Am J Transl Res 2025;17(4):2527-2540
www.ajtr.org /ISSN:1943-8141/AJTR0159540

Original Article

GLS1 promotes lipid metabolism in hepatocellular carcinoma by regulating the *PI3K/AKT/mTORC1* signaling pathway through *SREBP-1*

Yaocheng Sun[1,2*], Ying Shen[3*], Yongmin Yan[4,5], Wei Luo[4,5], Chuanlei Liu[1,2], Jianjun Tang[1,2]

[1]Department of General Surgery, The Wujin Clinical College of Xuzhou Medical University, Changzhou 213017, Jiangsu, China; [2]Department of General Surgery, Wujin Hospital Affiliated with Jiangsu University, Changzhou 213017, Jiangsu, China; [3]Department of Oncology, Wujin Hospital Affiliated with Jiangsu University, Changzhou 213017, Jiangsu, China; [4]Wujin Institute of Molecular Diagnostics and Precision Cancer Medicine of Jiangsu University, Changzhou 213017, Jiangsu, China; [5]Changzhou Key Laboratory of Molecular Diagnostics and Precision Cancer Medicine, Changzhou 213017, Jiangsu, China. [*]Equal contributors and co-first authors.

Received July 30, 2024; Accepted March 8, 2025; Epub April 15, 2025; Published April 30, 2025

Abstract: Objectives: Cancer cells exhibit altered metabolic profiles. Glutaminase 1 (*GLS1*), a key enzyme in cancer cells, promoting glutamine catabolism to glutamate and ammonia, is strongly associated with various human malignancies. Methods: *GLS1* promotes lipid accumulation and cell proliferation by upregulating the expression of sterol regulatory element-binding protein 1 (*SREBP-1*) and SREBP cleavage-activating protein (*SCAP*). Mechanistically, *GLS1* promotes lipid metabolism in HCC cells through the activation of the *PI3K/AKT/mTORC* pathway. Results: *GLS1*'s role in lipid metabolism in hepatocellular carcinoma (HCC) remains unexplored. Our findings indicate that *GLS1* is not only significantly overexpressed in HCC but also negatively correlates with clinical prognosis. Further investigation revealed that *GLS1* drives lipid accumulation and de novo fatty acid synthesis in HCC. Conclusions: Our study suggests that *GLS1* mediates *SREBP-1* to drive lipid metabolism in HCC via the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin complex 1 (*PI3K/AKT/mTORC1)* signaling pathway, thus we present *GLS1* as a potential biomarker and therapeutic target for HCC.

Keywords: Hepatocellular carcinoma, *GLS1*, lipid metabolism, *PI3K/AKT/mTORC1* signaling pathway, *SREBP-1*

Introduction

Hepatocellular carcinoma (HCC) accounts for approximately 80% of all primary liver cancers [1]. In China, HCC has an annual incidence of nearly 466,000 cases, leading to around 422,000 deaths each year. Surgical resection is feasible for only 10-15% of patients due to the advanced stage at which the disease is often diagnosed [2]. The etiology of HCC is multifactorial, involving variables such as hepatitis C virus (*HCV*), metabolic factors like severe obesity, hepatitis B virus (*HBV*), aflatoxins, and lifestyle factors including alcohol and tobacco use [3]. Early-stage HCC often presents with non-specific symptoms, resulting in late-stage diagnosis and limited treatment options. Despite advances in HCC prevention and treatment, long-term survival remains poor due to late detection and a high rate of recurrence and metastasis [4]. The pathogenesis and molecular mechanisms underlying HCC are still not fully understood [5]. Consequently, identifying effective therapeutic targets and understanding the molecular drivers of HCC are critical areas of research.

Upon ingestion, compounds such as nutrients and drugs undergo hepatic metabolism before entering systemic circulation, thereby controlling their bioavailability and maintaining their levels within the body [6]. The liver metabolizes dietary components, including lipids and carbohydrates, to generate energy and regulate physiological processes essential for homeostasis. However, impaired hepatic metabolism can lead to various liver diseases, including acute liver failure, viral hepatitis from *HBV* or *HCV*

https://doi.org/10.62347/ZTGP5030

infection, fibrosis/cirrhosis, non-alcoholic fatty liver disease (NAFLD), and, in some cases, hepatocellular carcinoma [7].

Glutamine, a versatile nutrient, plays an essential role in energy production, macromolecule synthesis, and cellular signaling while providing NADPH (nicotinamide adenine dinucleotide phosphate) and GSH (glutathione) to sustain redox balance [8]. Glutamine catabolism begins with the conversion by glutaminase (GLS) into two isozymes, *GLS1* and *GLS2*, in mammalian cells. *GLS1* mRNA produces two isoforms that vary only in their C-terminal regions due to alternative splicing. The longer isoform is known as kidney-type glutaminase (*KGA*), while the shorter is referred to as glutaminase C (*GAC*) [9]. *GLS1* has been identified as a tumor promoter across multiple cancers, whereas *GLS2* functions as a tumor suppressor [10]. *GLS1* is strongly linked to various human cancers, such as intrahepatic cholangiocarcinoma, ovarian carcinoma, colorectal cancer, and hepatocellular carcinoma, and is associated with poor clinical outcomes [11-14]. The regulatory mechanism of *GLS1* remains insufficiently understood, with limited information available. Specifically, the exact mechanism governing *GLS1* regulation is still unclear. Previous studies have offered some insights into specific regulatory pathways. For example, Gao et al. demonstrated that c-myc can increase *GLS1* levels by transcriptionally repressing miR-23a and miR-23b, leading to elevated glutamine metabolism [15]. Thangavelu et al. reported that epidermal growth factor (*EGF*) activates *GLS1* activity through the phosphorylation-dependent *Raf-Mek-Erk* signaling pathway [16]. Additionally, Liu HY et al. observed that the autophagy inducer rapamycin could counteract the inhibitory impact of *GLS1* depletion on the proliferation, colony formation, and migration of DLD1 and SW480 CRC cells [11]. Despite these findings, our understanding of the complex interactions between *GLS1*'s tumor-promoting properties and the broader metabolic network is still emerging. Moreover, whether *GLS1* plays other significant roles in regulating metabolism during tumor progression remains an open question.

Lipids contribute to biofilm formation by providing essential lipid components, regulating cell membrane fluidity, promoting lipid-mediated signal transduction, and promoting malignant biological behaviors such as tumor cell growth, invasion, and metastasis. Many tumor cells exhibit increased expression and activity of lipid metabolism-related enzymes, driving fatty acid synthesis within cancer cells. Numerous studies suggest that increased fatty acid synthesis within tumor cells is a critical factor in cancer initiation and progression, with activation of de novo synthesis correlating negatively with prognosis and disease-free survival in various tumors. This phenotype primarily arises from upregulated expression of adipogenesis-related genes at multiple levels, including transcriptional, translational, post-translational, and enzymatic modifications. Furthermore, alterations in these genes or in lipid metabolism influence oncogene expression. The surge in lipid production largely supports the rapid proliferation of cancer cells and their increased energy demands, necessitating the generation of additional cell membrane lipids. Targeting genes involved in lipogenesis, such as stearoyl-CoA desaturase (*SCD*), acetyl-CoA carboxylase (*ACC*), fatty acid synthase (*FASN*), ATP-citrate lyase (*ACLY*), and the key transcriptional regulator *SREBP-1*, has shown promise in inhibiting tumor angiogenesis, as these genes regulate lipid synthesis. Silencing these genes or applying pharmacological inhibitors can effectively suppress malignant cell growth. Despite the established role of fatty acid synthesis in cancer cell biology, the regulatory mechanisms driving this process in cancer cells remain largely unexplored.

Our research demonstrates that *GLS1* promotes HCC cell proliferation by activating the *AKT/GSK3/CyclinD1* signaling pathways [17]. We also observed a significant increase in lipid accumulation with *GLS1* overexpression, suggesting its influence on lipid metabolism in cancer progression. Additionally, our study reveals that persistent activation of the *PI3K-AKT-mTORC1* signaling pathway promotes cancer cell proliferation by upregulating *SREBP1*-mediated adipogenesis. Collectively, our findings highlight an overlooked regulatory mechanism of *SREBP-1*, indicating that *GLS1* drives de novo fatty acid synthesis and promotes cancer progression.

Materials and methods

Bioinformatics analysis

Several bioinformatics websites are being utilized to analyze gene expression and survival

outcomes in cancer research, such as Kaplan-Meier Plotter (https://kmplot.com/analysis/), the Human Protein Atlas (HPA) (https://www.proteinatlas.org/ENSG00000115419-GLS/pathology/liver+cancer), UALCAN (http://ualcan.path.uab.edu/index.html), GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=*GLS1*), and Linked Omics (http://www.linkedomics.org/admin.php).

