

ADAM12 Stabilizes EIF3B to Promote Glycolysis and Tumor Progression in Hepatocellular Carcinoma

Shengdong Wu¹, Yongfei Zhu¹, Yan Xia^{2,3}, Shengjun Wu^{2,3}, Caide Lu¹

¹Department of Hepatopancreatobiliary Surgery, The Affiliated Lihuili Hospital of Ningbo University, Ningbo, Zhejiang, 315040, People's Republic of China; ²Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, 310016, People's Republic of China; ³Zhejiang Provincial Engineering Research Center of Innovative Instruments for Precise Pathogen Detection, Hangzhou, People's Republic of China

Correspondence: Caide Lu, Department of Hepatopancreatobiliary Surgery, The Affiliated Lihuili Hospital of Ningbo University, Ningbo, Zhejiang, 315040, People's Republic of China, Email lucaide@nbu.edu.cn; Shengjun Wu, Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, 310016, People's Republic of China, Email 3202107@zju.edu.cn

Background: Hepatocellular carcinoma (HCC) is a highly aggressive malignancy with poor prognosis and limited therapeutic options. A disintegrin and metalloproteinase 12 (ADAM12) is aberrantly expressed in multiple cancers and has been implicated in tumor progression. However, its biological role and underlying mechanism in HCC remain unclear.

Methods: Public HCC datasets and bioinformatics analyses were used to evaluate ADAM12 expression and its clinical significance. The effects of ADAM12 on HCC cell viability, colony formation, migration, invasion, and apoptosis were assessed in vitro, and its role in tumor growth was examined in a xenograft model. The underlying mechanism was investigated by immunoprecipitation-mass spectrometry, co-immunoprecipitation, cycloheximide chase, ubiquitination, and metabolic assays.

Results: ADAM12 was significantly upregulated in HCC tissues and was associated with unfavorable overall survival. ADAM12 knockdown inhibited cell viability, colony formation, migration, and invasion, promoted apoptosis in vitro, and suppressed xenograft tumor growth in vivo without obvious body weight loss. Mechanistically, EIF3B was identified as an ADAM12-interacting protein. ADAM12 knockdown decreased EIF3B protein abundance without affecting its mRNA level, accelerated EIF3B degradation, and increased its ubiquitination, indicating that ADAM12 stabilizes EIF3B by limiting ubiquitin-proteasome-mediated degradation. Moreover, ADAM12 depletion reduced PKM2 and LDHA expression, decreased extracellular acidification rate, lactate production, and glucose uptake, and increased oxygen consumption rate, indicating a shift from glycolysis toward oxidative phosphorylation. These effects were largely rescued by EIF3B overexpression or PKM2 restoration.

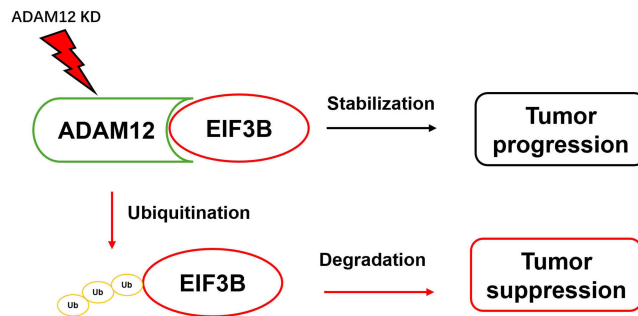
Conclusion: ADAM12 promotes glycolytic reprogramming and tumor progression in HCC by stabilizing EIF3B and regulating the EIF3B/PKM2 axis. The ADAM12-EIF3B pathway may therefore represent a potential therapeutic target in HCC.

Keywords: hepatocellular carcinoma, A disintegrin and metalloproteinase 12, eukaryotic translation initiation factor 3B, glycolysis inhibition, cancer therapy

Introduction

Liver cancer remains a pressing global health burden, with projections indicating annual incidence will surpass one million cases by 2025.¹ As the predominant form of liver malignancy, hepatocellular carcinoma (HCC) represents approximately 90% of cases and is closely linked to established risk factors including aflatoxin exposure, chronic HBV/HCV infections, and metabolic dysfunction.²⁻⁴ The absence of discernible clinical manifestations in early-stage disease frequently leads to delayed detection, with most patients presenting at advanced, treatment-refractory stages. HCC pathogenesis is characterized by widespread dysregulation of critical signaling pathways that drive malignant transformation, uncontrolled proliferation, and metastatic dissemination.²

Graphical Abstract



Despite significant advances in molecular oncology, current diagnostic modalities for HCC remain hampered by inadequate specificity and sensitivity, contributing to suboptimal early detection rates and consequent therapeutic delays.^{5–7} This diagnostic impasse has intensified the search for reliable biomarkers, with secreted proteins, including IL-6 and TNF- α , emerging as particularly promising candidates because of their potential for non-invasive detection and their functional roles in modulating the tumor microenvironment (TME).^{8,9} These proteins are actively involved in tumor initiation, progression, and angiogenesis, highlighting their value as potential diagnostic and therapeutic targets. Among these secreted proteins, the A Disintegrin and Metalloproteinase (ADAM) family has gained increasing attention for its critical role in cancer biology. As type I transmembrane glycoproteins, ADAM family members exhibit unique bifunctional capabilities encompassing proteolytic activity and cell adhesion.^{10–13} These proteins participate in diverse biological processes including ectodomain shedding, cellular migration, membrane fusion, and intracellular signaling.^{11,14} Among ADAM family members, ADAM12 demonstrates particular oncogenic relevance due to its tumor-restricted expression profile. While low expression in normal tissues, ADAM12 undergoes marked overexpression in multiple malignancies, including HCC, where its levels exhibit strong correlations with advanced disease stage and unfavorable clinical outcomes.^{15–17} Mechanistic studies have revealed that ADAM12 promotes tumor progression through induction of epithelial-mesenchymal transition (EMT) via cytoskeletal reorganization, thereby enhancing neoplastic proliferation and invasive capacity.¹⁸ Recent work has further identified TSPAN8-mediated ADAM12 upregulation as a key facilitator of HCC metastasis.¹⁹ Nevertheless, the complete spectrum of ADAM12's molecular mechanisms in HCC pathogenesis requires further elucidation.

Eukaryotic Translation Initiation Factor 3 Subunit B (EIF3B) is a core component of the eukaryotic translation initiation factor 3 (eIF3) complex, playing a pivotal role in protein synthesis initiation.^{20,21} Beyond its canonical role in translation initiation, EIF3B exerts precise control over gene expression through selective modulation of mRNA translation efficiency, thereby regulating essential cellular processes including proliferation, differentiation, and stress adaptation.²² Notably, EIF3B has emerged as a key player in oncogenesis, with its aberrant overexpression strongly correlated with tumor aggressiveness and poor prognosis in various malignancies including HCC, highlighting its potential as a therapeutic target and biomarker.^{21,23} Previous study demonstrated that EIF3B could accelerate HCC cell invasion, metastasis, and the epithelial-mesenchymal transition by stimulation of the TGFBI/MAPK/ERK signaling pathway via increasing the levels of pMEK and pERK.²⁴

In this study, we have established a significant association between elevated ADAM12 expression and poor prognosis in HCC patients, while genetic inhibition of ADAM12 produces multifaceted antitumor effects. This study provides mechanistic insights into the previously unexplored ADAM12-EIF3B regulatory axis, demonstrating that ADAM12-mediated stabilization of EIF3B drives glycolytic reprogramming and tumor progression. These findings not only advance our understanding of HCC pathogenesis but also unveil novel diagnostic and therapeutic opportunities for

ADAM12-expressing malignancies. In conclusion, the identification of this axis may facilitate the development of broad-spectrum molecular biomarkers and precision therapeutic strategies for HCC and other ADAM12-associated cancers.

