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RESEARCH ARTICLE

ILKAP drives hepatocellular carcinoma progression by modulating PGAM1-mediated glycolytic reprogramming

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Abstract **Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality, necessitating novel therapeutic targets. This study explores the oncogenic role of integrin-linked kinase-associated phosphatase (ILKAP) in HCC and its underlying mechanisms. Database analyses (TCGA, UALCAN) revealed ILKAP overexpression in HCC, correlating with poor prognosis. Functional assays demonstrated that *ILKAP* knockdown significantly suppressed HCC cell proliferation and migration *in vitro*, while xenograft models confirmed its role in tumor growth *in vivo*. RNA sequencing identified 357 differentially expressed genes (DEGs), including 48 protein-coding DEGs, with glycolytic enzyme PGAM1 notably downregulated upon ILKAP silencing. ILKAP and PGAM1 expression were positively correlated in HCC tissues, and elevated PGAM1 levels were linked to worse survival. Notably, restoring PGAM1 in *ILKAP*-knockdown cells rescued proliferation and invasion, underscoring PGAM1's critical role in ILKAP-mediated tumor progression. ILKAP depletion also reduced extracellular acidification rates and altered glycolysis-related gene expression, highlighting its role in metabolic reprogramming. These findings suggest that ILKAP drives HCC malignancy by modulating PGAM1 and glycolysis, providing a potential therapeutic target for HCC treatment. Further elucidation of the ILKAP-PGAM1 axis may offer new strategies for liver cancer management.**

Keywords ILKAP; PGAM1; HCC; glycolysis

Introduction

Hepatocellular carcinoma (HCC) is a prevalent and aggressive malignancy, representing a significant global health issue due to its high incidence and mortality rates. The pathogenesis of HCC is multifactorial, involving chronic liver diseases such as hepatitis B and C infections, alcoholic liver disease, and nonalcoholic fatty liver disease, which often lead to cirrhosis and eventual tumor development [1,2]. Despite advancements in treatment modalities, the prognosis for HCC patients remains poor, primarily due to late-stage diagnosis and the tumor's inherent resistance to conventional therapies [3,4]. Therefore, identifying novel biomarkers and therapeutic targets is crucial for improving patient outcomes.

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Recent studies have highlighted the importance of various molecular pathways and proteins in the development and progression of HCC. One such protein is integrin-linked kinase (ILK)-associated phosphatase (ILKAP), a member of the protein phosphatase 2C family, which has been implicated in regulating several cellular processes, including apoptosis, cell adhesion, and survival [5–8]. ILKAP's involvement in cancer biology has garnered attention, as it appears to inhibit ILK signaling, which is crucial for tumor growth and metastasis [9–11]. Elevated levels of ILKAP have been associated with poor prognosis in various cancers, including HCC, suggesting its potential as a therapeutic target [11–14].

Despite the established role of ILKAP in cancer, the specific mechanisms by which it influences HCC progression remain poorly understood. For instance, the relationship between ILKAP and metabolic reprogramming in HCC has not been extensively explored. Tumor cells often exhibit altered metabolic pathways, such as enhanced glycolysis, which supports

rapid proliferation and survival under hypoxic conditions [15,16]. Phosphoglycerate mutase 1 (PGAM1), a glycolytic enzyme, has been identified as a key regulator of tumor metabolism and aggressiveness in various cancers [17,18]. However, whether PGAM1 functions downstream of ILKAP in HCC remains to be elucidated. This interplay between ILKAP and PGAM1 could provide insights into the metabolic adaptations that facilitate HCC progression.

To investigate the role of ILKAP in HCC, we employed a combination of *in vitro* and *in vivo* approaches. We utilized human liver cancer cell lines and established knockdown models to assess the functional impact of ILKAP silencing on cell proliferation, migration, and invasion. Furthermore, we conducted RNA sequencing to identify differentially expressed genes (DEGs) and pathways affected by ILKAP modulation, focusing on those related to glycolysis and metabolic regulation. This approach not only elucidates the role of ILKAP in HCC but also highlights its potential as a therapeutic target.

Ultimately, our study aims to clarify the molecular mechanisms underpinning ILKAP's role in HCC progression and its interaction with glycolytic pathways. By delineating these processes, we hope to identify novel strategies for targeting ILKAP and associated metabolic pathways in HCC, potentially improving therapeutic outcomes for patients suffering from this aggressive malignancy. This research contributes to the growing body of literature exploring the complex relationships between metabolic reprogramming and cancer progression, underscoring the necessity for further investigation into potential therapeutic interventions.

Materials and methods

Cell lines and culture conditions

Human immortalized normal hepatocyte cell line (THLE-2) and HCC cell lines (Hep3B, SNU449, HCCLM3, and Huh7) were sourced from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco), 100 μg/mL penicillin, and 100 U/mL streptomycin (Sigma, St. Louis, MO, USA). Cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Transfection and plasmid construction

Small interfering RNAs (siRNAs) targeting ILKAP mRNA and short hairpin RNAs (shRNAs) for *ILKAP* knockdown, along with non-targeting shRNA controls, were obtained from Genepharma (Shanghai, China). Lentiviral infection of HCC cells was facilitated by polybrene (4 μg/mL) over one week to establish stable transfectants. The overexpression plasmid for PGAM1 and the vector control plasmid were purchased from Genepharma and transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. siRNA and shRNA sequences are listed in Table S2.

Clinical tissue samples

Human HCC tissues and corresponding adjacent non-tumor liver tissues were collected from patients undergoing surgical resection at Zhejiang Provincial People's Hospital (Hangzhou, China). All specimens were promptly harvested after surgery, snap-frozen in liquid nitrogen, and stored at –80 °C until further processing. The study protocol was reviewed and approved by the Ethics Committee of Zhejiang Provincial People's Hospital (Approval No. QT2023321). This study was granted exemption from written informed consent according to the clinical trial waiver application. All procedures were performed in accordance with the Declaration of Helsinki.

Western blot analysis

Protein from liver cancer cells was extracted using RIPA buffer (Beyotime), and concentrations were quantified with a BCA Protein Assay Kit. Lysates were denatured, separated by 10% SDS-PAGE gels, and transferred onto PVDF membranes (Millipore). The membranes were blocked in 5% skim milk and incubated with primary antibodies-ILKAP (16017-1-AP, Proteintech), GAPDH (HA721136, HUABIO), PGAM1 (ET7109-13, HUABIO), LDHA (ET1608-57, HUABIO), PFKP (HA500472, HUABIO), PFM2 (ER1901-90, HUABIO), PDHA1 (ET1702-75, HUABIO), Hexokinase 1 (ET1609-28, HUABIO), Hexokinase II (HA722933, HUABIO), P-AKT (HA722951, HUABIO), p-PI3K (HA721672, HUABIO), AKT (ET1609-51, HUABIO), PI3K (ET1610-36, HUABIO), each diluted at a 1:1000 working concentration. After washing, the blots were processed with appropriate secondary antibody (Anti-Rabbit IgG-HRP, HA1001, HUABIO) and visualized using ECL Substrate (1705061, Bio-Rad).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (15596018CN, Invitrogen) and reverse transcribed into cDNA using a reverse transcription kit (639505, Takara, Japan) according to the manufacturer's protocol. Quantitative real-time PCR was performed using TB Green (CN830S, Takara) on a real-time PCR system.

