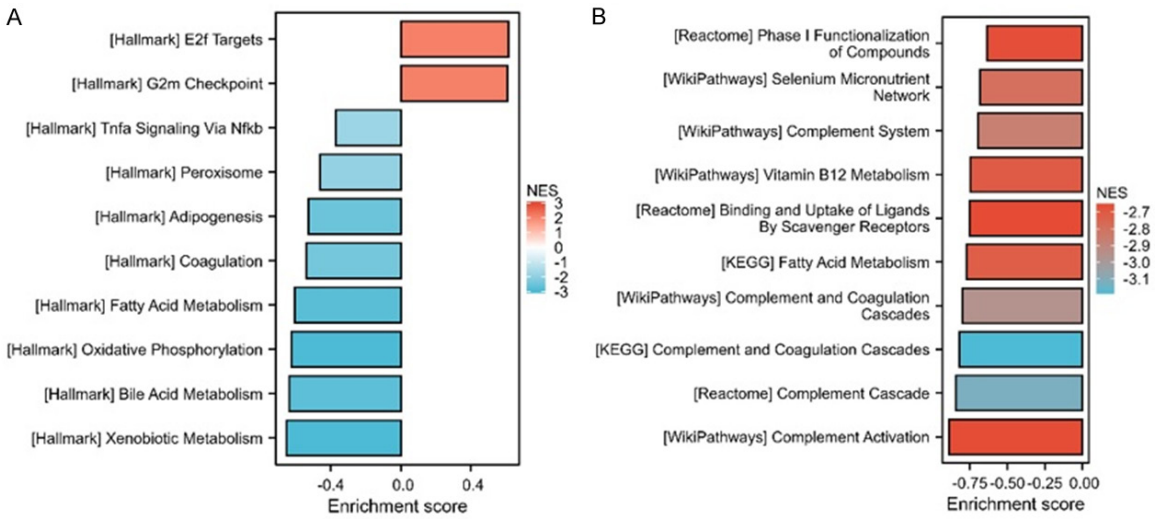

Figure 5. Gene set enrichment analysis (GSEA) of DEGs. A. GSEA analysis of the Hallmark gene sets from the Molecular Signatures Database (MSigDB). B. GSEA analysis of Reactome pathways, Wikipathways and KEGG pathways.