Materials and Methods

Chemicals

MG132 (Selleck Chemicals, USA) and cycloheximide (Selleck Chemicals, USA) were dissolved in DMSO to prepare stock solutions and stored at -20°C . Working concentrations were freshly diluted in culture medium before use.

Cell Culture

Bel-7402 and Huh-7 cells were obtained from the Meisen Chinese Tissue Culture Collections. Bel-7402 cells and Huh-7 cells were grown in 1640-RPMI and DMEM, respectively, supplemented with 10% FBS and 1% Penicillin-Streptomycin at 37°C with 5% CO_2 in humidified air.

Plasmid Constructs and Transfection

For ADAM12 knockdown experiments, two short hairpin RNAs targeting human ADAM12 were designed and cloned into the pLKO.1-puro vector (Addgene #8453), which contains a puromycin resistance gene and GFP reporter. The sequences were as follows: shCtrl (Scramble): 5'-CCTAAGGTTAAGTCGCCCTCG-3'; shADAM12-1 (sh1): 5'-GCGAGAGATGTTTGAGATTAT-3'; shADAM12-2 (sh2): 5'-GCATTGTTGAAGAACATTTA-3'. All constructs were verified by Sanger sequencing. To generate stable knockdown cell lines, cells were transduced with concentrated lentivirus and Polybrene (at final concentration of 10 $\mu\text{g/mL}$) and selected using 2 $\mu\text{g/mL}$ puromycin.

Cell Proliferation Assay

CCK8 assay was used to measure the cell viability. Transfected cells (2000 cells/well) were seeded in 96-well plates in 100 μL complete medium. After incubation with indicated time, 10 μL of CCK8 (Sigma, 96992) was added to each well according to manufacturer's protocol. Plates were incubated at 37°C for 4 h. Cells were incubated at different conditions with 1, 2, 3, 4, 5 days, respectively. The absorbance was determined at a wavelength of 450 nm using a plate reader (Tecan infinite, M2009PR).

Apoptosis Assay

The cell apoptosis assay was performed using eBioscience™ Annexin V-APC/PI Apoptosis Detection Kit (eBioscience, 88-8007-74). Lentivirus-transfected cells were incubated for 48 h. Cells were harvested after incubation, washed twice in cold PBS, centrifuged, and resuspended in 1X annexin-binding buffer. According to the manufacturer's instructions, cells were incubated with Annexin V and Propidium iodide (PI) sequentially. All samples were analyzed by NovoCyte Quanteon Flow Cytometer (Agilent Technologies, CA, USA).

Colony Formation Assay

The lentivirus-transfected cells were seeded in 6-well plates and each well contained 2 mL medium with 400–1000 cells. Then, cells were incubated continuously for 14 days. Finally, the cells were fixed with 4% paraformaldehyde for 30 minutes and stained with Giemsa stain for 10–20 minutes. After washing with ddH_2O , the image of each well was photographed.

Wound Healing Assay

The lentivirus-transfected cells were seeded in a 6-well plate. After the formation of a complete monolayer, cells were scratched using a wounding replicator. After scratching, cells were washed twice with PBS. Then, the cells were incubated in medium with 0.5% FBS. Images at different time points (0 and 48 hour) were taken on a Cellomics (Thermo, ArrayScan VT1). The relative wound area is calculated using Cellomics (Thermo, ArrayScan VT1).

Transwell Migration Assay

The required number of inserts were placed into a 24-well plate. Then, 100 μ L serum-free medium (RPMI-1640 for Bel-7402 cells and DMEM for Huh-7 cells, both containing phenol red; Gibco, USA) was added into each insert and incubated for 1–2 h. Then, the medium in the inserts was carefully removed, followed by the addition of 600 μ L medium with 30% FBS to the lower chamber. A total of 100 μ L of infected cells (100,000–200,000 cells) suspended in the corresponding serum-free medium was added to each insert. Then the inserts were placed into the lower chambers. After 24 h incubation, the inserts were inverted onto absorbent paper to remove medium, and non-migrated cells were removed with a cotton swab. Afterwards, 400 μ L staining solution was added to the empty wells in the 24-well plate. The inserts were immersed in staining solution for 5 min, then rinsed in a large water bath and dried for image capture.

Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Total RNA was isolated using TRIzol reagent (Sigma, T9424-100m) from indicated cell lines, and then cDNA synthesis and qRT-PCR were performed using the Hiscript QRT supermix for qPCR (+gDNA WIPER) (Vazyme, R123-01) and the SYBR Green Master Mix kit (Vazyme, Q111-02). Gene expression levels were normalized to the expression of the reference gene glyceralde-3-phosphate dehydrogenase (GAPDH), which was not influenced by the experimental conditions resulting in the Δ Ct value. Gene expression levels were calculated by the comparative Ct method ($2^{-\Delta\Delta C_t}$).

Western Blot Assay and Co-Immunoprecipitation (Co-IP)

After indicated treatment, cells were harvested and washed with cold 1X PBS twice, and subsequently lysed with RIPA lysis buffer supplemented with complete protease inhibitor cocktail, EDTA-free inside. Protein concentrations were determined by a BCA Protein Assay Kit (HyClone-Pierce, 23225) according to the manufacturer's protocol. Samples were separated by Bis-Tris gels and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked at room temperature for 1–2 hour in 0.1% TBST solution containing 5% skimmed milk, and subsequently incubated at 4°C overnight with one of the following primary antibodies. Membranes were washed three times in 0.1% TBST and incubated at room temperature for 1 hour with corresponding secondary antibodies. The bands were visualized via enhanced chemiluminescence (ECL) Western blot detection. For co-IP assay, proteins of indicated cells were collected and immunoprecipitated by anti-EIF3B, anti-ADAM12, anti-STUB1 and IgG antibodies and then subjected to Western blotting with antibody to EIF3B, ADAM12, and STUB1. The corresponding antibody is listed in [Table S1](#).

Glycolysis-Related Assay

To evaluate glycolytic activity, extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using the Seahorse XF Glycolysis Stress Test Kit and Mito Stress Test Kit (Agilent Technologies) on the Seahorse XF96 extracellular flux analyzer. Briefly, 1×10^4 Bel-7402 or Huh-7 cells per well were seeded into Seahorse XF96 microplates and cultured overnight. The next day, cells were washed and incubated with Seahorse XF assay medium (pH 7.4) supplemented with 2 mM glutamine (for ECAR) or 10 mM glucose, 1 mM pyruvate, and 2 mM glutamine (for OCR), in a non-CO₂ incubator for 1 h before measurement. For ECAR measurements, glucose (10 mM), oligomycin (1 μ M), and 2-deoxyglucose (2-DG, 100 mM) were sequentially injected to assess basal glycolysis, glycolytic capacity, and glycolytic reserve. For OCR measurements, oligomycin (1 μ M), FCCP (1 μ M), and rotenone/antimycin A (0.5 μ M) were added to measure basal respiration, maximal respiration, and non-mitochondrial respiration. Lactate production in cell culture supernatants was quantified using a Lactate Colorimetric Assay Kit (BioVision, K607-100) according to the manufacturer's instructions. Glucose uptake was assessed using the 2-NBDG fluorescent glucose analog (Cayman Chemical, Cat# 11046). Cells were incubated in glucose-free DMEM for 1 h, followed by incubation with 100 μ M 2-NBDG for 30 min at 37 °C. Fluorescence intensity was measured by flow cytometry (NovoCyte Quanteon, Agilent). All experiments were performed in triplicate and normalized to cell number or total protein content.