GAPDH was used as the internal control. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table S2.

RNA sequence (RNA-seq)

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Qualified RNA samples were sent to LC-Bio Technology Co., Ltd. (Hangzhou, China) for library preparation, high-throughput sequencing using the Illumina NovaSeq 6000 platform, and subsequent bioinformatics analysis, including differential gene expression and functional enrichment analysis.

EdU incorporation assay

The EdU assay was performed using a commercial kit (Beyotime, Cat No. C0071S) according to the manufacturer's instructions. Briefly, transfected HCC cells were incubated with 10 μmol/L EdU for 2 h at 37 °C, then fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.5% Triton X-100 for 10 min. Cells were stained using the Apollo reaction mixture, and nuclei were counterstained with Hoechst 33342. Images were captured with a fluorescence microscope, and EdU-positive cells were quantified using ImageJ software.

Colony formation assay

Post-transfection, HCC cells were seeded at 5×10^3 cells/well in 6-well plates. After 15 days of incubation, colonies were fixed with 4% paraformaldehyde, then stained with 0.1% crystal violet.

Wound healing assay

Transfected cells were cultured in 6-well plates with 10% FBS medium. A scratch was made using a 200 μL pipette tip, and cells were then incubated in serum-free medium. Wound closure was monitored at 0, 24, and 48 h.

Cell migration and invasion assays

Cell migration and invasion assays were performed using Transwell chambers (8.0 μm pore size, LABSELECT, Cat No. 14341). After 48 h of transfection, for migration assays, cells (5×10^4 per well) in serum-free medium were seeded into the upper chamber, while the lower chamber contained 10% FBS as a chemoattractant. For invasion assays, the upper chambers were pre-coated with Matrigel (1:8 dilution, BD Biosciences, USA), and 1×10^5 cells per well were seeded. After 48 h of incubation at 37 °C, non-migrated or non-invaded cells were removed with a cotton swab, and cells on the lower surface were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and quantified under a light microscope in three random fields.

Tumorigenesis in nude mice

Male BALB/c nude mice (4–6 weeks) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed under specific pathogen-free (SPF) conditions. Mice were randomly divided into two groups ($n = 8$ per group) and subcutaneously injected in the right axilla with 2×10^6 control or sh-ILKAP stably transfected cells suspended in 100 μL of phosphate-buffered saline (PBS). Tumor volume was measured every two days using a digital caliper and calculated using the formula: tumor volume = (length × width^2) / 2. At the endpoint, mice were euthanized, and tumors were excised, photographed, and weighed. All animal procedures were approved by the Animal Ethics Committee of Hangzhou Medical College, in accordance with institutional guidelines and national regulations (Approval No. 2021-106).

Glucose uptake and lactic acid production

HCC cells were seeded at 4×10^5 cells/well in 6-well plates. Culture medium was collected to measure extracellular lactate levels using a lactate assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and glucose uptake with a glucose assay kit (Beyotime, Shanghai, China).

Extracellular acidification rate (ECAR) measurement

Cells were seeded in XFe24 plates, then transfected. After transfection, ECAR was measured using a Seahorse XF glycolysis rate assay kit (Agilent Technologies) following the manufacturer's protocol.

Immunohistochemistry (IHC)

Paraffin-embedded tissue sections were deparaffinized, rehydrated, subjected to antigen retrieval, and blocked with 5% bovine serum albumin (BSA) at room temperature for 30 min. Sections were then incubated overnight at 4 °C with primary antibodies against ILKAP (16017-1-AP, Proteintech, 1:200), PGAM1 (ET7109-13, HUABIO, 1:200) and Ki-67 (9129S, CST, 1:400). After washing with PBS, the sections were incubated with HRP-conjugated secondary antibodies (HA1001, HUABIO, 1:1000) for 1 h at room temperature. Immunoreactivity was visualized using diaminobenzidine (DAB) substrate, followed by counterstaining with hematoxylin. Stained sections were imaged under a light microscope.

Statistical analysis

Data were analyzed using GraphPad Prism 8.0.0 software. Comparisons between two groups were performed using Student's *t*-test, while ANOVA was used for multiple group comparisons. Chi-square tests were applied for categorical data. Results are presented as mean ± standard deviation (SD), with *P*-values < 0.05 considered statistically significant. All tests were two-tailed.

Results

ILKAP is highly expressed in HCC and correlates with poor prognosis

To explore ILKAP expression across cancers, we analyzed data from the TCGA database and observed its upregulation in multiple tumor types (Fig. 1A). Focusing on HCC, analysis of the UALCAN and StarBase databases revealed significantly elevated ILKAP expression in HCC compared to normal tissues (Fig. 1B). Furthermore, ILKAP expression positively correlated with tumor grade and distant metastasis. Specifically, ILKAP expression progressively increased from Grade 1 to Grade 4 tumors and was further elevated in metastatic samples (Fig. 1C and 1D). Kaplan–Meier survival analysis demonstrated that HCC patients with high ILKAP expression had significantly shorter overall survival (Fig. 1E, $P < 0.05$). To validate these findings, we measured ILKAP mRNA and protein levels in four HCC cell lines (SNU449, HCCLM3, Bel7402, Huh7) and in immortalized normal liver cells (THLE-2). Consistent with the database results, ILKAP expression was markedly higher in HCC cells at both the mRNA and protein levels (Fig. 1F and 1G). Analysis of tissue microarray data from the Human Protein Atlas further confirmed a significant upregulation of ILKAP protein expression in liver cancer tissues (Fig. 1H). To further validate the expression pattern of ILKAP in HCC, we analyzed ILKAP protein levels in several pairs of tumor and adjacent non-tumor liver tissues. The results showed a consistent upregulation of ILKAP in tumor tissues compared to matched controls (Fig. 1I). Collectively, these results suggest that ILKAP overexpression is significantly associated with poor prognosis, making it a potential biomarker for HCC initiation and progression.

Knockdown of *ILKAP* inhibits HCC cell proliferation and migration *in vitro*

To elucidate the role of ILKAP in HCC progression, we performed functional assays using SNU449 and HCCLM3 cell lines, which displayed the highest levels of ILKAP expression. ILKAP expression was efficiently knocked down using siRNA, as confirmed by RT-qPCR and Western blot analysis (Fig. 2A). The effect of ILKAP depletion on cell proliferation was assessed using EdU staining and colony formation assays. *ILKAP* knockdown significantly reduced the number of proliferating cells and colony formation capacity in both HCC cell lines (Fig. 2B and 2C). To further evaluate the role of ILKAP in cell migration and invasion, we performed wound healing and Transwell assays. The results showed that ILKAP silencing led to a marked decrease in cell migration and invasive ability (Fig. 2D and 2E). These findings indicate that ILKAP promotes HCC cell proliferation, migration, and invasion *in vitro*, highlighting its role in driving the malignant phenotype of HCC cells.

