

Pressure suppresses hepatocellular glycogen synthesis through activating the p53/Pten pathway

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Abstract. Portal hypertension is the primary cause of complications in patients with chronic liver diseases, and markedly impacts metabolism within the nervous system. Until recently, the role of portal hypertension in hepatocellular metabolism was unclear. The present study demonstrated that an increase in extracellular pressure significantly decreased hepatocellular glycogen concentrations in HepG2 and HL-7702 cells. In addition, it reduced glycogen synthase activity, by inhibiting the phosphorylation of glycogen synthase 1. RNA-seq analysis revealed that mechanical pressure suppressed glycogen synthesis by activating the p53/phosphatase and tensin homolog pathway, further suppressing glycogen synthase activity. The present study revealed an association between mechanical pressure and hepatocellular glycogen metabolism, and identified the regulatory mechanism of glycogen synthesis under pressure.

Introduction

Numerous biochemical factors influence the extracellular microenvironment, including hormones and small molecules. Additionally, biophysical factors, including mechanical pressure, are notable components in the maintenance of the extracellular microenvironment; in particular, muscles, blood vessels, bone and dental tissues are under high degrees of pressure. The mechanical microenvironment serves a prominent regulatory role in cell proliferation, growth,

differentiation and metabolism, and it has been reported that external factors control mitotic spindle positioning to regulate cell proliferation (1). Notably, Lesman *et al* (2) demonstrated that contractile forces regulate cell division in three-dimensional environments. The effects of biomechanical factors are more profound on motor system-associated cells, including bone and muscle cells; moderate mechanical pressure regulates the proliferation and differentiation of osteoblasts (3). Furthermore, mechanical loading synergistically increases trabecular bone volume and improves the mechanical properties of mice (4). Fluid shear stress inhibits osteoblast apoptosis via the extracellular signal-regulated kinase 5 signaling pathway (5). It has also been reported that biophysical factors regulate the pluripotency of stem cells; specific pressures (300 and 600 Pa) accelerate the induction of pluripotency in defined three-dimensional microenvironments (6). Additional studies on the role of mechanical factors in cell function have focused on physiology (7,8). However, the number of studies on the role of mechanical factors in pathological conditions, such as hepatic portal hypertension, is limited.

Due to the absence of valves in the portal vein, retrograde blood flow cannot be prevented. When pre-hepatic, intrahepatic or post-hepatic obstruction occurs, retrograde flow is possible and may result in portal hypertension (9). Portal hypertension is the primary cause of complications in patients with chronic liver diseases (10). Such complications include esophageal and gastric varices, variceal bleeding, ascites, spontaneous bacterial peritonitis, splenomegaly and hepatic encephalopathy. Portal hypertension also has notable effects on metabolism (11-13); hepatic encephalopathy, a serious complication of portal hypertension, is a central nervous system dysfunction resulting from a metabolic disorder (14,15). Previous studies have revealed that portal hypertension inhibits the activity of cytochrome *c* oxidase in the nerve cells of the dentate gyrus and the basolateral, medial, lateral and central amygdala, thereby affecting the brain's memory function (14,16,17). Portal hypertension markedly influences metabolism within the nervous system; however, its effects on hepatocyte metabolism remain unclear.

Blood glucose equilibrium is one of the most notable metabolic balances of the human body, and the liver is the primary organ for carbohydrate metabolism and blood glucose maintenance. The liver maintains blood glucose levels by regulating

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glycogen synthesis and glycogenolysis (18). These processes are tightly controlled by various factors. In particular, insulin, a principle regulator of blood glucose, stimulates glycogen synthesis and inhibits glycogenolysis (19). Further studies have revealed that insulin promotes glycogen synthesis through binding of its receptor and activating the downstream protein kinase B/glycogen synthase kinase 3 β (Akt/GSK3 β) pathway (20). Numerous studies have been conducted on the regulation of glycogen synthesis in the liver (21-23). However, in pathological conditions, the mechanism underlying regulation of glycogen synthesis remains unidentified. In the present study, it was revealed that mechanical pressure inhibited hepatocellular glycogen synthesis, and the regulatory mechanism of glycogen synthesis under pressure was identified.