Cell culture

The Shanghai Institutes of Biological Sciences in Shanghai, China, provided normal LO2 cells and human HCC cell lines (MHCC97-H, HepG2, and SMMC-7721). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) at 37 degrees Celsius and 5% carbon dioxide in a humidified incubator. Gibco was the provider of DMEM, FBS, and trypsin (Carlsbad, CA, USA).

Plasmid transfection

The overexpressed plasmid pCDH-CMV-MCS-EF1-copGFP-T2A-Puro was generated by general biol. Plasmid sh-EGFP, pLKO.1-EGFP-puro-sh1-*GLS1*, and plko.1-EGFP-puro-sh2-*GLS1* were generated by general biol. Plasmids were transfected into HCC cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 4-6 h, the original liquid in the Petri dish was pipetted off and incubation was continued in the incubator for 24-48 h.

Western blotting

The cells were washed with cold PBS, and then treated with RIPA lysis buffer at 4°C for 10 minutes to extract total cellular proteins. The denatured proteins were boiled for 5 minutes and separated on 10% SDS-PAGE gels before being transferring onto PVDF membranes. After blocking with 5% skim milk powder at room temperature for one hour, the membranes were incubated overnight at 4°C with primary antibodies. Subsequently, secondary antibodies were added after washing off excess primary antibodies. For Western blotting, primary antibodies against *GLS1, FASN, SCD1, ACC1, ACLY, FABP1, FABP5, FATP2, CD36, CPT1A, MCAD, SREBP-1, SCAP, ChREBP, PI3K, p-AKT (ser473), AKT*, and *mTOR* (abclonal; 1:1,000) were used, with *β-Actin* (1:5000, Sangon Biotech) as a loading control.

Colony formation

Cells were harvested and suspended in medium in 6-well plates, followed by culturing for 10-14 days until the emergence of visible colonies. Subsequently, the colonies were fixed and stained with 0.05% crystal violet for 30 minutes, and images of the colonies were captured.

Cell proliferation

To evaluate cell viability, the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) was employed. Specifically, 2×10^3 cells per well were seeded in 96-well plates for cell proliferation assays. The microplate reader was used to measure the absorbance (optical density value) at 490 nm after 24, 48, 72, and 96 h of cell growth.

Real-time RT-PCR

Total RNA was extracted using RNAiso Plus (Takara, Shiga, Japan), followed by reverse transcription using the RevertAid First Strand cDNA Synthesis Kit (Thermo, Waltham, MA, USA) according to the manufacturer's instructions. Real-time PCR was performed in triplicate using SYBR-green-based detection and the CFX-96 sequence detection system (Bio-Rad Laboratories), with expression levels normalized to *GAPDH*. The PCR execution protocol and data processing methods were described in a previous publication. All primers synthesized by general biol are shown in **Table 1**.

Nile red staining and triglyceride measurement

To observe lipid deposition, cells were fixed in 4% paraformaldehyde in 6-well plates, stained with 0.05 g/ml Nile Red dye (Sigma) for 10 min, and nuclei were stained with DAPI. Using immunofluorescence microscopy, the pictures were visualized. For triglyceride determination, cells were lysed for 30 minutes in RIPA buffer containing 1% NP-40, and then a Biochemical Triglyceride Determination Kit was applied to cell lysates to quantify triglycerides (Sangon Biotech). Normalized to cellular protein levels.

Statistical analysis

By utilizing GraphPad Prism 5 software and an ANOVA or Student's *t*-test, the data were

Table 1. Synthesized primers

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
GAPDH	GGTGAAGGTCGGTGTGAACG	CTCGCTCCTGGAAGATGGTG
GLS1	AGTTGCTGGGGGCATTCTTTTAGTT	CCTTTGATCACCACCTTCTCTTCGA
FASN	AACTCCAAGGACACAGTCACCAT	CAGCTGCTCCACGAACTCAA
SCD1	CCGGACACGGTCACCCGTTG	CGCCTTGCACGCTAGCTGGT
ACC1	AATGTCCTTCTCCTCCAA	GAGTGAATGAGTTGTCCAA
ACLY	AACGCCAGCGGGAGCACATC	TTGCAGGCGCCACCTCATCG
SREBP-1	GACCACTGTCACTTCCAGCTA	CGCCGGTCTTAGGGTCAAGA
SCAP	TATCTCGGGCCTTCTACAACC	GGGGCGAGTAATCCTTCACA

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, *GLS1*: Glutaminase 1, *FASN*: fatty acid synthase, *SCD1*: stearoyl-CoA desaturase1, *ACC1*: acetyl-CoA carboxylase 1, *ACLY*: ATP-citrate lyase, *SREBP-1*: sterol regulatory element-binding protein 1, *SCAP*: SREBP cleavage-activating protein.

presented as the mean ± standard error of at least three independent experiments. Differences with *P* values under 0.05 were deemed statistically significant.

Results

GLS1 was highly-expressed in HCC and correlated with poor survival

We explored the relationship between *GLS1* expression and clinical prognosis by analyzing public databases. Our findings demonstrated significant upregulation of *GLS1* in HCC tissue samples compared to normal liver tissues at the mRNA level, as shown in the GEPIA and UALCAN databases (**Figure 1A**, **1B**). Notably, *GLS1* expression was significantly elevated in advanced and high-grade HCC tissues, while its expression was relatively low in early and low-grade HCC tissues (**Figure 1C**, **1D**). Additionally, survival analysis conducted using three bioinformatics databases, GEPIA, HPA, and Kaplan-Meier Plotter, indicated that high *GLS1* expression was associated with lower survival rates compared to low *GLS1* expression (**Figure 1E-G**).

GLS1 promotes lipid accumulation in hepatocellular carcinoma

Our study provided evidence that *GLS1* enhances HCC cell proliferation through activation of the *AKT/GSK3/CyclinD1* signaling pathway [17]. However, the influence of *GLS1* on lipid metabolism in HCC cells remains unclear. To investigate, we first assessed *GLS1* expression levels in four cell lines: LO2, HepG2, SMMC-7721, and MHCC97-H, using real-time PCR and Western blotting. As depicted in **Figure 2A** and **2B**, *GLS1* levels were significantly higher in the three HCC cell lines, HepG2, SMMC-7721, and MHCC97-H, compared to the normal human hepatocyte line (LO2). Furthermore, *GLS1* expression was higher in HepG2 cells than in SMMC-7721 and MHCC97-H cells. We then examined whether *GLS1* could stimulate lipid accumulation in HCC cells. *GLS1* was either knocked down or overexpressed in HCC cells, and Nile red staining along with triglyceride measurement assays were performed. Results indicated that overexpression of *GLS1* in SMMC-7721 and MHCC97-H cells led to increased cellular lipid accumulation and triglyceride (TG) levels (**Figure 2C** and **2D**). Conversely, *GLS1* knockout reduced cellular lipid accumulation and decreased TG levels (**Figure 2E** and **2F**). These findings suggest that *GLS1* promotes lipid accumulation in HCC.

GLS1 promotes de novo fatty acid synthesis in hepatocellular carcinoma

The mechanism through which *GLS1* regulates lipid metabolism has attracted significant interest. Researchers have identified several key enzymes involved in de novo fatty acid synthesis, fatty acid β-oxidation, and fatty acid uptake. Notably, Western blot analysis revealed that the expression of de novo fatty acid synthetic enzymes, including *FASN*, *SCD1, ACC1*, and *ACLY*, was upregulated in SMMC-7721 and MHCC97-H cells treated with *GLS1* (**Figure 3A**). In contrast, these enzymes showed reduced expression in HepG2 and SMMC-7721 cells treated with sh-*GLS1* compared to the sh-EGFP group (**Figure 3B**). The changes observed in de novo fatty acid synthetic enzymes were further