ILKAP regulates PGAM1 and enhances glycolysis to promote HCC malignancy

To investigate the molecular mechanisms driving ILKAP-mediated HCC progression, we conducted RNA sequencing on SNU449 cells with *ILKAP* knockdown and their controls. Differential expression analysis identified 357 DEGs, including 48 protein-coding DEGs ($|\log_2 FC| > 1$, $P < 0.05$), of which 26 were upregulated and 22 downregulated (Table S1). Among the downregulated genes, PGAM1, a key glycolytic enzyme, emerged as a potential downstream target (Fig. 3A and 3B). PGAM1 was prioritized due to its core role in glycolysis, pronounced dysregulation in RNA-seq, and independent RT-qPCR confirmation (Fig. 3C). Western blot confirmed that *ILKAP* knockdown significantly reduced PGAM1 expression at protein levels (Fig. 3D). Correlation analysis using the TCGA database demonstrated a significant positive correlation between ILKAP and PGAM1 expression in HCC tissues ($R = 0.20$, $P < 0.001$) (Fig. 3E). Further validation using UALCAN showed that PGAM1 expression was elevated in HCC tissues and associated with shorter overall survival (Fig. 3F and 3G). These findings suggest that ILKAP promotes HCC malignancy by regulating PGAM1 expression, potentially through glycolytic reprogramming.

PGAM1 is essential for ILKAP-mediated HCC progression

To determine the functional role of PGAM1 in ILKAP-mediated oncogenic effects, we overexpressed PGAM1 in *ILKAP*-knockdown cells. Western blot analysis confirmed successful PGAM1 restoration (Fig. 4A). EdU and colony formation assays revealed that PGAM1 overexpression rescued the proliferation defects caused by *ILKAP* knockdown (Fig. 4B and 4C). Similarly, Transwell and wound healing assays demonstrated that PGAM1 restoration reversed the inhibitory effects of

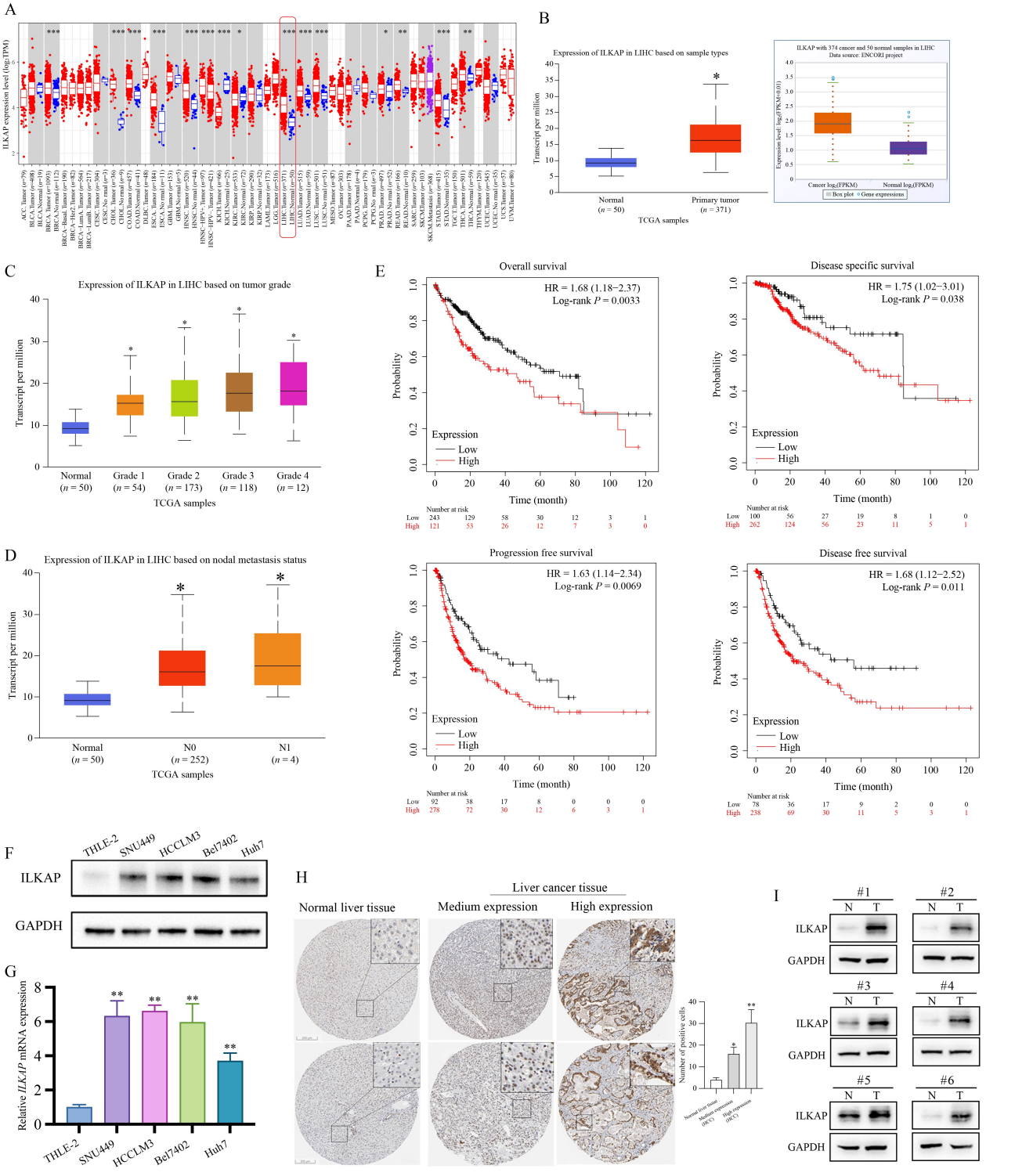

Fig. 1 ILKAP is highly expressed in liver cancer and is associated with poor prognosis. (A) ILKAP expression was analyzed across multiple cancer types using publicly available pan-cancer datasets. (B) UALCAN and StarBase analysis revealed significantly elevated ILKAP expression in HCC compared to normal liver tissues. (C, D) ILKAP expression levels were positively correlated with advanced tumor stages and lymph node metastasis in HCC. (E) Kaplan–Meier survival analysis demonstrated that HCC patients with high ILKAP expression exhibited significantly shorter overall survival compared to those with low ILKAP expression. (F) Western blot analysis showed that ILKAP protein levels were elevated in HCC cell lines compared to normal liver cells. (G) RT-qPCR analysis confirmed increased ILKAP mRNA expression in liver tumor cell lines relative to normal liver cells. (H) ILKAP protein expression was higher in liver cancer tissues compared to normal liver tissues, as determined by IHC. (I) Representative Western blot analysis of ILKAP expression in paired HCC and adjacent non-tumor liver tissues. T, paired HCC; N, adjacent non-tumor.