Materials and methods

Cell culture. The HL-7702 human hepatocyte cell line and HepG2 hepatoblastoma cell line were purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences, Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cells were cultured in high-glucose Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc) and 1% penicillin/streptomycin (Hyclone; GE Healthcare Life Sciences). Cells were maintained in a humidified atmosphere containing 5% CO₂ at 37°C in a cell incubator (Thermo Fisher Scientific, Inc.).

Pressure loading. The pressure-loading apparatus was set up as described previously, with some modifications (24,25), namely, the apparatus were composed of an air-tight steel chamber with inlet and outlet ports. To generate pressure, compressed helium gas was released into the chamber. The pressure was confirmed by a sphygmomanometer through a tube connected to the outlet. Cells used for experiments were placed in the chamber inside the incubator. After culturing at 37°C for 24 h, the cells were exposed to pressure between 0 and 15 mmHg for 24 h at 37°C for further experiments.

Glycogen analysis assay. For the Periodic Acid-Schiff (PAS) assay, the cells were seeded in 24-well plates at a density of 1x10⁴ cells/well, and were cultured for 24 h. The cells were exposed to different pressures and were stained using the Glycogen PAS Staining kit (cat. no. G1360; Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocol. The staining data were analyzed with Image-Pro Plus 6.0 software (Media Cybernetics, Inc.). The cell images were captured using a fluorescence microscope (Nikon ECLIPSE Ti; Nikon Corporation).

To further analyze glycogen concentration, cells were seeded into 24-well plates at a density of 1x10⁴ cells/well, cultured for 24 h and exposed to 0-15 mmHg pressure. The cells were washed with PBS and the glycogen content was determined using the Glycogen Content Detection kit (cat. no. BC0345; Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocol. Absorbance was determined at 620 nm using a SpectraMax M5 spectrophotometer (Thermo Fisher Scientific, Inc.).

Cell Counting kit (CCK)-8 assay. The cells were seeded in 96-well plates at a density of 1x10³ cells/well. Subsequently, the cells were exposed to different pressures for various durations and 10 μ l CCK-8 reagent (Dojindo Molecular Technologies, Inc.) was added to each well at 37°C for 1 h. Finally, absorbance was determined at 450 nm using a SpectraMax M5 spectrophotometer.

Apoptosis analysis. Cell apoptosis was detected using an Annexin V-FITC/propidium iodide (PI) cell apoptosis kit (cat. no. KGA108-1; KeyGen Biotech Co., Ltd.) according to the manufacturer's protocol. Briefly, cells reseeded at a density of 1x10⁵ cells/well in a 6-well plate were exposed to different pressures. Subsequently, the cells were washed with ice-cold PBS, collected in a 1.5 ml Eppendorf tube, and treated with Annexin V-FITC/PI for 10 min at room temperature according to the manufacturer's protocol. The cells were then analyzed using a flow cytometer (BD FACSVerse; BD Biosciences). Data were analyzed using FlowJo V10 Analysis software (FlowJo LLC).

Reverse transcription-quantitative PCR (RT-qPCR) and RNA-seq. Total RNA was isolated using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For each sample, 500 ng RNA was reverse transcribed to cDNA using a Prime-Script RT reagent kit (cat. no. RR037A; Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. The obtained cDNA was amplified using the Takara Ex Taq PCR kit (Takara Biotechnology Co., Ltd.) and qPCR amplification was conducted using the Stratagene Mx3000 QPCR system (Stratagene; Agilent Technologies, Inc.) and analyzed via the 2^{- $\Delta\Delta$ C_q} method (26). The thermocycling conditions were as follows: 95°C for 1 min, and 40 cycles of 95°C for 10 sec and 60°C for 30 sec. A melting curve was subsequently generated. The primer sequences used in these assays are shown in Table I.