Xenograft Subcutaneous Tumor Experiments

All experiments involving laboratory animals were performed with the approval of Laboratory Animal Center ethics committee (approval no. 2024SL527). Female BALB/c nude mice (4–6 weeks old, GemPharmatech) were randomly divided into two groups. Wild-type Bel-7402 cells (shCtrl) or ADAM12-knockdown (KD) Bel-7402 cells (1×10^7 cells in 200 μ L PBS) were subcutaneously injected into the right flank of each mouse. Beginning seven days post-inoculation, subcutaneous tumors were measured with a vernier caliper every three or four days. Tumor volumes (V) were estimated using the equation ($V = ab^2/2$, where a and b stand for the longest and shortest diameter, respectively). Mice body weights were also monitored regularly. At the end of experimental period, mice were euthanized, and tumors were harvested for analysis.

Statistical Analysis

Data was analyzed with GraphPad Prism software (San Diego, CA, USA). The data is presented as the mean $\pm$ SD of the number of experiments indicated as “n”. ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by *t* test or Anova.

Results

ADAM12 is Involved in the Pathogenesis of HCC and is a Potential Therapeutic Target for HCC

To elucidate the clinical significance of ADAM12 in hepatocellular carcinoma (HCC), we conducted comprehensive analyses of publicly available HCC datasets. Our investigation revealed consistent upregulation of ADAM12 expression in tumor tissues compared to adjacent normal liver tissues across multiple patient cohorts (Figure 1A). Importantly, survival analysis of the TCGA-LIHC dataset demonstrated that HCC patients with high ADAM12 expression had significantly poorer overall survival outcomes (Figure 1B), suggesting ADAM12 overexpression may contribute to HCC progression.

To functionally characterize ADAM12's role in HCC, we depleted ADAM12 from Bel-7402 HCC cells using short hairpin RNAs (shRNAs). Successful ADAM12 knockdown (ADAM12 KD) was confirmed by quantitative RT-PCR and Western blot (Figure 1C and D). A significant decrease in cell growth was observed upon ADAM12 knockdown (Figure 1E). ADAM12 knockdown also markedly induced apoptosis (Figure 1F) and effectively inhibited colony formation (Figure 1G) in Bel-7402 cells. In addition, ADAM12 depletion resulted in significant impairment of migratory and invasive capabilities in Bel-7402 cells (Figure 1H and I). These anti-tumor effects were consistently replicated in Huh-7 HCC cells, confirming the broad relevance of our findings (Figure S1). Together, these results establish ADAM12 as both a key mediator of HCC pathogenesis and a promising molecular target for therapeutic intervention.

ADAM12 Knockdown Promotes UPS-Dependent Degradation of EIF3B

To investigate the downstream molecules and regulatory mechanisms mediated by ADAM12, we performed immunoprecipitation mass spectrometry (IP-MS) to identify ADAM12-interacting proteins. Through systematic screening of E3 ubiquitin ligases and their potential substrates, we identified EIF3B as the most prominent ubiquitination substrate, exhibiting significantly higher binding scores compared to other candidate proteins (Figure S2). EIF3B is a key translation initiation factor that controls the production of numerous proteins. It has been reported that it plays a tumor-promoting role in multiple cancers.^{25–27} Notably, the E3 ubiquitin ligase STUB1 (also known as CHIP) was concurrently detected in the ADAM12 interactome. This finding is particularly intriguing given previous reports demonstrating STUB1-mediated ubiquitination of multiple proteins.²⁸ Based on these observations, we assumed that ADAM12 may functionally interact with EIF3B to modulate the STUB1-dependent ubiquitination regulatory axis.

To determine whether ADAM12 interacts with EIF3B, we performed Co-immunoprecipitation (Co-IP) analysis using an anti-ADAM12 antibody and Anti-EIF3B in lysates from Bel-7402 cells. Western blot analysis revealed that ADAM12 co-immunoprecipitated with EIF3B, but not in the IgG control (Figure 2A). The input lysate confirmed equal expression of both proteins. Similar results were also observed in Huh-7 cells (Figure S3A). These results demonstrate a protein-protein interaction between ADAM12 and EIF3B. Interestingly, we also observed protein-protein interaction between

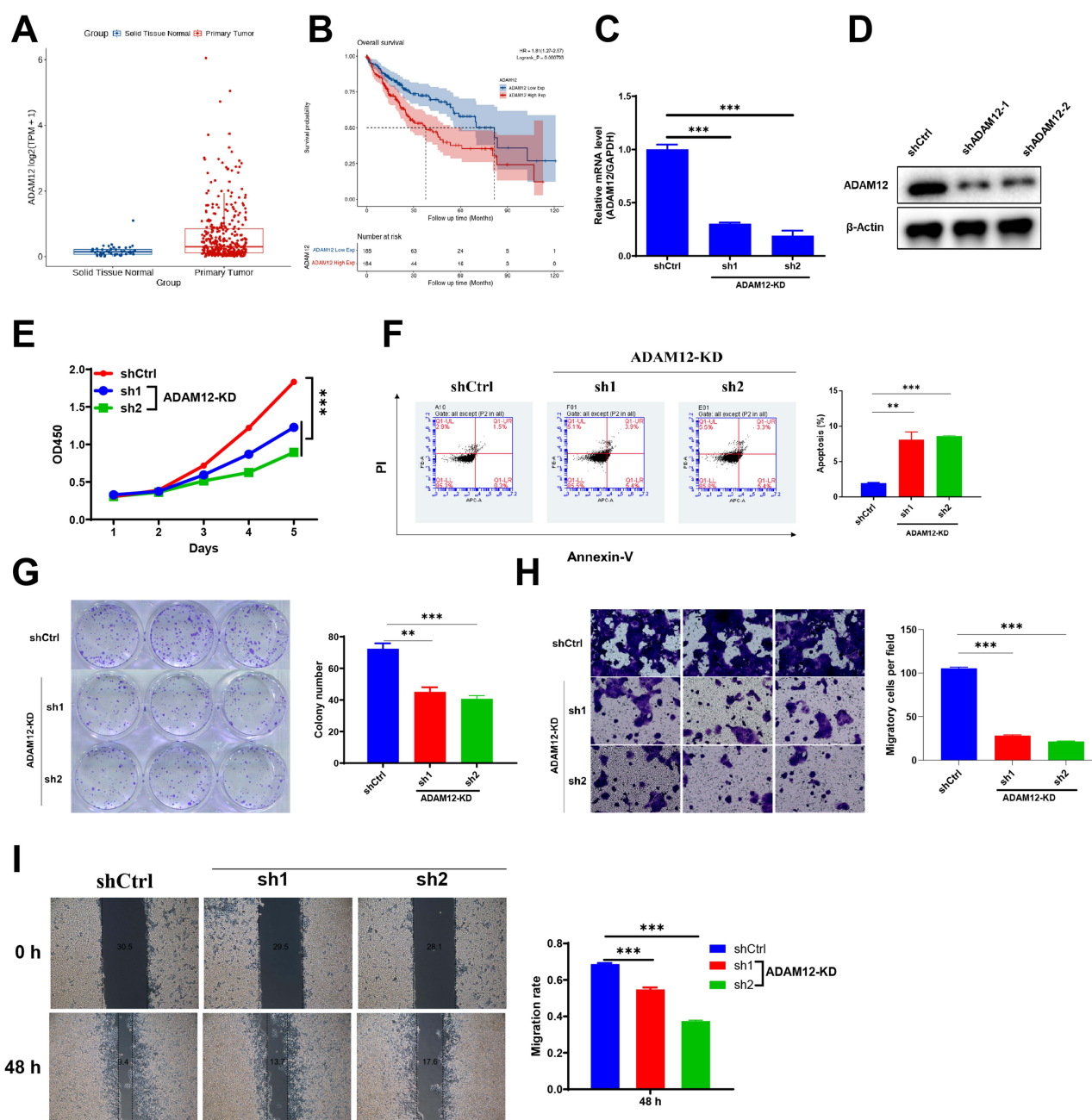


Figure 1 ADAM12 is involved in the pathogenesis of HCC and knockdown of ADAM12 showed antitumor effects in HCC cells. **(A)** ADAM12 expression in human HCC cases and normal controls. Data are from GDC (<https://portal.gdc.cancer.gov/>) **(B)** Overall survival of HCC patients from the TCGA HCC cohort separated by the top 50% and bottom 50% of ADAM12 expression (<http://ualcan.path.uab.edu/analysis.html>). p value was determined using the Log rank test. **(C)** The knockdown efficiency was detected by qRT-PCR on mRNA levels and **(D)** Representative Western blot analysis of the knockdown efficiency of Bel-7402 cells transduced with lentiviruses expressing control shRNA (shCtrl) or two independent shRNAs targeting ADAM12 (sh1 and sh2) (n = 3). **(E)** Cell viability of Bel-7402 cells upon ADAM12 KD, detected by CCK8 assay (n = 3). **(F)** Apoptosis assay of Bel-7402 cells upon ADAM12 KD. Histograms indicate the percentages of apoptotic (n = 3). **(G)** Colony formation of Bel-7402 cells upon ADAM12 KD (n = 3). **(H)** The invasive and **(I)** migrative capacity of Bel-7402 cells upon ADAM12 KD, detected by transwell assay and wound healing assay, respectively, (n = 3). **p < 0.01, ***p < 0.001.