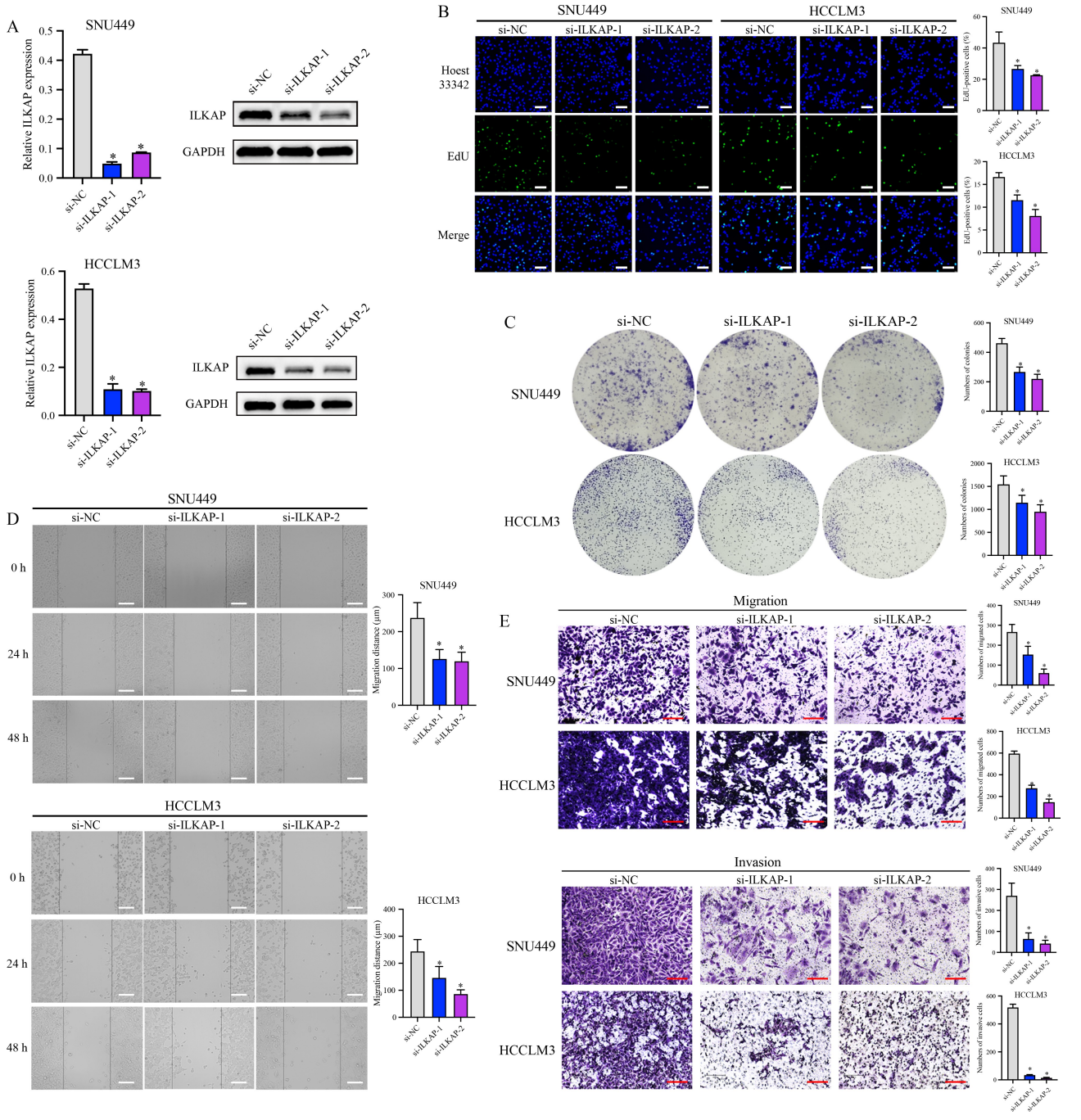

Fig. 2 Knockdown of *ILKAP* inhibits proliferation and migration of liver cancer cells *in vitro*. (A) Validation of *ILKAP* knockdown efficiency in SNU449 and HCCLM3 cells using RT-qPCR and Western blot. (B) EdU staining assays showed a significant decrease in the number of EdU-positive proliferating cells in the *ILKAP* knockdown group compared to the negative control (NC) group ($P < 0.05$) (scale bar = 50 μm). (C) Colony formation assays demonstrated reduced clonogenic capacity in *ILKAP*-silenced cells. (D) Wound healing assays indicated impaired migratory capacity in *ILKAP*-knockdown cells compared to controls (scale bar = 100 μm). (E) Transwell assays further confirmed that *ILKAP* knockdown significantly inhibited the migration and invasion of HCC cells (scale bar = 50 μm).

ILKAP knockdown on cell migration and invasion (Fig. 4D and 4E). These results confirm that PGAM1 is a critical mediator of ILKAP-driven HCC progression and that ILKAP exerts its oncogenic effects, at least in part, through PGAM1.

ILKAP enhances glycolysis through PGAM1 and activates the PI3K/Akt signaling pathway