For RNA-seq, 500 ng extracted total RNA from each sample was used for library construction. Libraries were constructed using Truseq RNA Sample Preparation kit V2 (Illumina, Inc; San Diego, USA) according to the manufacturer's protocol. Adapters with index sequences were attached to the libraries. After the average length of the libraries was confirmed, the concentration of each library was adjusted to 10 nM. Sequencing was performed for 100 bp using HiSeq2500 (Illumina, Inc.), with the single-read method. Mapping, data normalization and statistical analyses were performed using CLC Genomics Workbench (v. 8.5.1; Qiagen, Inc.). For volcano plotting, gene expression levels were averaged over the replicates for each cell line post-normalization and the cell lines were compared by calculating the fold change (FC). FC was defined as the ratio between the averages and P-values were determined for each gene expressed. In volcano plots, FC was plotted along the x-axis and P-value along the y-axis. Significantly altered genes were defined as those with thresholds of FC>2 and P<0.01. Gene set enrichment analysis (GSEA) was performed at www.broadinstitute.org/gsea.

Western blot analysis. Cultured cells were lysed in strong RIPA buffer (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.) containing Halt Protease Inhibitor

Table I. Primer sequences used in reverse transcription-quantitative PCR.

Gene	Sequence (5'-3')
Gys1-F	ACAACCTGGAGAACTTCAAC
Gys1-R	ATCTGGGACACAGTAGTGAA
Gsk3 β -F	CTCCTCATGCTCGGATTCA
Gsk3 β -R	TGCAGAAGCAGCATTATTGG
Agl-F	CATTTAATAGAGAAAATTC
Agl-R	CATGATTATCAGCACCAACACGT
G6pc3-F	GATGCCTAGCCTGGCTTATT
G6pc3-R	CAGGACAGCGCCAGTTATTA
p53-F	CAAACCCCTGGTTTAGCACTTC
p53-R	TGTCCTTCCTGGAGCGATCT
Pten-F	CCGAAAGGTTTTGCTACCATTCT
Pten-R	AAAATTATTTCCTTTCTGAGCATTCC
Akt-F	GCAGCACGTGTACGAGAAGA
Akt-R	GGTGTCACTCTCCGACGTG
Pygl-F	TGCCCGGCTACATGAATAACA
Pygl -R	TGTCATTGGGATAGAGGACCC
Gapdh-F	CCCATCACCATCTTCCAGGAGC
Gapdh-R	CAGTGAGCTTCCCGTTTCAGC

F, forward; R, reverse; Agl, amylo- α -1, 6-glucosidase, 4-alpha-glucanotransferase; Akt, protein kinase B; G6pc3, glucose-6-phosphatase catalytic subunit 3; Gsk3 β , glycogen synthase kinase 3 β ; Gys1, glycogen synthase 1; Pygl, glycogen phosphorylase L; Pten, phosphatase and tensin homolog.

Cocktails (cat. no. 78430; Thermo Fisher Scientific, Inc.). Protein concentrations were measured using a BCA protein assay kit (cat. no. 23235; Pierce; Thermo Fisher Scientific, Inc.). Subsequently, 50 μ g/lane of protein was separated via 10% SDS-PAGE and then transferred into a polyvinylidene difluoride membrane (cat. no. 03010040001; Sigma-Aldrich; Merck KGaA). The membranes were immersed in TBS-0.1% Tween 20 (cat. no. T8220-100ml; Beijing Solarbio Science & Technology Co., Ltd.) solution and blocked with 5% skimmed milk at room temperature for 1 h. Primary antibodies targeting glycogen synthase 1 (GYS1; 1:1,000; cat. no. ab40810; Abcam), phosphorylated (p)-GYS1 (S641; 1:1,000; cat. no. ab81230; Abcam), p53 (1:1,000; cat. no. ab26; Abcam), phosphatase and tensin homolog (Pten; 1:1,000; cat. no. ab32199; Abcam), caspase-3 (1:1,000; cat. no. ab13847; Abcam), Akt (1:1,000; cat. no. ab8805; Abcam), p-Akt (S473; 1:1,000; cat. no. ab81283; Abcam) and GAPDH (1:2,000; cat. no. sc-47724; Santa Cruz Biotechnology, Inc.) were incubated with the membranes overnight at 4°C, followed by incubation with the horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:3,000; cat. no. ab97051; Abcam) and goat anti-mouse IgG (1:3,000; cat. no. ab6789; Abcam) for 1 h at room temperature. Detection and analysis of HRP was performed using the Super Signal West Pico Chemiluminescent Substrate (cat. no. 34580; Pierce; Thermo Fisher Scientific, Inc.). Quantitative analysis was performed using Alpha View Analysis Tools (AlphaViewSA v.3.2.2 software; ProteinSimple).