EIF3B with the ubiquitin E3 ligase STUB1 in both Bel-7402 and Huh-7 cells, which was confirmed by Co-IP assay (Figures 2B and S3B). Based on the Co-IP results, we propose that the ADAM12-EIF3B interaction might modulate STUB1-dependent ubiquitination of EIF3B.

To investigate the effects of ADAM12 on the cellular levels of EIF3B, we performed knockdown assay. In contrast to scrambled control (shCtrl) group, ADAM12 KD resulted in the decreased levels of ADAM12 in Bel-7402 cells (Figure 2C)

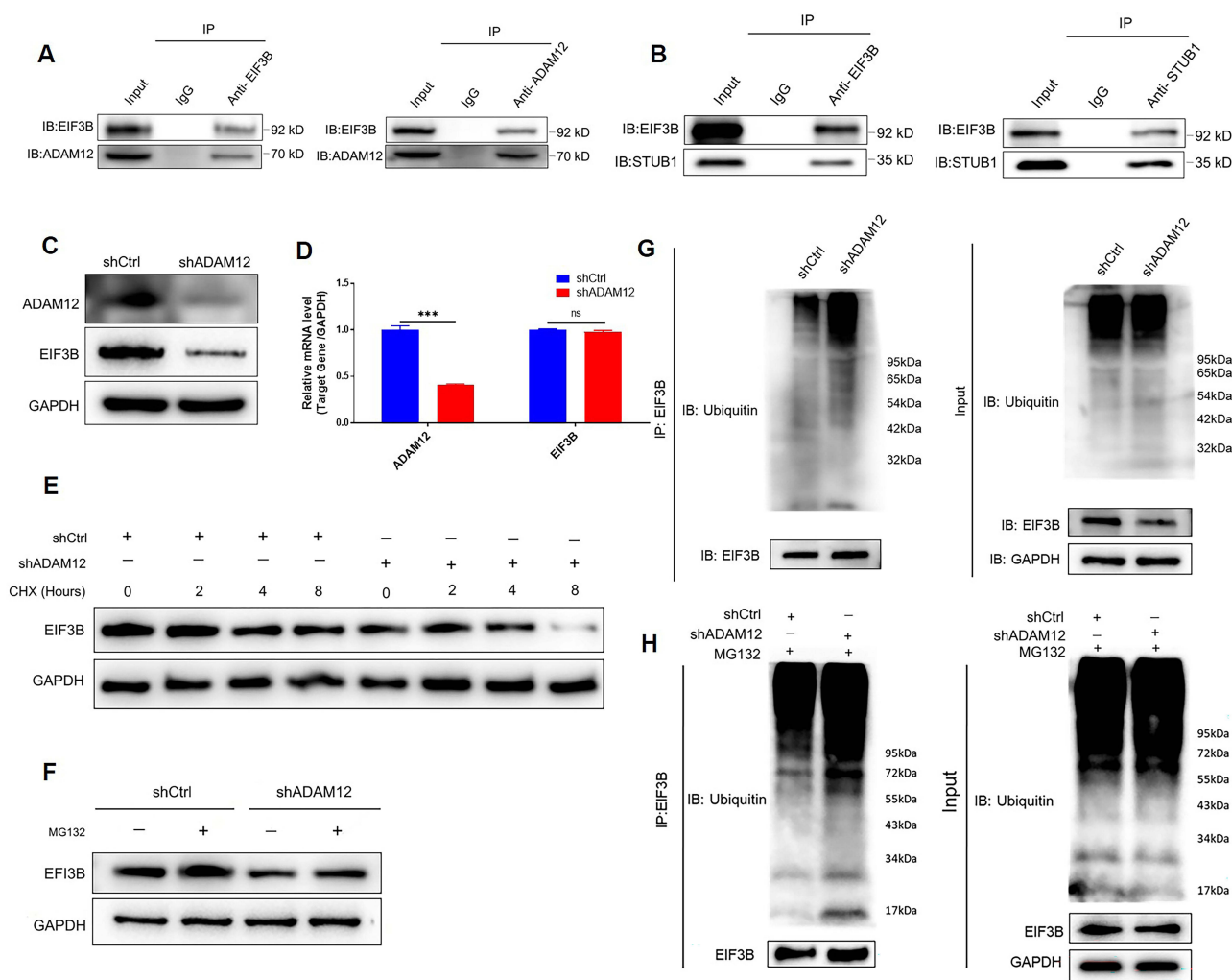


Figure 2 ADAM12 knockdown induces UPS-mediated EIF3B degradation. Co-immunoprecipitation (Co-IP) analysis of protein interactions involving ADAM12 and EIF3B (A), EIF3B and STUB1 (B) in Bel-7402 cells. IP was performed using antibodies against EIF3B, ADAM12, or STUB1, with IgG serving as a negative control. Input lysates represent total protein levels. (C) Protein levels of ADAM12 and EIF3B in ADAM12-knockdown Bel-7402 cells. (D) mRNA levels of ADAM12 and EIF3B in ADAM12-knockdown Bel-7402 cells, measured by qRT-PCR (mean $\pm$ SD, $n = 3$). (E) Western blot analysis of EIF3B protein levels in ADAM12-knockdown Bel-7402 cells treated with cycloheximide (CHX, 50 μ g/mL), a protein synthesis inhibitor. (F) Western blot analysis of EIF3B protein levels in ADAM12-knockdown Bel-7402 cells treated with or without MG132 (1 μ M), a proteasome inhibitor. (G) Western blot analysis of EIF3B ubiquitination levels in ADAM12-knockdown Bel-7402 cells. (H) Western blot analysis of EIF3B ubiquitination levels in ADAM12-knockdown Bel-7402 cells treated with MG132, a proteasome inhibitor. *** $p < 0.001$.

and Huh-7 cells (Figure S3C). Notably, the obvious decrease in levels of EIF3B was also observed (Figures 2C and S3C). These results indicate that ADAM12 might regulate EIF3B stability. To investigate the possible transcriptional effects of shADAM12, RT-qPCR assays were performed to examine the mRNA levels of ADAM12 and EIF3B. The ADAM12 mRNA level was significantly decreased, while no significant effect was observed on EIF3B mRNA level after ADAM12 KD suggesting that the cellular downregulation of EIF3B is owing to direct protein degradation and not owing to transcriptional downregulation (Figures 2D and S3D). Additionally, CHX chase assay showed time-dependent degradation of EIF3B following translational inhibition by CHX (μ g/mL) (Figures 2E and S3E). Notably, ADAM12 KD cells exhibited significantly accelerated EIF3B degradation compared to control cells (Figures 2E and S3E). However, pretreatment with the proteasome inhibitor MG132 effectively blocked shADAM12-induced EIF3B degradation (Figures 2F and S3F), suggesting that this process is dependent on proteasomal activity, to a large extent, mediated by the ubiquitin-proteasome system (UPS).