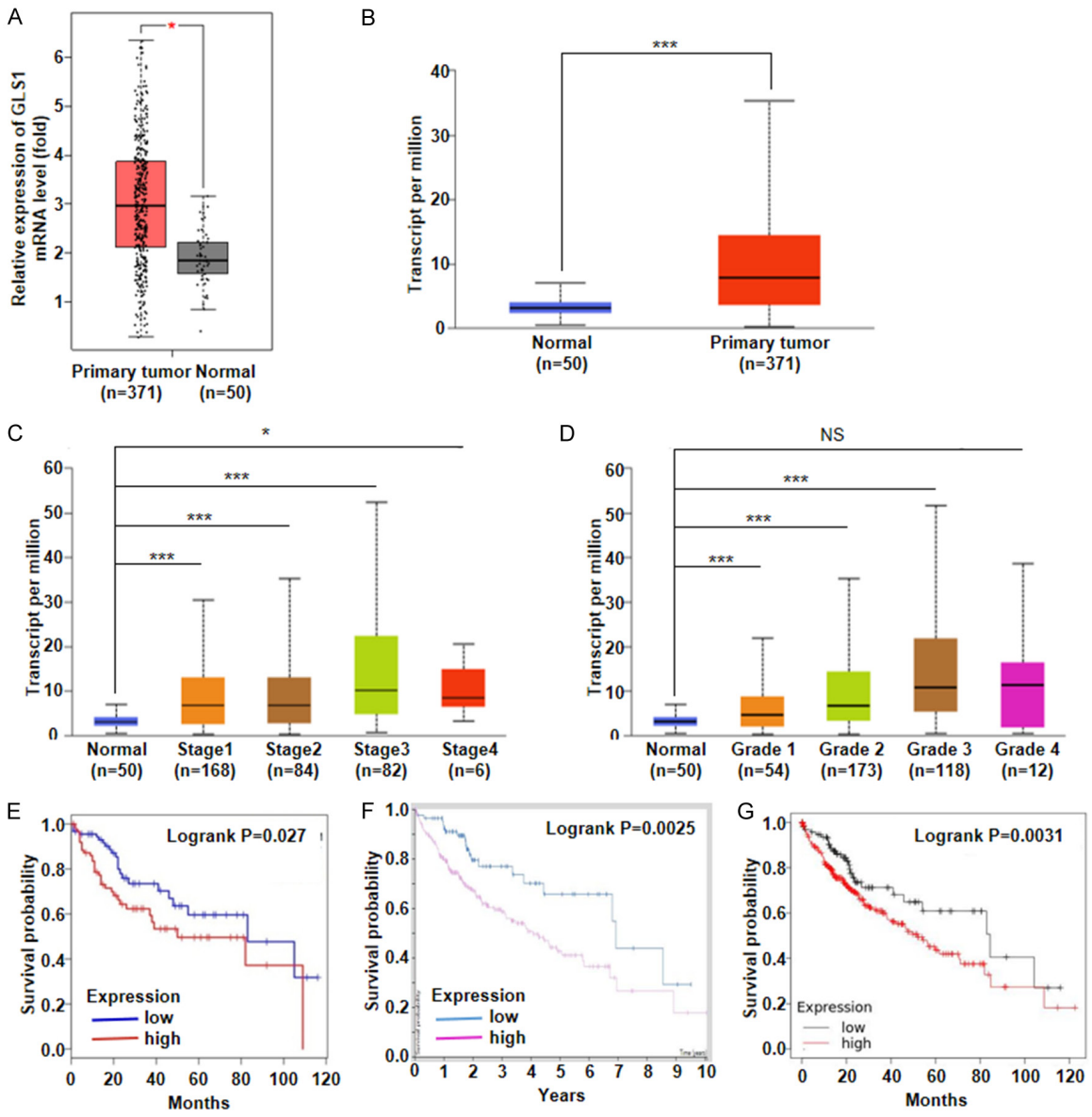

Figure 1. Bioinformatics analysis indicated that Glutaminase 1 *(GLS1)* was high-expressed in hepatocellular carcinoma (HCC) and was correlated with poor survival. (A, B) Gene Expression Profiling Interactive Analysis (GEPIA) (A) and UALCAN (http://ualcan.path.uab.edu/index.html) (B) Two databases were used to analyze the mRNA level of *GLS1* in HCC samples and normal liver samples. (C) UALCAN database was used to analyze the mRNA level of *GLS1* in different stages of HCC. (D) The mRNA level of *GLS1* in different grades of HCC was analyzed in the UALCAN database. (E-G) GEPIA, The Human Protein Atlas (HPA), and Kaplan-Meier Plotter databases were used for survival analysis to evaluate the influence of *GLS1* on the overall survival of HCC patients. **P*<0.05, ****P*<0.001, NS: no significance.

confirmed at the mRNA level, aligning with the protein-level data (Figure S1). However, *GLS1* overexpression did not impact the protein levels of fatty acid β-oxidase enzymes, such as carnitine palmitoyl transferase 1A (*CPT1A),* medium-chain acyl-CoA dehydrogenase (*MCAD),* and short-chain acyl-CoA dehydrogenase (*SCAD)*, nor fatty acid uptake proteins, including fatty acid binding protein 1 (*FABP1),* fatty acid binding protein 2 (*FATP2),* fatty acid binding protein 5 (*FABP5),* and Cluster of differentiation 36 (*CD36)* (**Figure 3A**).

Given that *GLS1* significantly influences de novo fatty acid synthesis and brings about distinct changes in related metabolic enzymes, it

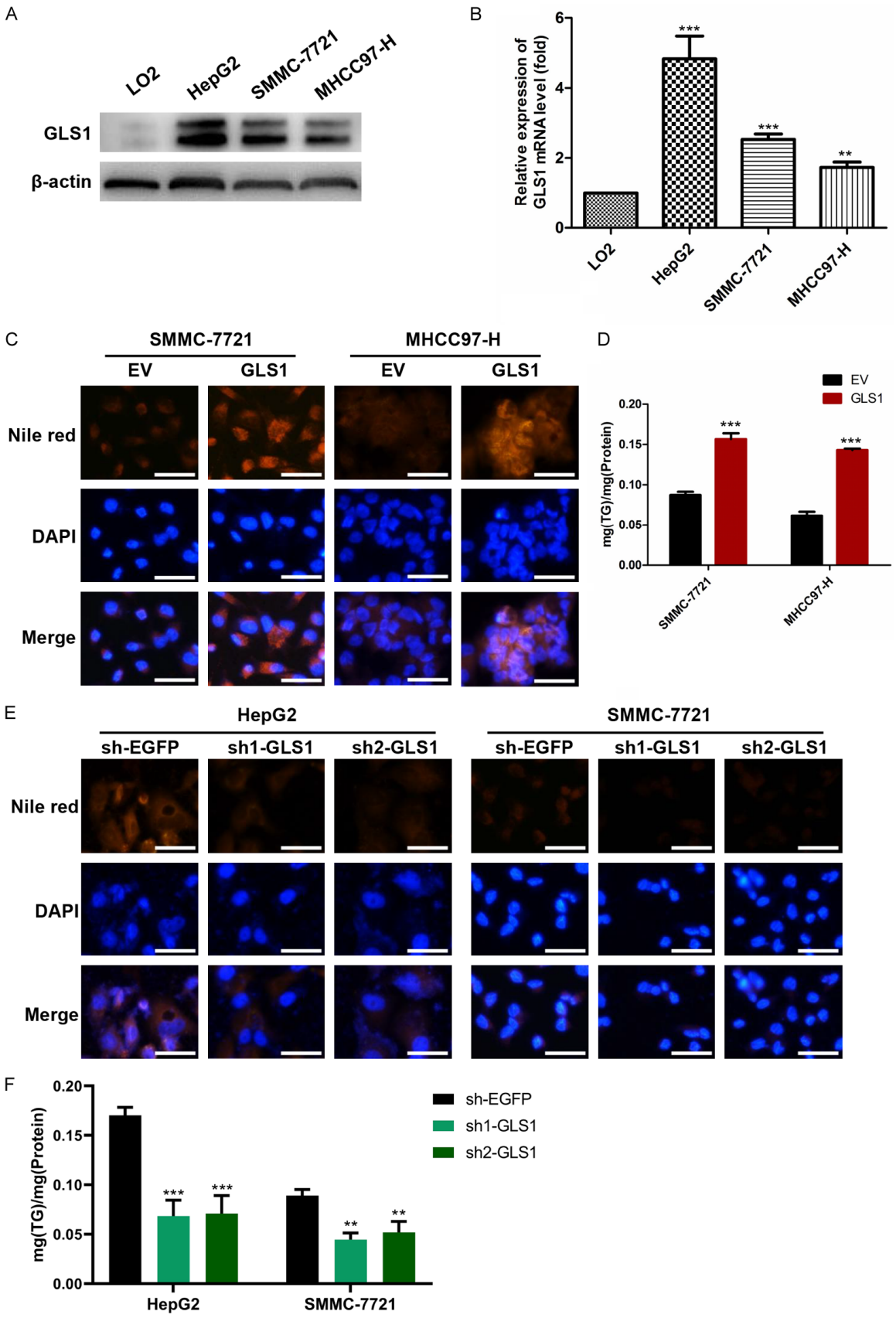
A
LO2
HepG2
SMMC-7721
MHCC97-H
GLS1
β-actin
B
Relative expression of GLS1 mRNA level (fold)
LO2
HepG2
SMMC-7721
MHCC97-H
C
SMMC-7721
MHCC97-H
EV
GLS1
Nile red
DAPI
Merge
D
EV
GLS1
mg(TG)/mg(Protein)
SMMC-7721
MHCC97-H
E
HepG2
SMMC-7721
sh-EGFP
sh1-GLS1
sh2-GLS1
Nile red
DAPI
Merge
F
sh-EGFP
sh1-GLS1
sh2-GLS1
mg(TG)/mg(Protein)
HepG2
SMMC-7721