Statistical analysis. Experimental results were analyzed using GraphPad Prism 5 software (GraphPad Software, Inc.). The data obtained from each group were compared with the control group. Independent samples t-tests were performed to compare the difference between two groups. The average of multiple groups was analyzed by one way analysis of variance followed by Dunnett's post hoc test. Data are presented as the mean \pm standard error of the mean of three independent experiments. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

An increase in pressure decreases hepatocellular glycogen concentration. To evaluate the effects of extracellular pressure on hepatocellular glycogen metabolism, PAS staining was used to analyze the intracellular glycogen concentration of HepG2 cells treated with 0, 5, 10 and 15 mmHg pressure for 24 h. A pressure of 10 mmHg significantly decreased the intracellular glycogen concentration of HepG2 cells compared with the control group (0 mmHg; Fig. 1A and C); however, the decrease in glycogen concentration between 10 and 15 mmHg was not substantial, indicating that the effect of pressure had reached saturation at 15 mmHg. A similar result was observed in the normal hepatic cell line HL-7702 (Fig. 1B and D), suggesting that an increase in extracellular pressure inhibited intracellular glycogen synthesis. To support this result, glycogen concentration was detected using the Glycogen Content Detection kit; when the pressure was increased, glycogen concentration gradually decreased in HepG2 and HL-7702 cells (Fig. 1E and F). The results indicated that specific pressure values decreased hepatocellular glycogen concentration, which may be associated with a decrease in glycogen synthase activity.

An increase in pressure does not affect cell viability and apoptosis. The present study detected the effects of pressure on cell viability and apoptosis. Previous data revealed that the glycogen content of hepatocytes was significantly reduced when pressure reached 10 mmHg, with no considerable difference compared with that at 15 mmHg. Therefore, 0 and 10 mmHg pressures were employed for further study. There was no significant alteration in HepG2 or HL-7702 cell viability between the 0 and 10 mmHg groups (Fig. 2A). Similarly, cell apoptosis was not affected by 10 mmHg pressure (Fig. 2B and C). In addition, the protein expression levels of active caspase-3, an apoptosis marker, were not affected by pressure (Fig. 2D). These results indicated that mechanical pressure did not affect cell viability and apoptosis.

An increase in pressure suppresses glycogen synthesis by inhibiting GYS1 phosphorylation. Numerous studies have indicated that glycogen synthesis is regulated by various genes, including Gys1, GSK3 β , glucose-6-phosphatase catalytic subunit 3, amylo- α -1, 6-glucosidase, 4-alpha-glucanotransferase and glycogen phosphorylase. Therefore the expression of these genes was detected in HepG2 and HL-7702 cells following treatment with different pressures. The expression levels of genes of interest were evaluated using RT-qPCR. Following treatment with 0 and 10 mmHg pressure for 24 h, the mRNA expression levels