To further validate this mechanism, we performed Co-IP assays to assess the ubiquitination levels of EIF3B. As demonstrated in the Figure 2G, ADAM12 KD led to a marked increase in EIF3B ubiquitination in Bel-7402 cells. Consistent with the proteasomal degradation pathway, MG132 pretreatment did not abolish EIF3B ubiquitination, but its

degradation (Figure 2H). Similar results were also observed in ADAM12 KD Huh-7 cells (Figure S3G and H). Taken together, these results demonstrate that ADAM12 knockdown promotes UPS-dependent degradation of EIF3B by enhancing its ubiquitination.

ADAM12 Knockdown Suppresses Cancer Cell Growth via Glycolysis Inhibition

Previous studies showed that EIF3B activates the PI3K/AKT/mTOR pathway to promote PKM2/LDHA-driven glycolysis,^{24,25,29} and our results demonstrate that ADAM12 KD accelerates EIF3B degradation (Figure 2), we propose that ADAM12 sustains tumor metabolic reprogramming by stabilizing EIF3B, thereby maintaining high PKM2/LDHA expression and glycolytic flux.

To test this hypothesis, we first constructed stable cell lines overexpressing EIF3B (Figure S4). Then we examined the protein levels of the glycolytic enzymes PKM2 and LDHA following ADAM12 KD. Western blot analysis revealed a significant reduction in both PKM2 and LDHA in Bel-7402 and Huh-7 cells upon ADAM12 depletion (Figures 3A and S5A). To determine the EIF3B's role in regulating PKM2 and LDHA expression, subsequent analysis revealed that EIF3B overexpression significantly restored PKM2 and LDHA protein levels, confirming its functional involvement in their regulation (Figures 3B and S5B). We next characterized the metabolic phenotype of ADAM12-deficient Bel-7402 cells by measuring extracellular acidification rate (ECAR), lactate production, glucose uptake, and oxygen consumption rate (OCR). ADAM12 knockdown induced a striking metabolic shift, marked by suppressed glycolysis (decreased ECAR and lactate production), impaired glucose uptake, and enhanced mitochondrial oxidative phosphorylation (increased OCR) (Figures 3C–F and S5C–F). However, these effects could be significantly reversed by EIF3B overexpression (Figures 3G–J and S5G–J). Similar results were also observed in the recovery of PKM2 of ADAM12-KD cells (Figure S6). Based on these results, we can conclude that ADAM12 knockdown induces a metabolic shift from glycolysis to oxidative phosphorylation (OXPHOS).

To determine the functional effects of this metabolic shift, we assessed cell viability and migration. CCK-8 assays showed that ADAM12 knockdown significantly reduced Bel-7402 cell viability (Figure 4A), while wound healing assays demonstrated a significant suppression of migration compared to control (Figure 4B and C). Both phenotypes were largely reversed by EIF3B overexpression and PKM2 recovery (Figure S7), further underscoring the pivotal role of the ADAM12-EIF3B axis in promoting oncogenic growth and migration. Importantly, these results can also be reproduced in ADAM12-KD Huh-7 cells (Figure S8). Based on these results above, our findings reveal that ADAM12 drives cancer cell proliferation and migration by sustaining EIF3B-mediated glycolytic metabolism. Targeting this axis via ADAM12 depletion can effectively suppresses tumor growth and migration.

ADAM12 Knockdown Demonstrates Potent Anti-Tumor Potency in vivo

Enlightened by the promising in vitro anti-tumor potency, we evaluated the in vivo anti-tumor potency of ADAM12 knockdown. As the results shown in Figure 5A–C, ADAM12 depletion could effectively delay tumor growth in vivo. In addition, no significant body weight loss was observed in both treatment group mice, indicating acceptable toxicity (Figure 5D). In addition, the IHC analysis and Western blot analysis showed that ADAM12 knockdown led to the decreased level of ADAM12, PKM2, and LDHA, confirming in vivo target engagement and glycolysis inhibition (Figure 5E and F).

Discussion

The clinical and mechanistic significance of ADAM12 in HCC pathogenesis is underscored by its tumor-specific overexpression, strong correlation with poor prognosis, and functional indispensability for maintaining oncogenic phenotypes. The discovery of the ADAM12-EIF3B axis reveals a previously unrecognized regulatory mechanism in HCC, where ADAM12 stabilizes EIF3B by preventing it from STUB1-mediated ubiquitination and proteasomal degradation. This interaction not only expands the known repertoire of ADAM12's non-proteolytic functions but also positions it as a central orchestrator of metabolic reprogramming. By sustaining EIF3B levels, ADAM12 drives PKM2/LDHA-dependent glycolysis, thereby fueling the Warburg effect, a metabolic hallmark of aggressive tumors.³⁰ The consequent shift to oxidative phosphorylation upon ADAM12 depletion suggests that targeting this axis could reverse the