Figure 2. *GLS1* promotes lipid accumulation in hepatocellular carcinoma. A, B. Relative expression levels of *GLS1* protein and mRNA were assessed in LO2, HepG2, SMMC-7721, and MHCC97-H. C. Cellular neutral lipids were measured in SMMC-7721 and MHCC97-H cells overexpressing *GLS1* by Nile red staining. Scale bars, 50 μm. D. Cellular triglyceride (TG) was measured in SMMC-7721 and MHCC97-H cells overexpressing *GLS1* by Biochemical Triglyceride Determination Kit. E. Cellular neutral lipids were measured in HepG2 and SMMC-7721 cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1* by Nile red staining. Scale bars, 50 μm. F. Cellular TG was measured in SMMC-7721 and MHCC97-H cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1* by Biochemical Triglyceride Determination Kit. The data are shown as the mean ± s.d. of three independent experiments, $^{**}P<0.01$, $^{***}P<0.001$.

is likely that *GLS1* regulates this pathway by modulating critical regulatory factors. To investigate this possibility, we examined the effect of *GLS1* on the principal regulators of de novo fatty acid synthesis, including *SREBP-1*, *SCAP*, and carbohydrate-responsive element-binding protein *(ChREBP)*. Interestingly, overexpression of *GLS1* in SMMC-7721 and MHCC97-H cells led to a pronounced increase in both *SREBP-1* and *SCAP* protein levels (**Figure 3C**), while *GLS1* interference resulted in an opposite effect (**Figure 3D**). The effects of *GLS1* overexpression or interference on *SREBP-1* and *SCAP* mRNA levels were consistent with those observed at the protein level (Figure S1). These findings indicate that *GLS1* promotes de novo fatty acid synthesis in hepatocellular carcinoma cells.

GLS1 promotes lipid accumulation and cell proliferation via SREBP-1 and SCAP

To investigate whether *GLS1* regulates de novo fatty acid synthesis and cell proliferation through *SREBP-1* and *SCAP*, we assessed the effects of suppressing these regulatory factors using shRNAs. Western blot analysis showed that knockdown of *SREBP-1* or *SCAP* significantly inhibited the expression of de novo fatty acid synthesis enzymes *FASN*, *SCD1, ACC1*, and *ACLY* in SMMC-7721 cells (**Figure 4A**). Interestingly, *SREBP-1* or *SCAP* knockdown also notably reduced the *GLS1*-induced increase in fatty acid synthetic enzyme protein levels in SMMC-7721 cells (**Figure 4B**). Conversely, overexpression of *SREBP-1* or *SCAP* in SMMC-7721 cells yielded the opposite outcome, as expected (Figure S2). Additionally, knockdown of *SREBP-1* or *SCAP* significantly reduced the *GLS1*-induced rise in cellular lipid accumulation and TG levels (**Figures 4C**, **5A**, **5B**). These results confirm that inhibition of *SREBP-1* or *SCAP* via shRNAs can significantly reduce fatty acid synthase expression in HCC cells. Moreover, knockdown of *SREBP-1* or *SCAP* effectively suppressed the *GLS1*-driven increase in fatty acid synthetic enzyme levels and mitigated *GLS1*'s stimulatory effect on neutral lipid accumulation and TG levels. Our findings suggest that *SREBP-1* and *SCAP* are essential mediators of *GLS1*-induced fatty acid synthase production and lipid metabolism.

In a previous study, we demonstrated that *GLS1* promotes both proliferation and colony formation in HCC cells [17]. To further confirm these findings, a rescue assay was conducted to evaluate cell proliferation and colony formation abilities. As shown in **Figure 5C** and **5D**, knockdown of *SREBP-1* or *SCAP* significantly reduced the *GLS1*-mediated promotion of proliferation and colony formation in SMMC-7721 cells. Collectively, these results indicate that *SREBP-1* and *SCAP* are crucial for *GLS1*'s role in promoting de novo fatty acid synthesis, ultimately influencing the proliferation of HCC cells.

GLS1 affects PI3K-AKT-mTORC1 signaling mediated SREBP-1 in HCC cells

Recent studies have connected mTORC1 (mammalian target of rapamycin complex 1) with lipogenesis through *SREBP-1*. As illustrated in **Figure 6**, *GLS1* knockdown reduced levels of *PI3K, p-AKT,* and *p-mTOR* in HepG2 and SMMC-7721 cells, whereas *GLS1* overexpression increased these proteins in SMMC-7721 and MHCC97-H cells. Total *AKT* and *mTOR* protein levels remained unaffected by *GLS1* knockdown or overexpression. These results suggest that *GLS1* modulates the *PI3K-AKT-mTORC1* signaling pathway, which regulates *SREBP-1* in HCC cells.

Discussion

Our study reveals a significant role of *GLS1* in driving lipid accumulation in hepatocellular carcinoma (HCC). Specifically, *GLS1* promotes de novo fatty acid synthesis by modulating key metabolic enzymes. Our findings demonstrate that *SREBP-1* and *SCAP* are essential for *GLS1*-

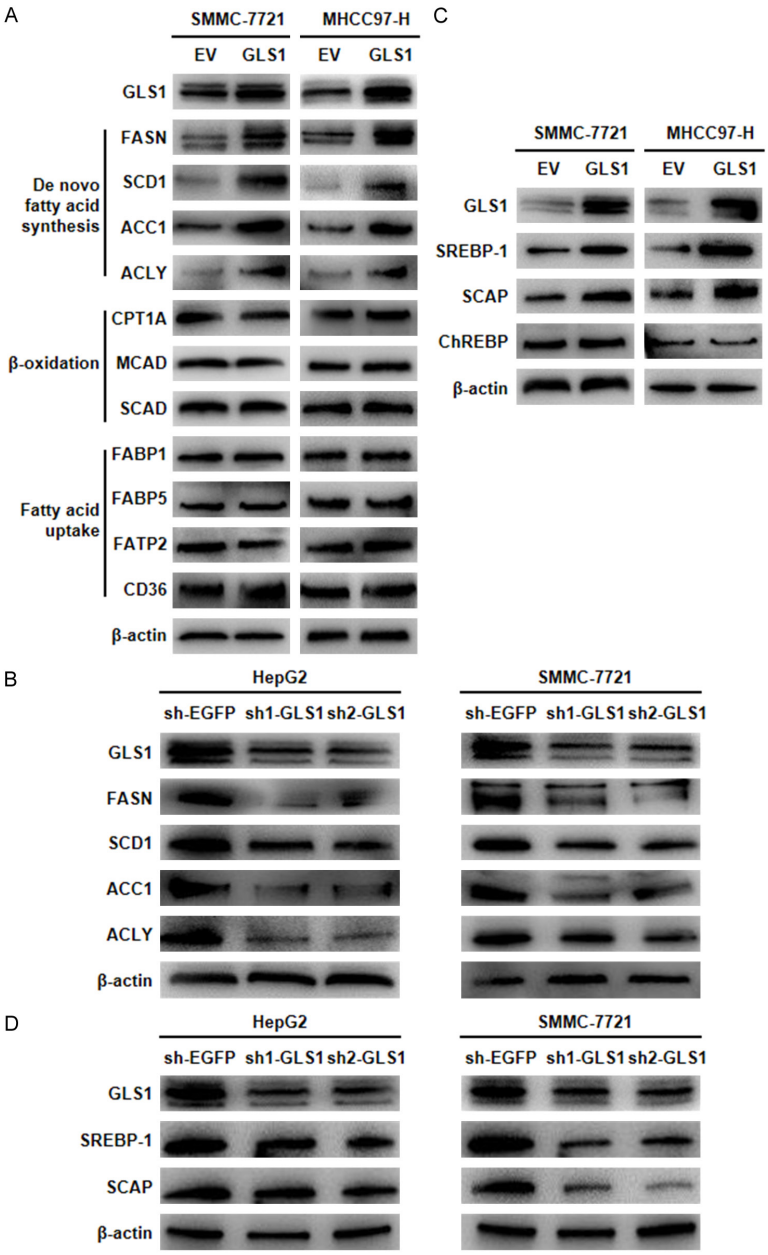

Figure 3. *GLS1* promotes de novo fatty acid synthesis in hepatocellular carcinoma. A. Protein levels of metabolic enzymes were determined by Western blot in SMMC-7721 and MHCC97-H cells overexpressing *GLS1*. β-Actin served as loading control. B. Protein levels of metabolic enzymes in de novo fatty acid synthesis were determined by Western blot in HepG2 and SMMC-7721 cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1*. β-Actin served as loading control. C. Protein levels of a transcription factor in de novo fatty acid synthesis and *SCAP* were determined by Western blot in SMMC-7721 and MHCC97-H cells overexpressing *GLS1*. β-Actin served as loading control. D. Protein levels of *SREBP-1* and *SCAP* were determined by Western blot in HepG2 and SMMC-7721 cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1*. β-Actin served as loading control. Each experiment was examined in triplicate.

mediated upregulation of fatty acid synthase expression and subsequent lipid metabolism.