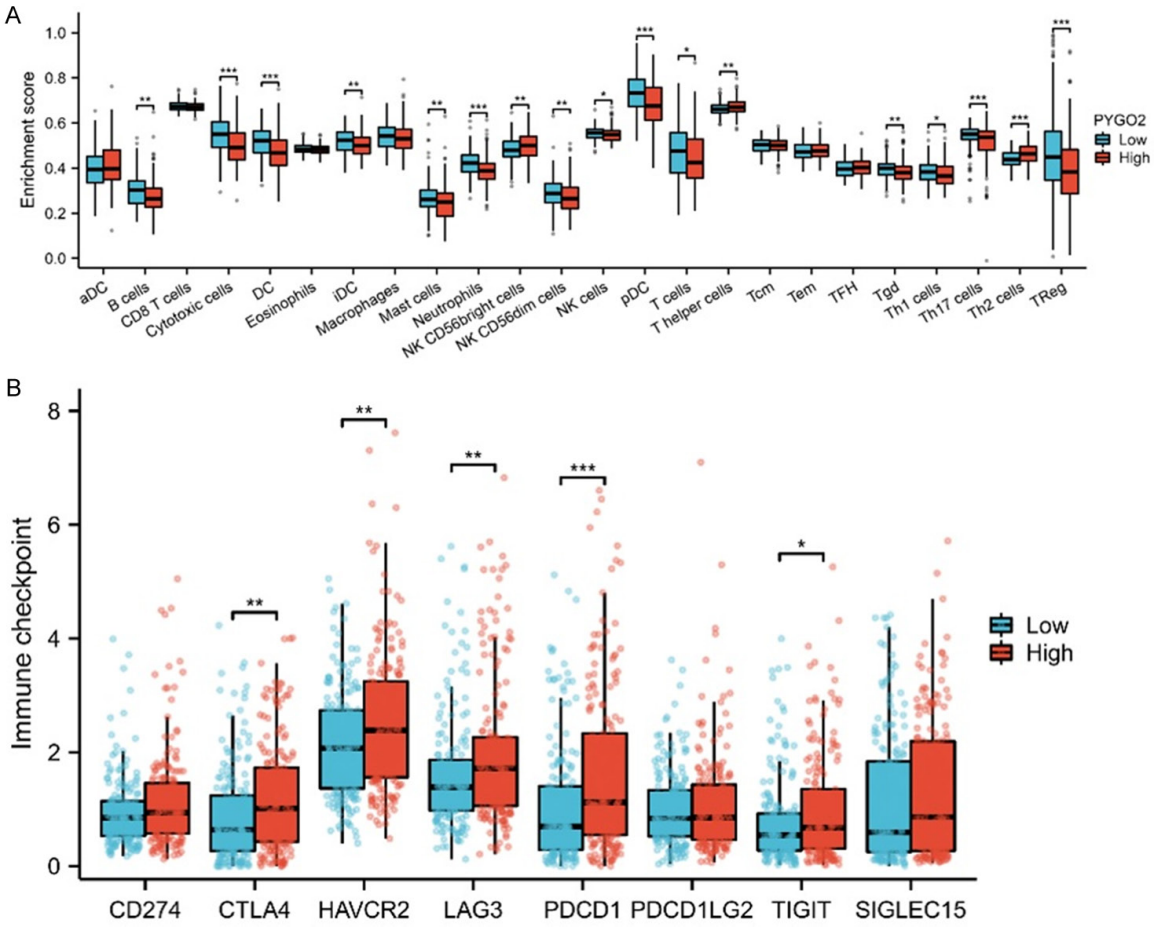

Figure 6. Analysis of PYGO2 expression and immune infiltration in liver cancer. A. Proportions of immune cell infiltration in the high- and low-PYGO2 expression groups based on ssGSEA in the TCGA cohort. B. Differences in immune checkpoint gene expression between the high- and low-PYGO2 expression groups.

[14-17]. Therefore, identifying new biomarkers to predict prognosis and improve individualized treatment is essential. Accurately predicting liver cancer prognosis can significantly enhance the survival rate of LIHC patients.

In this study, through analysis of the TCGA and GTEx databases, we found that PYGO2 expression was significantly upregulated in LIHC. Research suggests that PYGO2 may activate Wnt target genes, contributing to abnormalities in the Wnt pathway and tumor progression. It is also involved in rRNA transcription, promoting cancer cell growth [18, 19]. These findings indicate that PYGO2 plays a role in the occurrence and development of LIHC. Furthermore, our results show that high PYGO2 expression is significantly correlated with pathological T stage, pathological stage, tumor status, and race, aligning with previous studies [20-22]. Kaplan-Meier survival analysis demonstrated that low PYGO2 expression was associated with favorable overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) in liver cancer. Similarly, prior studies suggest that PYGO2 expression is a potential prognostic indicator of poor survival in various solid tumors, including ovarian, breast, cervical, lung, colorectal, brain, and liver cancers [5, 23-28]. Although limited studies have explored its function in tumors, our findings suggest that PYGO2 can serve as an effective prognostic marker for LIHC and may provide novel insights for its treatment.