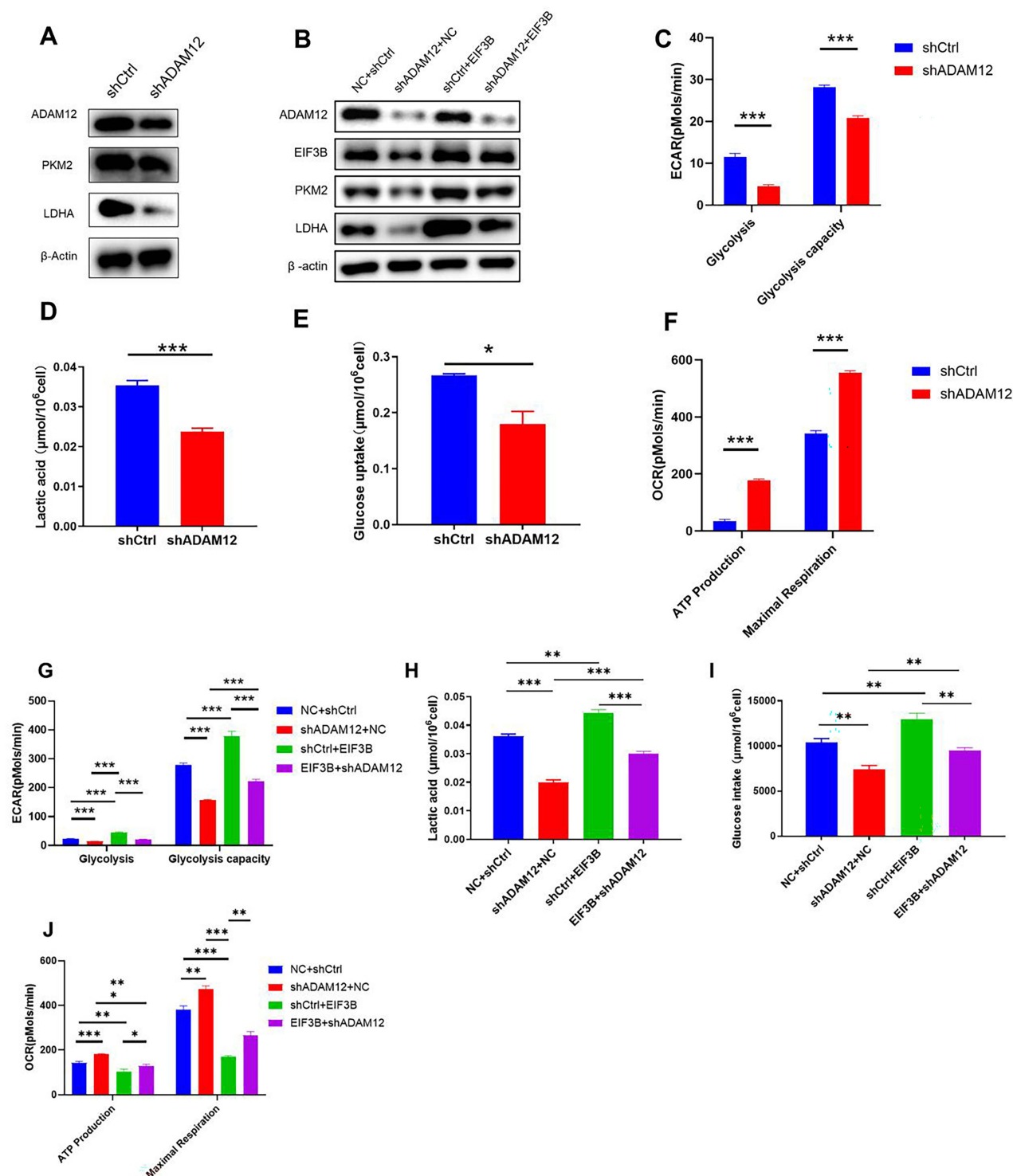


Figure 3 ADAM12 knockdown inhibits glycolysis. **(A)** Protein levels of PKM2 and LDHA in ADAM12-knockdown Bel-7402 cells, detected by Western blot. **(B)** Effect of EIF3B overexpression on PKM2 and LDHA protein levels in ADAM12-KD cells, detected by Western blot. **(C)** Glycolytic Stress Test parameters for ADAM12-KD Bel-7402 cells (mean $\pm$ SD, $n = 3$). Quantification of lactate production **(D)** and Glucose uptake **(E)** in ADAM12-KD Bel-7402 cells (mean $\pm$ SD, $n = 3$). **(F)** Mito Stress Test parameters for ADAM12-KD Bel-7402 cells (mean $\pm$ SD, $n = 3$). The results of EIF3B gene rescue experiment in Glycolytic Stress **(G)**, lactate production **(H)** and Glucose uptake **(I)** and Mito Stress **(J)** in ADAM12-KD Bel-7402 cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

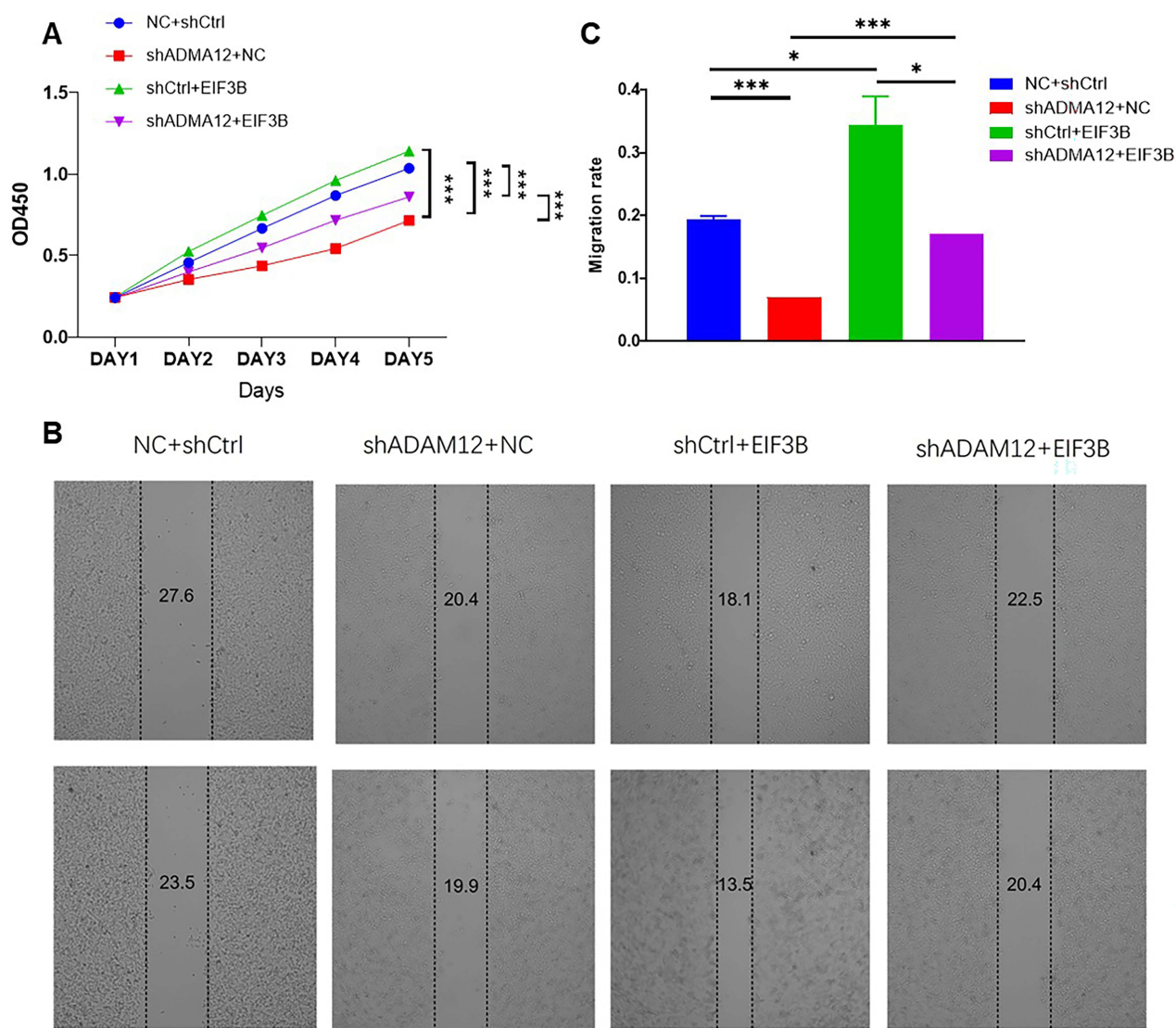


Figure 4 ADAM12 knockdown suppresses Bel-7402 cells growth and migration in vitro. **(A)** Cell viability of ADAM12-knockdown Bel-7402 cells was determined by CCK8 assay. Cell migration of ADAM12-knockdown Bel-7402 cells was measured by wound healing assay **(B)** and quantitatively analyzed **(C)**. * $p < 0.05$, *** $p < 0.001$.

metabolic plasticity that often underlies therapeutic resistance. Importantly, the in vivo efficacy of ADAM12 knockdown, coupled with its negligible systemic toxicity, highlights its translational potential as a therapeutic vulnerability in HCC.

Although ADAM12 has been implicated in tumor progression in several malignancies, its mechanistic contribution to HCC has remained insufficiently defined. Previous studies have primarily emphasized the proteolytic and extracellular functions of ADAM12, including its involvement in growth factor signaling, extracellular matrix remodeling, and tumor invasion. In contrast, our findings support a distinct role for ADAM12 in post-translational regulation within tumor cells. Specifically, we identify EIF3B as a functionally relevant downstream effector whose protein stability is maintained by ADAM12, thereby linking ADAM12 to the control of glycolytic metabolism. This observation broadens the current understanding of ADAM12 biology beyond its conventional metalloproteinase-associated functions and suggests that ADAM12 may act not only as a membrane-associated protease, but also as a regulator of intracellular oncogenic signaling networks. Moreover, by connecting ADAM12 to the EIF3B-PKM2/LDHA glycolytic program, our study provides a mechanistic explanation for how ADAM12 overexpression may translate into aggressive tumor behavior in HCC.