To our knowledge, this is the first study to offer detailed insights into how *GLS1* coordinates both proliferation and lipid metabolism in HCC cells. By identifying the mechanism through which the oncogenic PI3K-AKT-mTORC1 pathway promotes de novo fatty acid synthesis via *SREBP-1*-mediated enzyme activity, we establish a critical link between *GLS1* and lipid metabolism. These findings, combined with our previous work, suggest that targeting *GLS1* may provide new avenues for the diagnosis and treatment of HCC.

Metabolic abnormalities play a central role in carcinogenesis, allowing tumors to adapt to the local microenvironment, which in turn supports cancer cell survival [18, 19]. This adaptive process, often referred to as “metabolic reprogramming”, encompasses a series of clonal adaptations during carcinogenesis, producing metabolites with various functions at multiple levels [20, 21]. Increasing evidence suggests that elevated de novo fatty acid synthesis is a prominent feature in cancer development, with activation of this pathway negatively correlated with disease-free survival across multiple tumor types. This phenotype largely results from the upregulation of lipid-related genes through transcriptional, translational, post-translational modifications, and enzyme activity improvement. These gene alterations, along with changes in lipid metabolism, influence oncogene expression, further supporting tumor progression [22]. Furthermore, the increased lipid production in cancer cells is primarily driven by the need for increased cell membrane

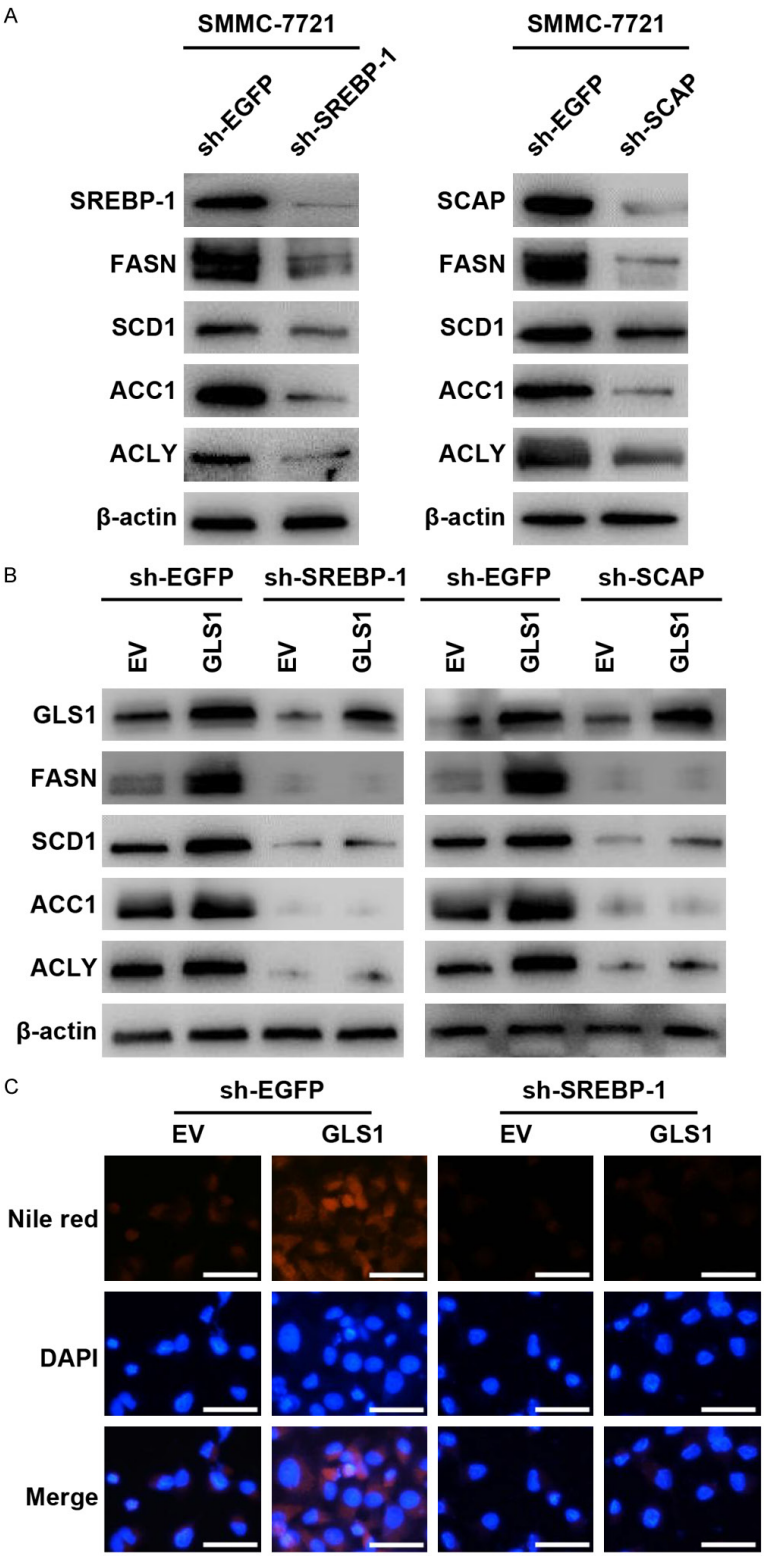

Figure 4. *GLS1* promotes fatty acid synthetic enzymes protein and lipid accumulation via *SREBP-1* and *SCAP*. A. Protein levels of *SREBP-1*, *SCAP*, and metabolic enzymes in de novo fatty acid synthesis were determined by Western blot in SMMC-7721 expressing sh-EGFP, sh-*SREBP-1*, or sh-*SCAP*. β-Actin served as loading control. B. Protein levels of *GLS1* and metabolic enzymes in de novo fatty acid synthesis were determined after knocking down *SREBP-1* or sh-*SCAP* in SMMC-7721 overexpressing *GLS1* by Western blot. β-Actin served as loading control. C. Cellular neutral lipids were measured after overexpressing *GLS1* and knocking down *SREBP-1* in SMMC-7721 cells by Nile red staining. Scale bars, 50 μm. Each experiment was examined in triplicate.

synthesis to meet the rapid proliferation and energy demands of these cells. This activation of lipid synthesis is strongly associated with tumor progression and poor prognosis across various cancer types, highlighting its negative correlation with disease-free survival.

Hepatocellular carcinoma (HCC) is often characterized by the upregulation of genes involved in fatty acid (FA) synthesis, including *ACLY*, *ACC*, and *FASN*, which promote the conversion of citrate to acetyl-CoA, malonyl-CoA, and FA, respectively. Targeting key genes involved in lipogenesis, such as *FASN*, *ACC*, *ACLY*, and the primary transcriptional regulator SREBP1, can effectively suppress tumor neogenesis and reduce tumor cell growth, either through gene knockdown or chemical inhibitors. For example, inhibiting *FASN* has been shown to deactivate *AKT* and induce apoptosis in non-small cell lung cancer (NSCLC) cells at the EC1 and EC5 growth-inhibitory concentrations [23]. Similarly, *ACLY* inhibitors can trigger apoptosis and increase the cytotoxic effects of sorafenib in thyroid cancer cells [24]. The desaturase *SCD1* is a vital enzyme in lipid metabolism, responsible for converting saturated fatty acids (SFA) to monounsaturated fatty acids (MUFA), and is closely linked to cancer progression. Dysregulated *SCD1*