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis indicated that *ILKAP*

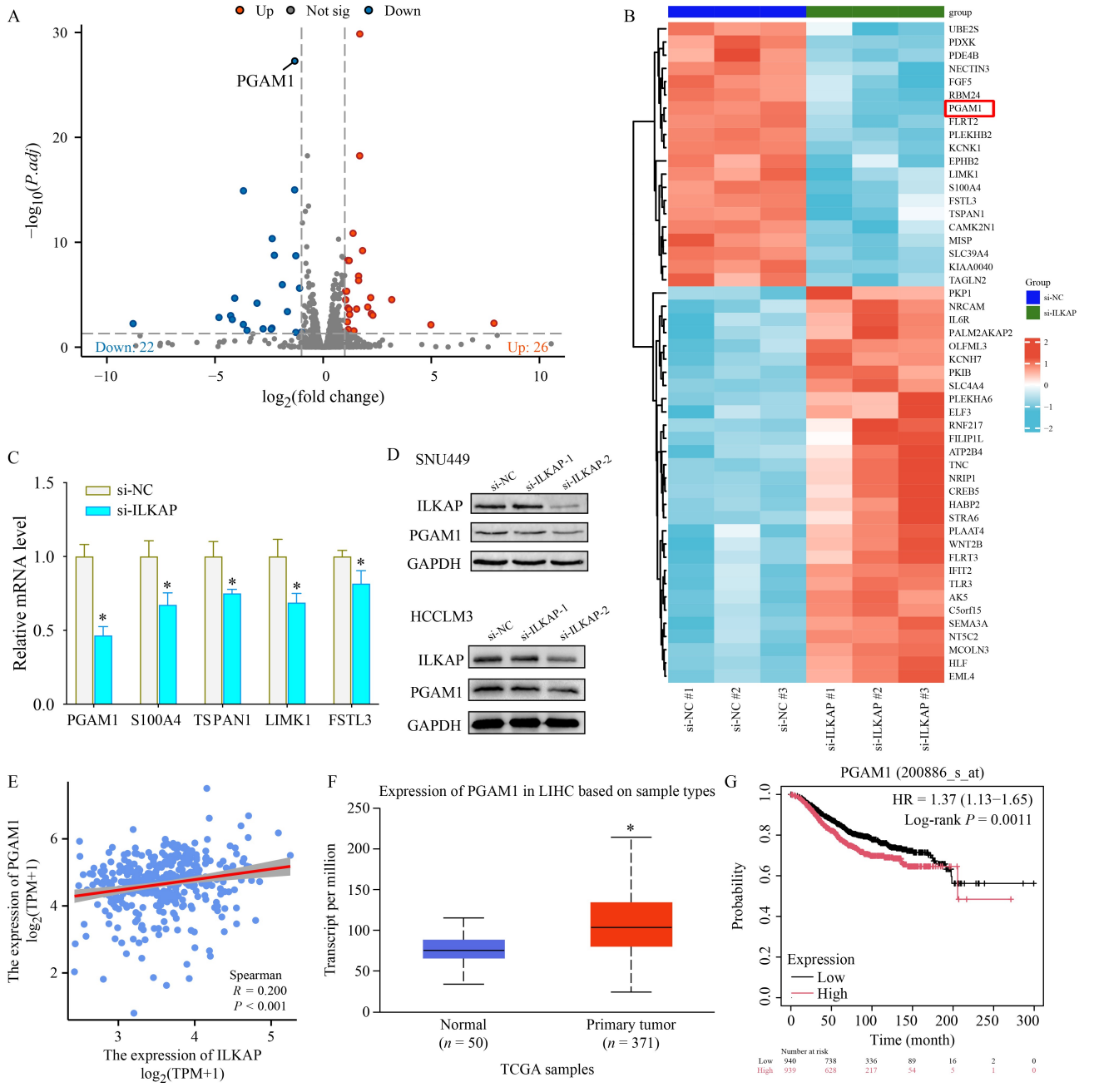

Fig. 3 ILKAP regulates PGAM1 to promote liver cancer malignancy via enhanced glycolysis. (A, B) Heatmap and volcano plot showing DEGs in SNU449 cells with *ILKAP* knockdown compared to control cells. (C) RT-qPCR validation of representative DEGs identified by RNA-seq. (D) Western blot analysis confirmed reduced PGAM1 expression at the protein levels upon *ILKAP* knockdown. (E) Correlation analysis demonstrated a significant positive correlation between ILKAP and PGAM1 expression ($P < 0.001$). (F) UALCAN analysis showed elevated PGAM1 expression in liver cancer tissues compared to normal tissues. (G) Kaplan–Meier survival analysis indicated that higher PGAM1 expression was associated with poorer prognosis in HCC patients.

knockdown primarily affected metabolic pathways, including glycolysis (Fig. 5A). ECAR measurements demonstrated reduced glycolytic activity in *ILKAP*-depleted cells, which was restored by PGAM1 overexpression (Fig. 5B). Consistently, glucose uptake and lactate production were significantly reduced in *ILKAP*-knockdown cells, whereas PGAM1 restoration reversed these effects (Fig. 5C–5F).

Correlation analysis from TCGA database revealed positive associations between ILKAP and glycolysis-related genes (*HK1*, HK2, *ENO1*, *PFKP*, *LDHA*, *PDHA1*, *PGK1*) (Fig. 6A). Western blot analysis confirmed reduced expression of key glycolytic enzymes, including HK1, HK2, PKM2, and LDHA, in *ILKAP*-depleted cells

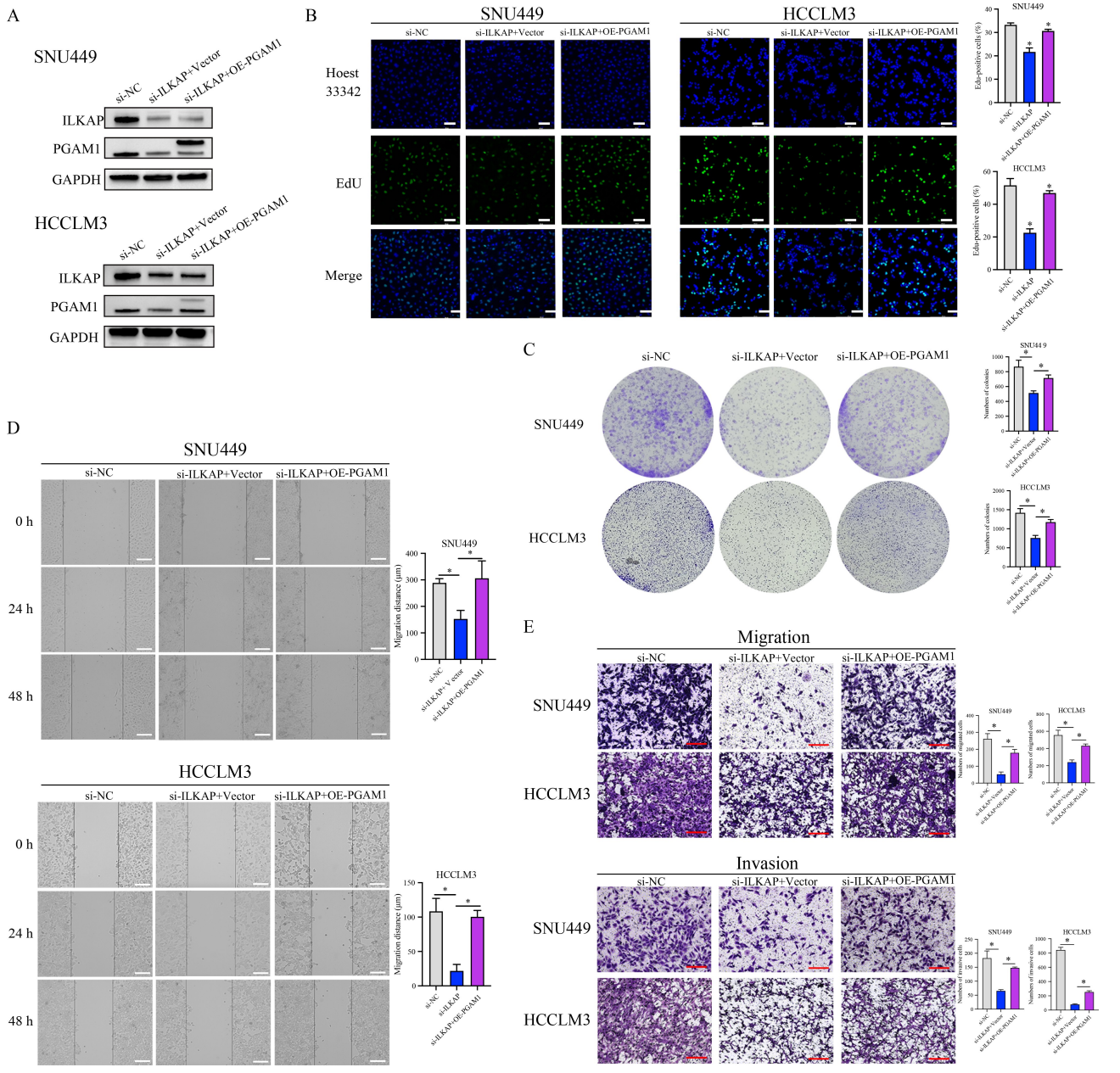

Fig. 4 PGAM1 is critical for ILKAP-mediated progression of HCC. (A) Western blot analysis verified the overexpression of PGAM1 in *ILKAP*-knockdown cells transfected with a PGAM1 overexpression plasmid. (B, C) EdU staining and colony formation assays demonstrated that PGAM1 overexpression rescued the *ILKAP* knockdown-induced suppression of cell proliferation (scale bar = 50 μm). (D, E) Wound healing and Transwell assays showed that PGAM1 overexpression reversed the inhibitory effects of *ILKAP* knockdown on cell migration and invasion (wound healing scale bar = 100 μm, Transwell scale bar = 50 μm).