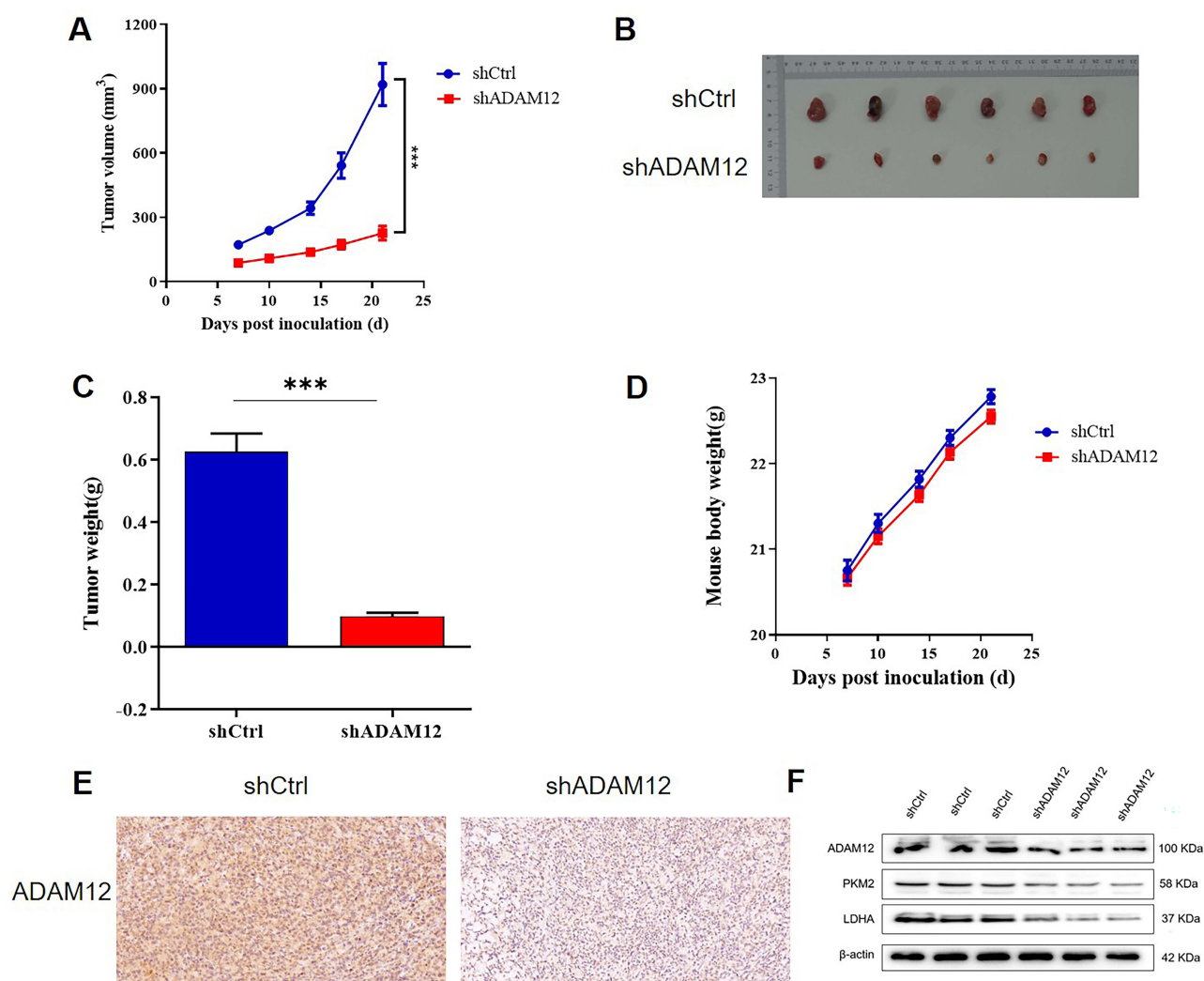


Figure 5 ADAM12 Knockdown Suppresses Tumor Growth in vivo. **(A)** Tumor growth curve, **(B)** photo image, **(C)** tumor weight, and **(D)** body weight curve of each mice group in ADAM12-knockdown Bel-7402 xenograft model. **(E)** Representative images of IHC analysis of ADAM12 in different groups; scale bar = 50 μ m. **(F)** Expression levels of ADAM12, PKM2, and LDHA in ADAM12-knockdown Bel-7402 xenograft tumor tissues. *** $p < 0.001$.

Beyond cell-intrinsic effects, the role of ADAM12 as a metalloproteinase raises intriguing questions about its impact on the tumor immune microenvironment. While this study focuses on its cell-autonomous functions, ADAM family members have been reported to modulate immune responses through ectodomain shedding of cytokines and immune checkpoint ligands.^{31,32} The observed glycolytic inhibition following ADAM12 depletion could indirectly reshape immune surveillance through multiple mechanisms. First, by reducing lactate accumulation, a known suppressor of cytotoxic T-cell activity and promoter of Treg differentiation.^{33,34} Second, through potential modulation of chemokine gradients that govern immune cell trafficking, as ADAM12-mediated cleavage of membrane-anchored chemokines has been reported in other malignancies.³⁵ Third, by altering extracellular matrix stiffness through fibronectin processing, which may influence CD8⁺ T-cell infiltration and macrophage polarization toward anti-tumor M1 phenotypes.³⁶ These immunomodulatory possibilities warrant investigation in immunocompetent models, particularly given the emerging importance of combining metabolic and immune therapies in HCC treatment.

From a therapeutic standpoint, the ADAM12-EIF3B axis presents a compelling target for precision medicine strategies. Small-molecule modulators disrupting this interaction or monoclonal antibodies neutralizing ADAM12's extracellular domain could be explored, leveraging its tumor-selective expression to minimize off-target effects. Given EIF3B's broader role in PI3K/AKT signaling and protein synthesis, combination therapies with kinase inhibitors or

mTOR pathway blockers may enhance the therapeutic efficacy. Clinically, stratifying patients by ADAM12/EIF3B expression could identify those most likely to respond, while circulating ADAM12 levels might serve as a non-invasive biomarker for early detection or therapeutic monitoring. In conclusion, this work not only advances our understanding of HCC pathogenesis but also provides a framework for targeting ADAM12-driven malignancies across diverse cancer types.

Several limitations of the present study should also be acknowledged. First, although our in vitro and in vivo data consistently support a tumor-promoting role of the ADAM12–EIF3B axis, the downstream signaling events linking EIF3B stabilization to metabolic enzyme regulation require further investigation. In particular, the precise contribution of intermediate pathways involved in PKM2 and LDHA regulation remains to be clarified. Second, most mechanistic experiments in this study were performed in established HCC cell lines, and therefore additional validation in patient-derived models and larger clinical cohorts would further strengthen the translational relevance of our findings. Third, while our data suggest that ADAM12 may represent a promising therapeutic target, the feasibility and efficacy of directly targeting ADAM12 or disrupting its interaction with EIF3B remain to be determined in preclinical settings. Future studies using orthotopic models, immunocompetent systems, and therapeutic intervention strategies will be important to define the full biological and clinical significance of this axis in HCC.

Statement of Ethics

This study involving human tissue specimens was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of The Affiliated Lihuili Hospital of Ningbo University (approval no. 2024SL527). Written informed consent was obtained from all patients prior to sample collection and use for research purposes.