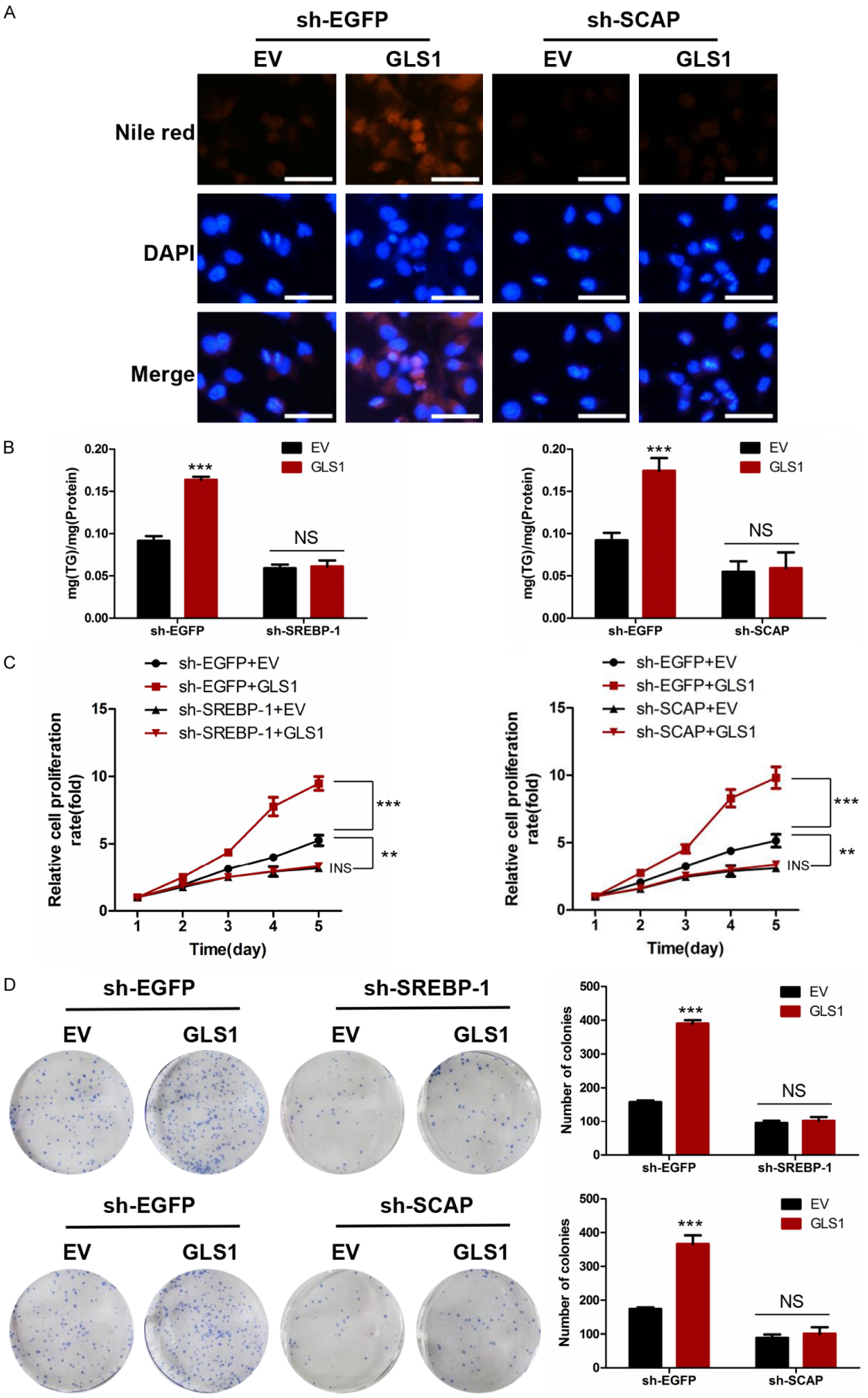
A
sh-EGFP
sh-SCAP
EV
GLS1
EV
GLS1
Nile red
DAPI
Merge
B
mg(TG)/mg(Protein)
EV
GLS1
***
NS
sh-EGFP
sh-SREBP-1
sh-SCAP
C
sh-EGFP+EV
sh-EGFP+GLS1
sh-SREBP-1+EV
sh-SREBP-1+GLS1
sh-SCAP+EV
sh-SCAP+GLS1
Relative cell proliferation rate(fold)
Time(day)
***
**
INS
D
sh-EGFP
sh-SREBP-1
sh-SCAP
EV
GLS1
Number of colonies
***
NS

Figure 5. *GLS1* promotes lipid accumulation and cell proliferation via *SREBP-1* and *SCAP*. A. Cellular neutral lipids were measured after overexpressing *GLS1* and knocking down *SCAP* in SMMC-7721 cells by Nile red staining. Scale bars, 50 μm. B. Cellular TG was measured after knocking down *SREBP-1* or sh-*SCAP* in SMMC-7721 overexpressing *GLS1* by Biochemical Triglyceride Determination Kit. C. Relative cell proliferation rate was measured after knocking down *SREBP-1* or sh-*SCAP* in SMMC-7721 overexpressing *GLS1* by CCK-8 assay. D. Colony forming assay was performed after knocking down *SREBP-1* or sh-*SCAP* in SMMC-7721 overexpressing *GLS1*. The data are shown as the mean ± s.d. of three independent experiments, ***P*<0.01, ****P*<0.001, NS: no significance.

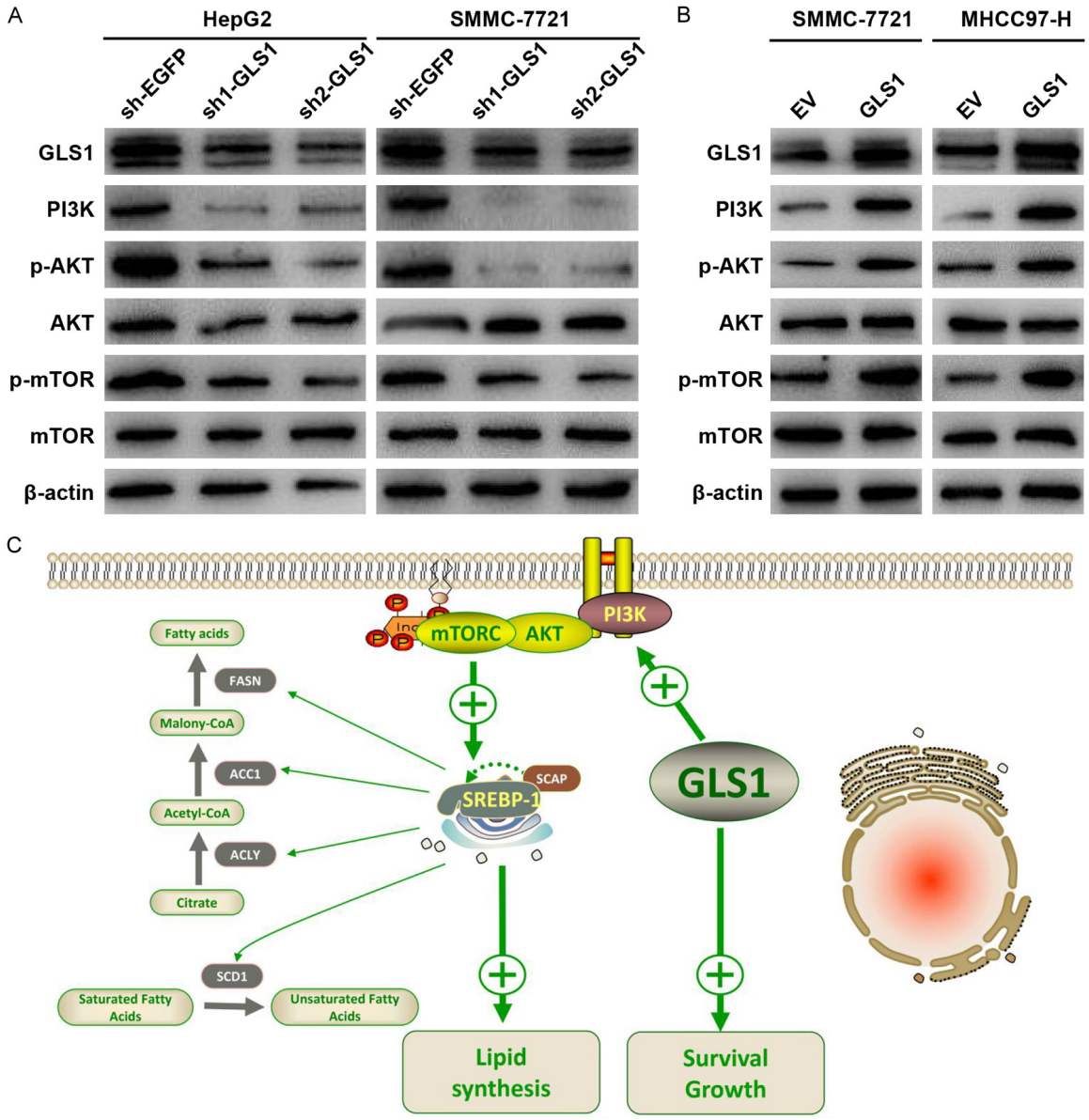

Figure 6. *GLS1* affects *PI3K-AKT-mTORC1* signaling mediated *SREBP-1* in HCC cells. A. Protein levels of *PI3K, p-AKT, AKT, p-mTOR*, and *mTOR* were determined in HepG2 and SMMC-7721 cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1*. β-Actin served as loading control. B. Protein levels of *PI3K, p-AKT, AKT, p-mTOR*, and *mTOR* were determined in SMMC-7721 and MHCC97-H cells overexpressing *GLS1*. β-Actin served as loading control. C. Schematic model illustrating the effect of *GLS1* on PI3K-AKT-mTORC1 signaling and lipid metabolism in HCC. Each experiment was examined in triplicate.

expression is commonly observed across various cancers. Recent studies indicate that *SCD1* functions as an oncogene in gastric cancer, promoting metastasis, anti-ferroptotic cell

death, and the proliferation of gastric cancer cells both in vitro and in vivo [25]. Several lipid synthesis inhibitors have shown promising anticancer effects in preclinical research [23, 24]. However, developing cancer therapies that target variations in lipid metabolism poses significant challenges, primarily due to a limited understanding of the lipid metabolic mechanisms in cancer cells.