(Fig. 6B). Additionally, *ILKAP* knockdown led to reduced phosphorylation levels of PI3K and AKT, suggesting inactivation of the PI3K/Akt signaling pathway, which is known to regulate glycolysis and tumor progression (Fig. 6C). These results indicate that ILKAP promotes glycolytic reprogramming and HCC malignancy by upregulating PGAM1 and activating the PI3K/Akt signaling pathway.

Knockdown of ILKAP suppresses tumor growth *in vivo*

To validate the *in vitro* findings, we established a xenograft tumor model using HCCLM3 cells stably transfected with ILKAP shRNA (Fig. 7A). Tumor-bearing nude mice were divided into the sh-ILKAP group and the control (sh-NC) group. Tumor growth was

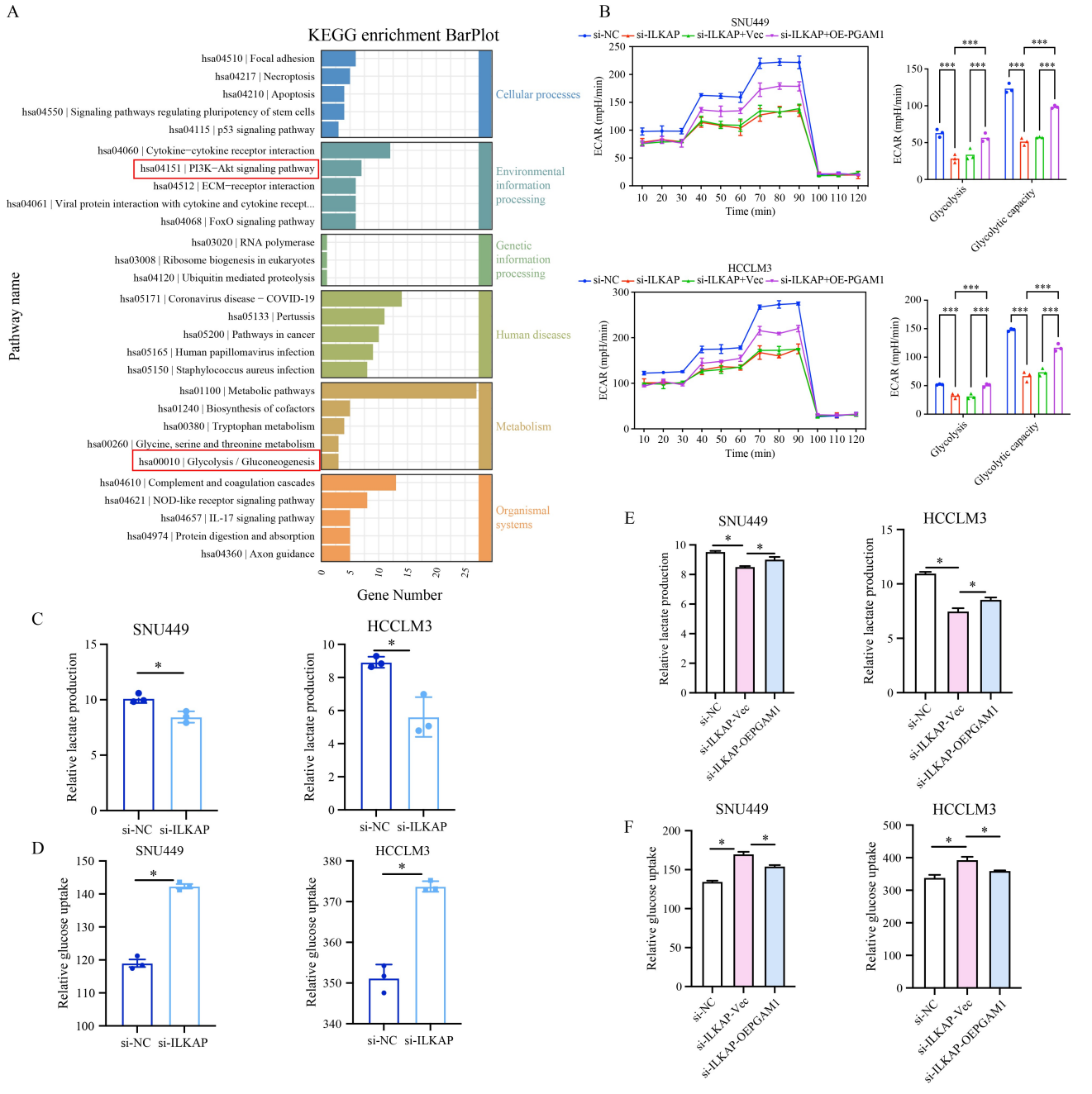

Fig. 5 ILKAP enhances glycolysis through PGAM1 in liver cancer cells. (A) KEGG pathway enrichment analysis of DEGs in *ILKAP*-knockdown cells indicated a significant involvement of glycolytic pathways. (B) ECAR assays revealed reduced glycolytic activity in *ILKAP*-knockdown cells, which was restored by PGAM1 overexpression. (C, D) Knockdown of *ILKA*P significantly decreased glucose uptake and lactate production in liver cancer cells. (E, F) PGAM1 overexpression rescued glucose uptake and lactate secretion defects caused by *ILKAP* knockdown.

monitored over 14 days, and the results revealed a significant reduction in tumor volume and weight in the *ILKAP* knockdown group compared to controls (Fig. 7B–7D, $P < 0.05$). IHC analysis further confirmed that the expression of ILKAP protein in tumors of the sh-ILKAP group was decreased, which significantly inhibited the expression of the proliferation marker Ki-67 (Fig. 7E). Meanwhile, the knockdown of *ILKAP* markedly suppressed the expression of the downstream target PGAM1 as well as the expression levels of p-AKT and p-PI3K (Fig. 7F). Together, these results provide strong evidence that ILKAP promotes HCC tumor growth *in vivo*.