We performed functional enrichment analysis of PYGO2 in LIHC. Gene Set Enrichment Analysis (GSEA) revealed that PYGO2 high-expression groups showed significant enrichment in pathways such as E2F targets, G2M checkpoint,

Phase I Functionalization of Compounds, Selenium Micronutrient Network, and Complement System. E2F targets and G2M checkpoint pathways are crucial for regulating genes involved in DNA replication and cell cycle progression and are significantly enriched in various tumors E2F targets and G2M checkpoint pathways are crucial for regulating genes involved in DNA replication and cell cycle progression and are significantly enriched in various tumors [29, 30]. The E2F family of transcription factors plays a key role in cell cycle regulation, apoptosis, and DNA damage response, contributing to tumor pathogenesis and progression The E2F family of transcription factors plays a key role in cell cycle regulation, apoptosis, and DNA damage response, contributing to tumor pathogenesis and progression [31]. Moreover, E2F expression has prognostic value in cancer, influencing liver cancer progression and survival outcomes [32]. Understanding different E2F expression patterns may aid in developing more effective treatment strategies [33]. The G2-M checkpoint is critical for maintaining genomic stability by ensuring proper DNA repair before mitosis. In many cancers, G1-S checkpoint defects force tumor cells to rely on an abnormally regulated G2-M checkpoint, promoting unchecked proliferation and affecting prognosis [34]. These results suggest that PYGO2 may contribute to tumor progression by modulating these pathways. However, further research is needed to fully elucidate the mechanisms underlying PYGO2's role in liver cancer.

Immune cells in the tumor microenvironment play a crucial role in tumor progression, prognosis, and response to immunotherapy, including immune checkpoint inhibition (ICI) treatment [31]. Our study found that PYGO2 is highly expressed in liver cancer, potentially promoting tumor progression by altering immune cell infiltration and leading to poor patient prognosis. However, the precise mechanism underlying immune infiltration in LIHC remains unclear. Assessing immune cell infiltration in LIHC is critical for predicting response to ICI therapy. Our results showed a positive correlation between PYGO2 expression and Th2 cell infiltration. Studies have demonstrated that Th2 cells are predominantly localized within tumors, where they suppress immune responses and promote tumor growth and metastasis [32, 33]. Conversely, PYGO2 expression negatively correlated with activated plasmacytoid dendritic cell-like cells and cytotoxic cells. Activated plasmacytoid dendritic cell-like cells are innate immune cells that suppress liver cancer cell growth [34]. Cytotoxic cells are crucial for antitumor immunity, and their presence is associated with better prognosis in liver cancer patients [35]. These findings suggest that PYGO2 expression may influence the immune microenvironment of LIHC, impacting immune regulation and tumor progression.

Our results also indicate that high PYGO2 expression is associated with increased expression of immune checkpoint genes, particularly PDCD1. PDCD1 encodes for Programmed Cell Death Protein 1 (PD-1), a key immune checkpoint receptor. PD-1 (CD279) is widely expressed in tumor tissues and interacts with PD-L1/PD-L2 on tumor cells, facilitating immune evasion [36]. Although our findings do not conclusively demonstrate that PYGO2 directly promotes PD-1-mediated tumor immune escape in LIHC, they suggest a potential link between PYGO2 and immunosuppressive mechanisms, offering new perspectives for immunotherapy research. Further investigation is required to determine how PYGO2 influences immune checkpoint pathways and its implications for immune-targeted therapies.

Although our study provides new insights into the relationship between PYGO2 expression and LIHC prognosis, it has several limitations: Most data were obtained from online databases, preventing access to detailed patient information and treatment histories. Further experimental validation and clinical studies are needed to explore the biological functions and molecular mechanisms of PYGO2 in LIHC.

Conclusion, our findings suggest that PYGO2 expression in liver cancer may impact disease progression and prognosis by modulating immune cell infiltration. PYGO2 is implicated in LIHC pathogenesis, influencing tumor growth, metastasis, and the tumor microenvironment. Additionally, PYGO2 negatively regulates immune cell infiltration, potentially affecting disease outcomes. Given its association with immune suppression and tumor progression, PYGO2 holds promise as a novel biomarker for LIHC prognosis. However, further research is necessary to fully elucidate its biological role and its potential in targeted cancer therapies.

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Disclosure of conflict of interest

The authors state that they conducted the research without any commercial or financial relationships that could be considered a potential conflict of interest.

Address correspondence to: Zhongping Qian, Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. E-mail: moony_u@163.com

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