Sterol regulatory element-binding proteins (SREBPs) are essential nuclear transcription factors. The HUMAN GENOME encodes two SREBP genes, *SREBP-1* and srebp-2, which directly regulate the transcription of over 30 genes involved in fatty acid, triglyceride, cholesterol, and phospholipid synthesis and uptake [26]. Research has demonstrated a strong association between *SREBP-1* and various cancers. Abnormal expression of *SREBP-1* has been identified in multiple tumors, including colorectal, prostate, breast, and hepatocellular cancers [27]. As one of the primary transcription factors governing endogenous lipid synthesis, *SREBP-1*, along with its downstream target gene *FASN*, has been referred to as a metabolic proto-oncogene in some studies. Stimulated by growth factors such as insulin, *EGF*, and platelet-derived growth factor (*PDGF*), *SREBP-1* directly activates a range of target genes related to lipid synthesis, including *FASN*, *ACLY*, *ACC*, and *SCD1*. This activation promotes endogenous lipid production, supports energy supply for membrane signaling lipids and membrane structures, and drives tumor initiation and progression [28]. *SCAP* is a critical maturation factor for *SREBP-1*. Under normal conditions, *SCAP* binds to *SREBP-1* in the endoplasmic reticulum. In sterol-deficient conditions, *SREBP-1* is transported to the Golgi for maturation, after which the mature *SREBP-1* is delivered to the nucleus to function as a transcription factor [29]. In our study, *GLS1* overexpression not only significantly increased *SREBP-1* and *SCAP* protein expression but also enhanced *SCAP*-mediated protein maturation. This dual regulation strengthens the link between *GLS1* and *SREBP-1*, leading to robust control of fatty acid synthesis by *GLS1* and ultimately resulting in lipid accumulation. Thus, our findings suggest that *GLS1* promotes lipid accumulation through *SREBP-1*, which holds significant implications for cancer cell proliferation.

Lipids are fundamental components of cellular membranes and play critical roles as signaling molecules. Among these, phosphatidylinositol (3,4,5)-triphosphate (*PIP3*) acts as an essential second messenger, produced by phosphatidylinositol 3-kinase (*PI3K*) in response to growth factor signals, which facilitates *AKT* recruitment and activation. Studies have shown that activation of the proto-oncogene RAS or loss of the tumor suppressor protein phosphatase (*PTEN*) can activate the *PI3K-AKT* pathway, increasing *SREBP-1* maturation. Consequently, the *AKT-mTORC1* signaling pathway is activated through *SREBP-1*-mediated lipogenesis. Notably, inhibition of upstream factors such as the epidermal growth factor receptor (*EGFR*), *PI3K*, and *AKT* significantly reduces *SREBP-1* nuclear content and nitrogen terminal levels [30]. *mTORC1*, a major downstream effector of *AKT*, is regulated by specific amino acids and also requires mature nuclear *SREBP-1* to drive hepatic lipid production by regulating *SREBP-1* expression [31]. Additionally, our findings indicate that activation of de novo fatty acid synthesis enzymes is a key pathway within the *PI3K-AKT-mTORC1* axis in human HCC. However, the precise molecular mechanism by which *GLS1* contributes to this pathway requires further exploration.

In summary, our study establishes a link between *GLS1* and lipid metabolism in HCC through the *PI3K-AKT-mTORC1* signaling pathway (**Figure 6C**). Our results indicate that targeting the regulation of de novo fatty acid synthesis and lipid homeostasis may offer a promising therapeutic strategy for malignancies with abnormal *GLS1* expression, as suggested by ongoing clinical trials investigating fatty acid-blocking agents [32, 33]. Overall, our findings highlight the potential clinical implications of *GLS1*-targeted therapies and highlight the need for further investigation into *GLS1* and its related pathways for cancer treatment.

Acknowledgements

This study was supported by several grants, including the Changzhou Sci & Tech Program (No. CJ20220005), the Science and Xuzhou Medical University Hospital Development Fund Project (No. XYFY202465), and the Young Talent Development Plan of Changzhou Health Commission (No. CZQM2021027).

Disclosure of conflict of interest

None.

Address correspondence to: Jianjun Tang, Department of General Surgery, Wujin Hospital Affiliated with Jiangsu University, Changzhou 213017, Jiangsu, China. Tel: +86-13961185561; E-mail: tangjianjun@wjrmyy.cn

References

[1] Xing M, Wang X, Kiken RA, He L and Zhang JY. Immunodiagnostic biomarkers for hepatocellular carcinoma (HCC): the first step in detection and treatment. Int J Mol Sci 2021; 22: 6139.

[2] Wang J, Li J, Tang G, Tian Y, Su S and Li Y. Clinical outcomes and influencing factors of PD-1/PD-L1 in hepatocellular carcinoma. Oncol Lett 2021; 21: 279.

[3] Giraud J, Chalopin D, Blanc JF and Saleh M. Hepatocellular carcinoma immune landscape and the potential of immunotherapies. Front Immunol 2021; 12: 655697.

[4] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A and Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16: 589-604.

[5] Kanwal F, Khaderi S, Singal AG, Marrero JA, Loo N, Asrani SK, Amos CI, Thrift AP, Gu X, Luster M, Al-Sarraj A, Ning J and El-Serag HB. Risk factors for HCC in contemporary cohorts of patients with cirrhosis. Hepatology 2023; 77: 997-1005.

[6] Trefts E, Gannon M and Wasserman DH. The liver. Curr Biol 2017; 27: R1147-R1151.

[7] Ding HR, Wang JL, Ren HZ and Shi XL. Lipometabolism and glycometabolism in liver diseases. Biomed Res Int 2018; 2018: 1287127.

[8] Hensley CT, Wasti AT and DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Invest 2013; 123: 3678-84.

[9] Elgadi KM, Meguid RA, Qian M, Souba WW and Abcouwer SF. Cloning and analysis of unique human glutaminase isoforms generated by tissue-specific alternative splicing. Physiol Genomics 1999; 1: 51-62.

[10] Katt WP, Lukey MJ and Cerione RA. A tale of two glutaminases: homologous enzymes with distinct roles in tumorigenesis. Future Med Chem 2017; 9: 223-43.

[11] Liu HY, Zhang HS, Liu MY, Li HM, Wang XY and Wang M. GLS1 depletion inhibited colorectal cancer proliferation and migration via redox/Nrf2/autophagy-dependent pathway. Arch Biochem Biophys 2021; 708: 108964.

[12] Cao J, Zhang C, Jiang GQ, Jin SJ, Gao ZH, Wang Q, Yu DC, Ke AW, Fan YQ, Li DW, Wang AQ and Bai DS. Expression of GLS1 in intrahepatic cholangiocarcinoma and its clinical significance. Mol Med Rep 2019; 20: 1915-24.

[13] Li B, Cao Y, Meng G, Qian L, Xu T, Yan C, Luo O, Wang S, Wei J, Ding Y and Yu D. Targeting glutaminase 1 attenuates stemness properties in hepatocellular carcinoma by increasing reactive oxygen species and suppressing Wnt/beta-catenin pathway. EBioMedicine 2019; 39: 239-54.

[14] Wu S, Fukumoto T, Lin J, Nacarelli T, Wang Y, Ong D, Liu H, Fatkhutdinov N, Zundell JA, Karakashev S, Zhou W, Schwartz LE, Tang HY, Drapkin R, Liu Q, Huntsman DG, Kossenkov AV, Speicher DW, Schug ZT, Van Dang C and Zhang R. Targeting glutamine dependence through GLS1 inhibition suppresses ARID1A-inactivated clear cell ovarian carcinoma. Nat Cancer 2021; 2: 189-200.

[15] Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT and Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 2009; 458: 762-5.

[16] Thangavelu K, Pan CQ, Karlberg T, Balaji G, Uttamchandani M, Suresh V, Schuler H, Low BC and Sivaraman J. Structural basis for the allosteric inhibitory mechanism of human kidney-type glutaminase (KGA) and its regulation by Raf-Mek-Erk signaling in cancer cell metabolism. Proc Natl Acad Sci U S A 2012; 109: 7705-10.

[17] Xi J, Sun Y, Zhang M, Fa Z, Wan Y, Min Z, Xu H, Xu C and Tang J. GLS1 promotes proliferation in hepatocellular carcinoma cells via AKT/GSK3beta/CyclinD1 pathway. Exp Cell Res 2019; 381: 1-9.

[18] Drapela S, Ilter D and Gomes AP. Metabolic reprogramming: a bridge between aging and tumorigenesis. Mol Oncol 2022; 16: 3295-318.

[19] Iorio M, Umesh Ganesh N, De Luise M, Porcelli AM, Gasparre G and Kurelac I. The neglected liaison: targeting cancer cell metabolic reprogramming modifies the composition of non-malignant populations of the tumor microenvironment. Cancers (Basel) 2021; 13: 5447.

[20] Navarro C, Ortega A, Santeliz R, Garrido B, Chacin M, Galban N, Vera I, De Sanctis JB and Bermudez V. Metabolic reprogramming in cancer cells: emerging molecular mechanisms and novel therapeutic approaches. Pharmaceutics 2022; 14: 1303.