All animal experiments were conducted under the same ethics protocol (approval no. 2024SL527). All procedures complied with institutional guidelines for the care and use of laboratory animals, relevant national regulations, and the AVMA Guidelines for the Euthanasia of Animals (2020). Female BALB/c nude mice (4–6 weeks old) were anesthetized with isoflurane (2–3% in oxygen, inhalation) prior to any surgical or invasive procedures. At the experimental endpoint, mice were euthanized by intraperitoneal injection of sodium pentobarbital (150 mg/kg), and death was confirmed by cessation of heartbeat and respiration. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Rumgay H, Arnold M, Ferlay J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol.* 2022;77(6):1598–1606. doi:10.1016/j.jhep.2022.08.021

2. Zheng J, Wang S, Xia L, et al. Hepatocellular carcinoma: signaling pathways and therapeutic advances. *Signal Transduct Target Ther.* **2025**;10(1):35. doi:10.1038/s41392-024-02075-w
3. Balogh J, Victor D, Asham EH, et al. Hepatocellular carcinoma: a review. *J Hepatocell Carcinoma.* **2016**;3:41–53. doi:10.2147/JHC.S61146
4. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers.* **2021**;7(1):6. doi:10.1038/s41572-020-00240-3
5. Schutte K, Schulz C, Link A, Malfertheiner P. Current biomarkers for hepatocellular carcinoma: surveillance, diagnosis and prediction of prognosis. *World J Hepatol.* **2015**;7(2):139–149. doi:10.4254/wjh.v7.i2.139
6. Borchers A, Pieler T. Programming pluripotent precursor cells derived from *Xenopus* embryos to generate specific tissues and organs. *Genes.* **2010**;1(3):413–426. doi:10.3390/genes1030413
7. Pan Y, Chen H, Yu J. Biomarkers in hepatocellular carcinoma: current status and future perspectives. *Biomedicines.* **2020**;8(12):576. doi:10.3390/biomedicines8120576
8. Jia D, Jiang Z, Cui M, Ding X. Proteomics efforts for hepatocellular carcinoma drug development. *Clin Cancer Bull.* **2024**;3(1):22. doi:10.1007/s44272-024-00027-7
9. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol.* **2018**;19(3):222–232. doi:10.1038/s41590-018-0044-z
10. Wang Z, Li W, Chen S, Tang XX. Role of ADAM and ADAMTS proteases in pathological tissue remodeling. *Cell Death Discov.* **2023**;9(1):447. doi:10.1038/s41420-023-01744-z
11. Mochizuki S, Okada Y. ADAMs in cancer cell proliferation and progression. *Cancer Sci.* **2007**;98(5):621–628. doi:10.1111/j.1349-7006.2007.00434.x
12. Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. *Mol Aspects Med.* **2008**;29(5):258–289. doi:10.1016/j.mam.2008.08.001
13. Oh S, Lee SS, Jin H, et al. A disintegrin and metalloproteinase domain (ADAM) 12, ADAM 17 mRNA and ADAM10 protein hold potential as biomarkers for detection of early gastric cancer. *Sci Rep.* **2025**;15(1):763. doi:10.1038/s41598-024-84237-y
14. Murphy G. The ADAMs: signalling scissors in the tumour microenvironment. *Nat Rev Cancer.* **2008**;8(12):929–941. doi:10.1038/nrc2459
15. Lv P, Zhang Y, Wu W, Jiang X, Xiang W. Pan-cancer analysis identifies ADAM12 as a prognostic biomarker and indicator of immune infiltration in glioma. *Sci Rep.* **2025**;15(1):6314. doi:10.1038/s41598-025-90121-0
16. Torabinejad S, Miro C, Nappi A, et al. Obesity alters the fitness of peritumoral adipose tissue, exacerbating tumor invasiveness in renal cancer through the induction of ADAM12 and CYP1B1. *Mol Oncol.* **2025**;19(6):1612–1632. doi:10.1002/1878-0261.13782
17. Nyren-Erickson EK, Jones JM, Srivastava DK, Mallik S. A disintegrin and metalloproteinase-12 (ADAM12): function, roles in disease progression, and clinical implications. *Biochim Biophys Acta.* **2013**;1830(10):4445–4455. doi:10.1016/j.bbagen.2013.05.011
18. Xu J, Wang Y, Jiang J, Yin C, Shi B. ADAM12 promotes clear cell renal cell carcinoma progression and triggers EMT via EGFR/ERK signaling pathway. *J Transl Med.* **2023**;21(1):56. doi:10.1186/s12967-023-03913-1
19. Fang T, Lin J, Wang Y, et al. Tetraspanin-8 promotes hepatocellular carcinoma metastasis by increasing ADAM12m expression. *Oncotarget.* **2016**;7(26):40630.
20. Kolupaeva VG, Unbehauen A, Lomakin IB, Hellen CU, Pestova TV. Binding of eukaryotic initiation factor 3 to ribosomal 40S subunits and its role in ribosomal dissociation and anti-association. *RNA.* **2005**;11(4):470–486. doi:10.1261/rna.7215305
21. Zang Y, Zhang X, Yan L, et al. Eukaryotic translation initiation factor 3b is both a promising prognostic biomarker and a potential therapeutic target for patients with clear cell renal cell carcinoma. *J Cancer.* **2017**;8(15):3049–3061. doi:10.7150/jca.19594
22. Sharma DK, Bressler K, Patel H, Balasingam N, Thakor N. Role of Eukaryotic Initiation Factors during Cellular Stress and Cancer Progression. *J Nucleic Acids.* **2016**;2016:8235121. doi:10.1155/2016/8235121
23. Feng X, Li J, Liu P. The biological roles of translation initiation factor 3b. *Int J Biol Sci.* **2018**;14(12):1630–1635. doi:10.7150/ijbs.26932
24. Wang L, Huang C, Lin W, et al. EIF3B affects the invasion and metastasis of hepatocellular carcinoma cells via the TGFBI/MAPK/ERK pathway. *Ann Hepatol.* **2025**;30(1):101564. doi:10.1016/j.aohep.2024.101564
25. Wang L, Wen X, Luan F, et al. EIF3B is associated with poor outcomes in gastric cancer patients and promotes cancer progression via the PI3K/AKT/mTOR signaling pathway. *Cancer Manag Res.* **2019**;11:7877–7891. doi:10.2147/CMAR.S207834
26. Fang P, Han Y, Qu Y, et al. EIF3B stabilizes PTGS2 expression by counteracting MDM2-mediated ubiquitination to promote the development and progression of malignant melanoma. *Cancer Sci.* **2022**;113(12):4181–4192. doi:10.1111/cas.15543
27. Zhu H, Shan Y, Ge K, Lu J, Kong W, Jia C. EIF3B promotes cancer progression in pancreatic cancer. *Scand J Gastroenterol.* **2021**;56(3):281–288. doi:10.1080/00365521.2020.1868566
28. Bhuripanyo K, Wang Y, Liu X, et al. Identifying the substrate proteins of U-box E3s E4B and CHIP by orthogonal ubiquitin transfer. *Sci Adv.* **2018**;4:e1701393. doi:10.1126/sciadv.1701393
29. Cheng S-C, Quintin J, Cramer RA, et al. mTOR- and HIF-1 α -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science.* **2014**;345:6204. doi:10.1126/science.1250684
30. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci.* **2016**;41(3):211–218. doi:10.1016/j.tibs.2015.12.001
31. Lichtenthaler SF, Lemberg MK, Fluhrer R. Proteolytic ectodomain shedding of membrane proteins in mammals—hardware, concepts, and recent developments. *EMBO J.* **2018**;37(15):EMBJ201899456. doi:10.15252/embj.201899456
32. Scheller J, Chalaris A, Garbers C, Rose-John S. ADAM17: a molecular switch to control inflammation and tissue regeneration. *Trends Immunol.* **2011**;32(8):380–387. doi:10.1016/j.it.2011.05.005
33. Watson MJ, Vignali PDA, Mullett SJ, et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature.* **2021**;591(7851):645–651. doi:10.1038/s41586-020-03045-2
34. Multhoff G, Vaupel P. Lactate-avid regulatory T cells: metabolic plasticity controls immunosuppression in tumour microenvironment. *Signal Transduct Target Ther.* **2021**;6(1):171. doi:10.1038/s41392-021-00598-0
35. Murphy G. Regulation of the proteolytic disintegrin metalloproteinases, the ‘Sheddases’. *Semin Cell Dev Biol.* **2009**;20(2):138–145. doi:10.1016/j.semedb.2008.09.004
36. Zhu H, Jiang W, Zhu H, et al. Elevation of ADAM12 facilitates tumor progression by enhancing metastasis and immune infiltration in gastric cancer. *Int J Oncol.* **2022**;60(5):1–3. doi:10.3892/ijo.2022.5341

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