Discussion

HCC is the most prevalent form of primary liver cancer, responsible for a significant proportion of cancer-related

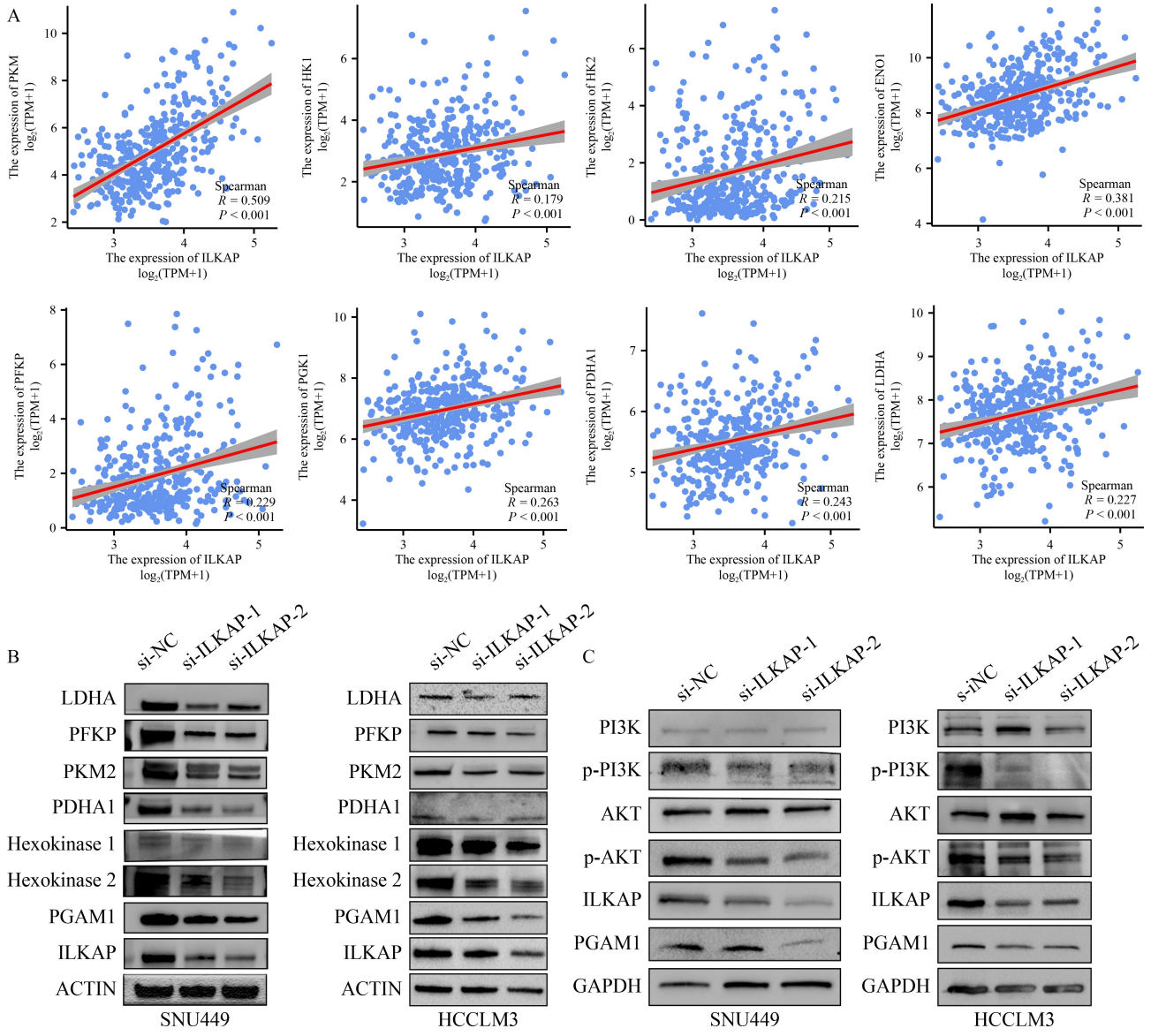

Fig. 6 ILKAP promotes glycolysis and activates the PI3K/Akt signaling pathway in liver cancer cells. (A) Correlation analysis revealed significant positive associations between ILKAP expression and multiple glycolysis-related genes, including *HK1*, *HK2*, *ENO1*, *PFKP*, *LDHA*, *PDHA1*, and *PGK1*, in liver cancer tissues. (B) Western blot analysis confirmed decreased expression of key glycolytic enzymes (HK1, HK2, PKM2, LDHA, and PFKP) following *ILKAP* knockdown. (C) *ILKAP* knockdown led to a significant reduction in PI3K and Akt phosphorylation levels, indicating suppression of the PI3K/Akt signaling pathway, which is known to regulate glycolysis and tumor progression.

mortality worldwide. It is often associated with underlying conditions such as chronic hepatitis B and C infections, alcoholic liver disease, and non-alcoholic fatty liver disease, which lead to liver cirrhosis, a major risk factor for HCC development [19–21]. The aggressive nature of HCC is compounded by its late diagnosis and limited treatment options, emphasizing the need for a deeper understanding of the molecular mechanisms driving its progression and metastasis [22]. Recent studies have identified various biomarkers and signaling pathways that contribute to the malignancy of HCC, presenting potential targets for therapeutic intervention aimed at improving patient outcomes and survival rates [23–25].

In this study, we explored the role of ILKAP in HCC progression, highlighting its upregulation in HCC tissues and cell lines. Our findings indicate that ILKAP promotes cancer cell proliferation, migration, and glycolysis, which are critical factors for tumor growth and metastasis. Utilizing various *in vitro* and *in vivo* models, we demonstrated that silencing ILKAP significantly impedes HCC cell proliferation and migration. Additionally, we identified PGAM1 as a downstream target of ILKAP, linking its expression to enhanced glycolytic activity and