[21] Bao MH and Wong CC. Hypoxia, metabolic reprogramming, and drug resistance in liver cancer. Cells 2021; 10: 1715.

[22] Liu X, Zhang P, Xu J, Lv G and Li Y. Lipid metabolism in tumor microenvironment: novel therapeutic targets. Cancer Cell Int 2022; 22: 224.

[23] Tan YJ, Ali A, Tee SY, Teo JT, Xi Y, Go ML and Lam Y. Galloyl esters of trans-stilbenes are inhibitors of FASN with anticancer activity on non-small cell lung cancer cells. Eur J Med Chem 2019; 182: 111597.
[24] Huang SS, Tsai CH, Kuo CY, Li YS and Cheng SP. ACLY inhibitors induce apoptosis and potentiate cytotoxic effects of sorafenib in thyroid cancer cells. Endocrine 2022; 78: 85-94.
[25] Wang C, Shi M, Ji J, Cai Q, Zhao Q, Jiang J, Liu J, Zhang H, Zhu Z and Zhang J. Stearoyl-CoA desaturase 1 (SCD1) facilitates the growth and anti-ferroptosis of gastric cancer cells and predicts poor prognosis of gastric cancer. Aging (Albany NY) 2020; 12: 15374-91.
[26] Ferre P, Phan F and Foufelle F. SREBP-1c and lipogenesis in the liver: an update1. Biochem J 2021; 478: 3723-39.
[27] Zhao Q, Lin X and Wang G. Targeting SREBP-1-mediated lipogenesis as potential strategies for cancer. Front Oncol 2022; 12: 952371.
[28] Shimano H and Sato R. SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. Nat Rev Endocrinol 2017; 13: 710-30.
[29] Kawamura S, Matsushita Y, Kurosaki S, Tange M, Fujiwara N, Hayata Y, Hayakawa Y, Suzuki N, Hata M, Tsuboi M, Kishikawa T, Kinoshita H, Nakatsuka T, Sato M, Kudo Y, Hoshida Y, Umemura A, Eguchi A, Ikenoue T, Hirata Y, Uesugi M, Tateishi R, Tateishi K, Fujishiro M, Koike K and Nakagawa H. Inhibiting SCAP/SREBP exacerbates liver injury and carcinogenesis in murine nonalcoholic steatohepatitis. J Clin Invest 2022; 132: e151895.
[30] Zhang M, Pan J and Huang P. Interaction between RAS gene and lipid metabolism in cancer. Zhejiang Da Xue Xue Bao Yi Xue Ban 2021; 50: 17-22.
[31] Aoki M and Fujishita T. Oncogenic roles of the PI3K/AKT/mTOR Axis. Curr Top Microbiol Immunol 2017; 407: 153-89.
[32] Bacci M, Lorito N, Smiriglia A and Morandi A. Fat and furious: lipid metabolism in antitumoral therapy response and resistance. Trends Cancer 2021; 7: 198-213.
[33] Corn KC, Windham MA and Rafat M. Lipids in the tumor microenvironment: from cancer progression to treatment. Prog Lipid Res 2020; 80: 101055.

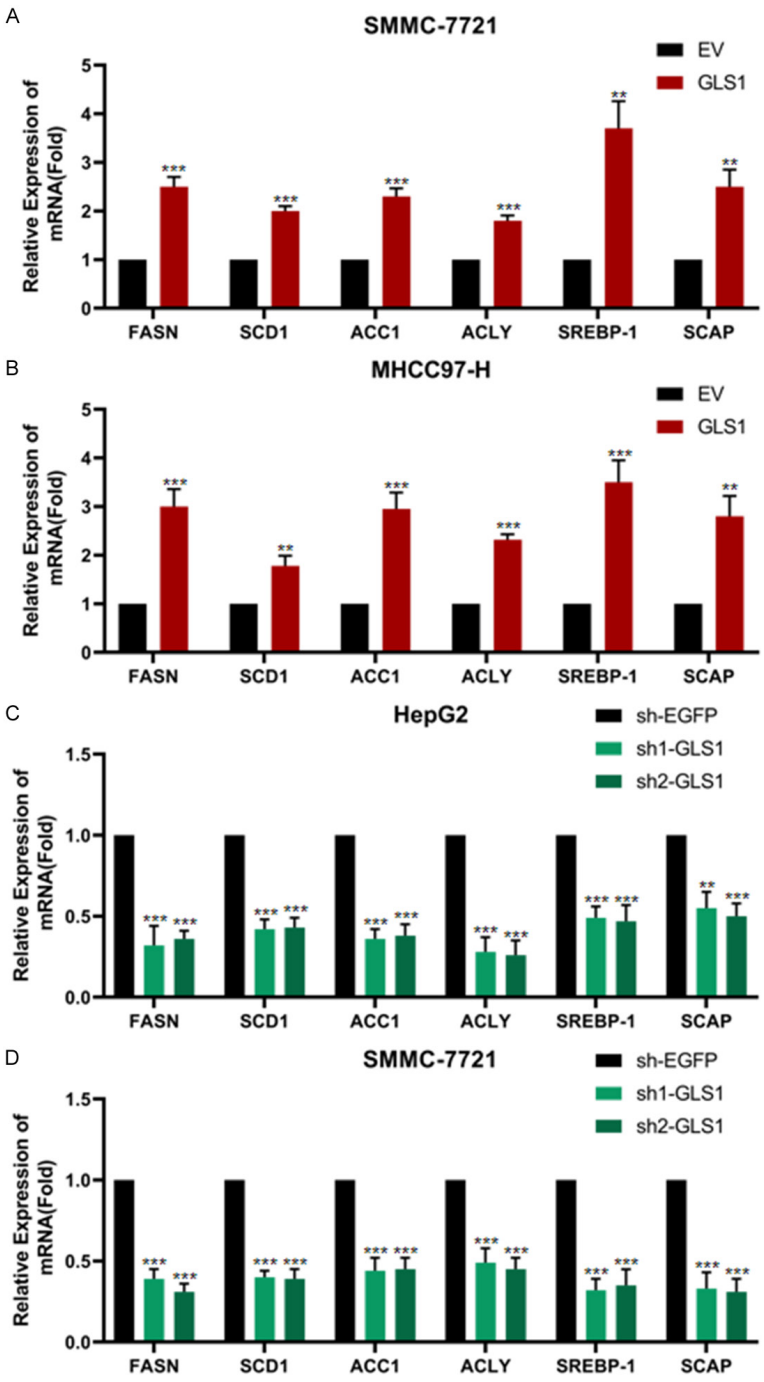

Figure S1. A, B. Relative mRNA levels of fatty acid synthase (*FASN*), stearoyl-CoA desaturase1 (*SCD1*), acetyl-CoA carboxylase 1(*ACC1*), ATP-citrate lyase (*ACLY*), *SREBP-1* and *SCAP* were determined in SMMC-7721 and MHCC97-H cells overexpressing *GLS1*. C, D. Relative mRNA levels of *FASN*, *SCD1, ACC1, ACLY*, *SREBP-1* and *SCAP* were determined in HepG2 and SMMC-7721 cells expressing sh-EGFP, sh1-*GLS1*, or sh2-*GLS1*. The data are shown as the mean ± s.d. of three independent experiments, ***P*<0.01, ****P*<0.001.

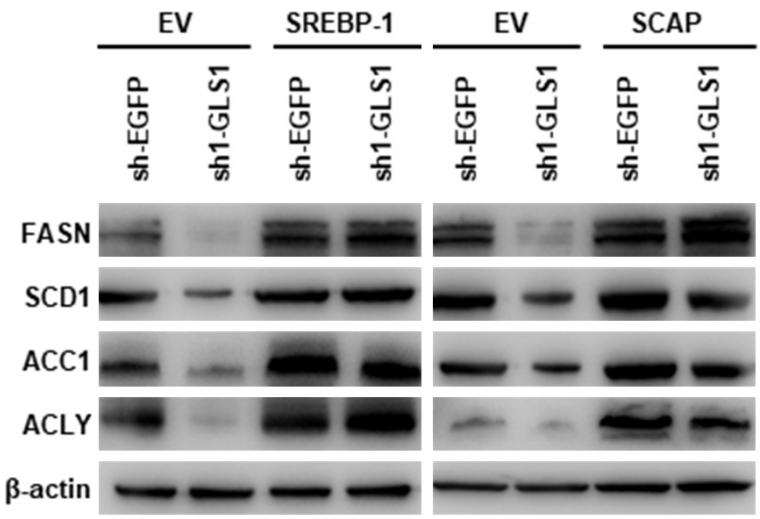

Figure S2. Protein levels of *GLS1* and metabolic enzymes in de novo fatty acid synthesis were determined after overexpressing *SREBP-1* or sh-*SCAP* in SMMC-7721 knocking down *GLS1* by Western blot. β-Actin served as loading control. Each experiment was examined in triplicate.