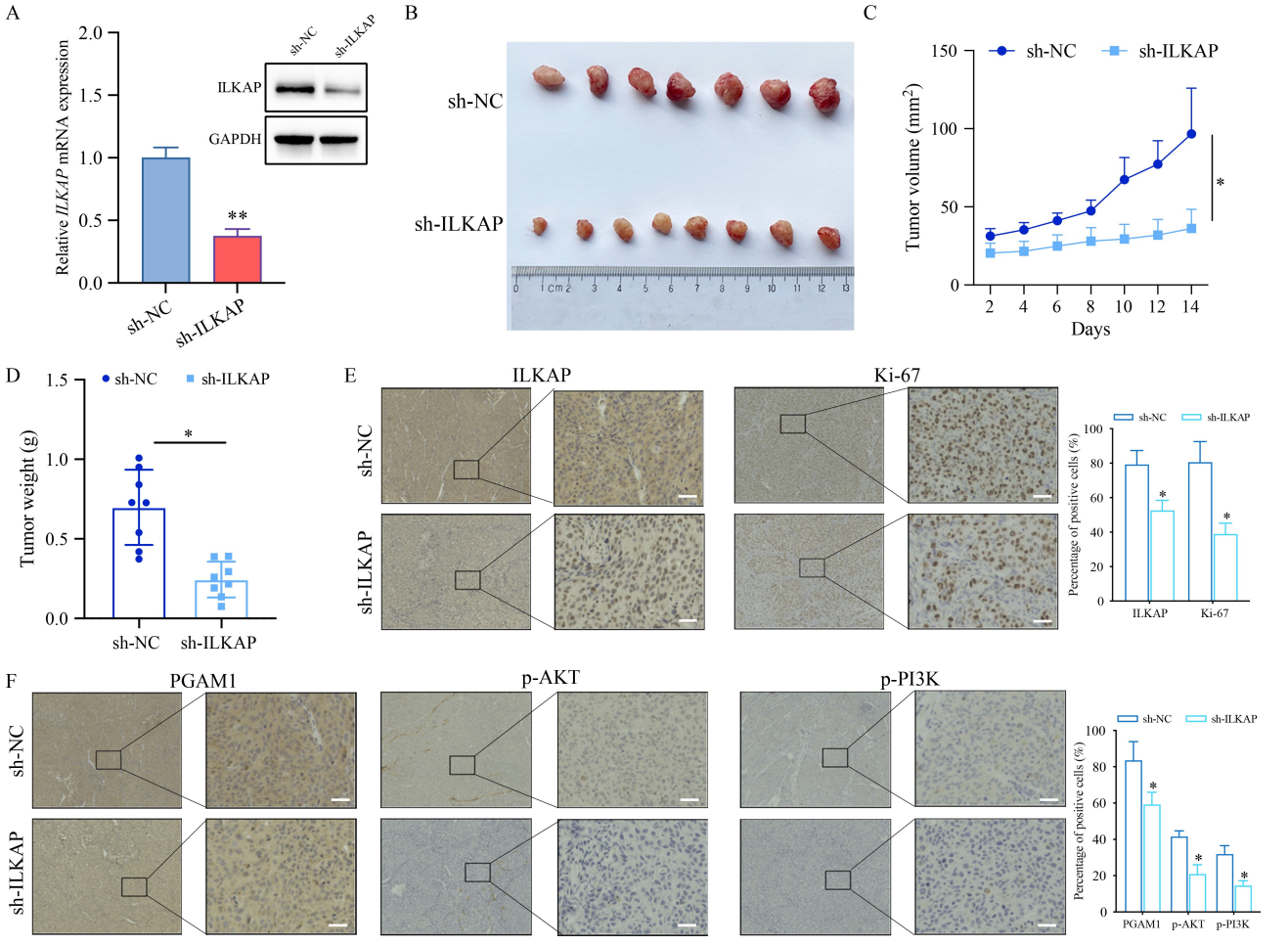

Fig. 7 Knockdown of *ILKAP* suppresses tumor growth in a mouse xenograft model. (A) Validation of *ILKAP* knockdown efficiency in stably transfected HCCLM3 cells. (B) Representative images of subcutaneous tumors formed in nude mice inoculated with HCCLM3 cells transfected with sh-ILKAP or control vectors. (C) Tumor volumes were recorded every two days and showed significant growth suppression in the *ILKAP* knockdown group. (D) Tumor weights were significantly lower in the sh-ILKAP group compared to the control group. (E, F) IHC analysis showed reduced ILKAP expression in the sh-ILKAP group, accompanied by decreased Ki-67, PGAM1, p-AKT, and p-PI3K levels (scale bar = 50 μm).

HCC malignancy. These results suggest that targeting the ILKAP-PGAM1 axis may provide a novel therapeutic approach for combating HCC.

The findings of this study elucidate the molecular mechanisms underlying the role of ILKAP in HCC. Our data indicate that ILKAP is significantly upregulated in HCC tissues and cell lines, correlating with poor prognosis and aggressive tumor characteristics. Through the regulation of PGAM1, a key glycolytic enzyme, ILKAP influences the metabolic reprogramming of cancer cells, promoting aerobic glycolysis—a hallmark of cancer metabolism. The interaction between ILKAP and PGAM1 highlights a critical axis in HCC, where ILKAP-mediated signaling not only enhances glycolytic activity but also supports tumor cell proliferation, migration, and invasion. This finding is consistent with previous reports indicating that dysregulation of glycolytic enzymes contributes to the malignant phenotype of various cancers, providing a potential therapeutic target for intervention in HCC progression.

Moreover, our study reveals that the knockdown of *ILKAP* leads to decreased PGAM1 expression, which in turn reduces glycolytic flux and impairs the invasive capabilities of HCC cells. This relationship underscores the importance of ILKAP in orchestrating the glycolytic switch, which is crucial for cancer cell survival and growth under hypoxic conditions often found within the tumor microenvironment. The observed alteration in glucose uptake and lactic acid production further supports the notion that ILKAP plays a pivotal role in maintaining the metabolic demands of rapidly proliferating tumor cells. These insights expand our understanding of the metabolic dependencies of HCC and suggest that targeting the ILKAP-PGAM1 pathway may enhance therapeutic efficacy, particularly in patients with advanced disease.

The interplay between tumor cells and immune components is critical for shaping the tumor microenvironment and can significantly affect patient responses to immunotherapy [26–30]. The upregulation of glycolysis, facilitated by ILKAP, may promote an immunosuppressive environment that fosters tumor progression and resistance to immune-mediated destruction. Therefore, understanding the ILKAP-mediated metabolic reprogramming in HCC not only provides insights into tumor biology but also opens avenues for developing novel therapeutic strategies that could enhance the effectiveness of existing treatments by targeting metabolic vulnerabilities within the tumor microenvironment.

One notable limitation of this study is the reliance on *in vitro* and xenograft models, which may not fully recapitulate the complexity of liver cancer in human patients. While our findings provide valuable insights into the role of ILKAP and its downstream target PGAM1 in liver cancer progression, the translation of these results to clinical settings requires caution. The biological behavior of cancer cells in a controlled laboratory environment can differ significantly from that in a heterogeneous tumor microenvironment. Additionally, the study primarily focuses on specific cell lines, which may exhibit unique characteristics that do not represent the broader spectrum of liver cancer. Future studies should aim to validate these findings in an expanded cohort of patient-derived samples to enhance the generalizability of our conclusions.

In conclusion, our research establishes a critical role for ILKAP in promoting the malignant characteristics of liver cancer via the regulation of PGAM1 and glycolysis. The upregulation of ILKAP correlates with poor prognosis and increased tumor aggressiveness, positioning it as a potential therapeutic target. Furthermore, the elucidation of ILKAP's involvement in glycolytic metabolism underscores its significance in the metabolic reprogramming of liver cancer cells. Collectively, our findings provide a foundation for future investigations into the therapeutic implications of targeting ILKAP in liver cancer treatment, which may ultimately contribute to improved patient outcomes.

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Compliance with ethics guidelines

Conflicts of interest Juejiashan Li, Yihong Chen, Qiyi Qian, Yating Gao, Nana Zhou, Xiaoyan Li, Qiuran Xu, Dongsheng Huang, and Wenhu Chen declare that they have no conflict of interest.

Institutional Animal Care and Use Committee of Hangzhou Medical College approved all animal procedures, which followed ARRIVE guidelines. All institutional and national guidelines for the care and use of laboratory animals were followed.

Electronic supplementary material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-025-1178-7 and is accessible for authorized users.

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