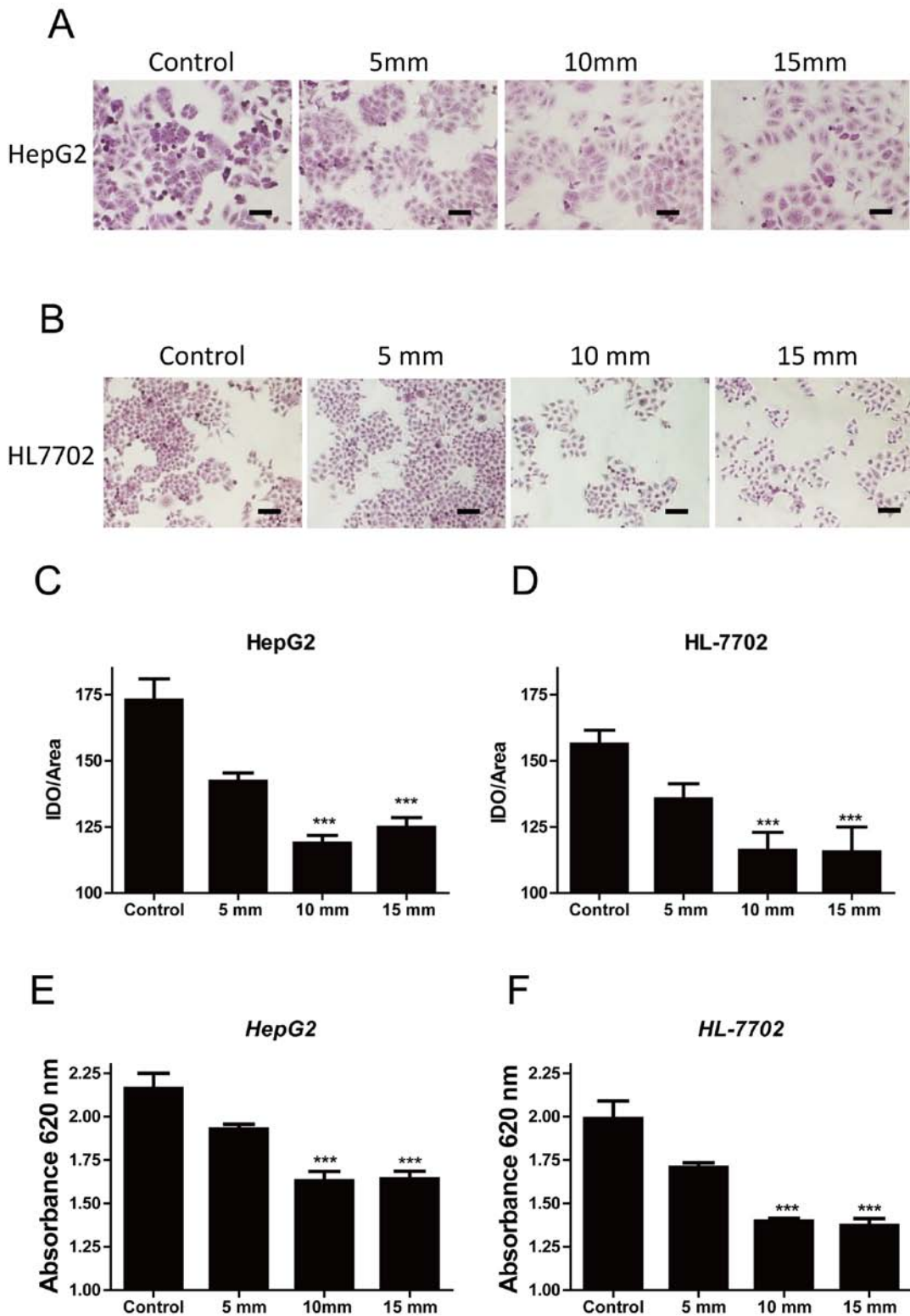


Figure 1. Increasing pressure decreases glycogen concentration in HepG2 and HL-7702 cells. Periodic acid-Schiff analysis of the glycogen concentration in (A) HepG2 and (B) HL-7702 cells treated with different pressures [Control (0), 5, 10 and 15 mmHg] (magnification, x100; scale bar, 60 μm). Quantification of glycogen staining in (C) HepG2 and (D) HL-7702 cells. Glycogen concentration analysis of (E) HepG2 and (F) HL-7702 cells treated with different pressures (0, 5, 10 and 15 mmHg). Data are presented as the mean ± standard error of the mean, n=3. ***P<0.001 vs. control. IDO, integrated option density; mm, mmHg.

remained unchanged (Fig. 3A and B). It has been reported that p-GYS1 (S641) is the activated isoform of the enzyme and is closely associated with glycogen synthesis. Therefore, the protein expression levels of GYS1 and p-GYS1 (S641) were detected using western blotting. The protein expression

levels of total GYS1 remained unchanged (Fig. 3C and D); however, p-GYS1 (S641) expression was significantly decreased under 10 mmHg pressure. These results suggested that mechanical pressure suppressed glycogen synthesis by inhibiting the phosphorylation of GYS1.

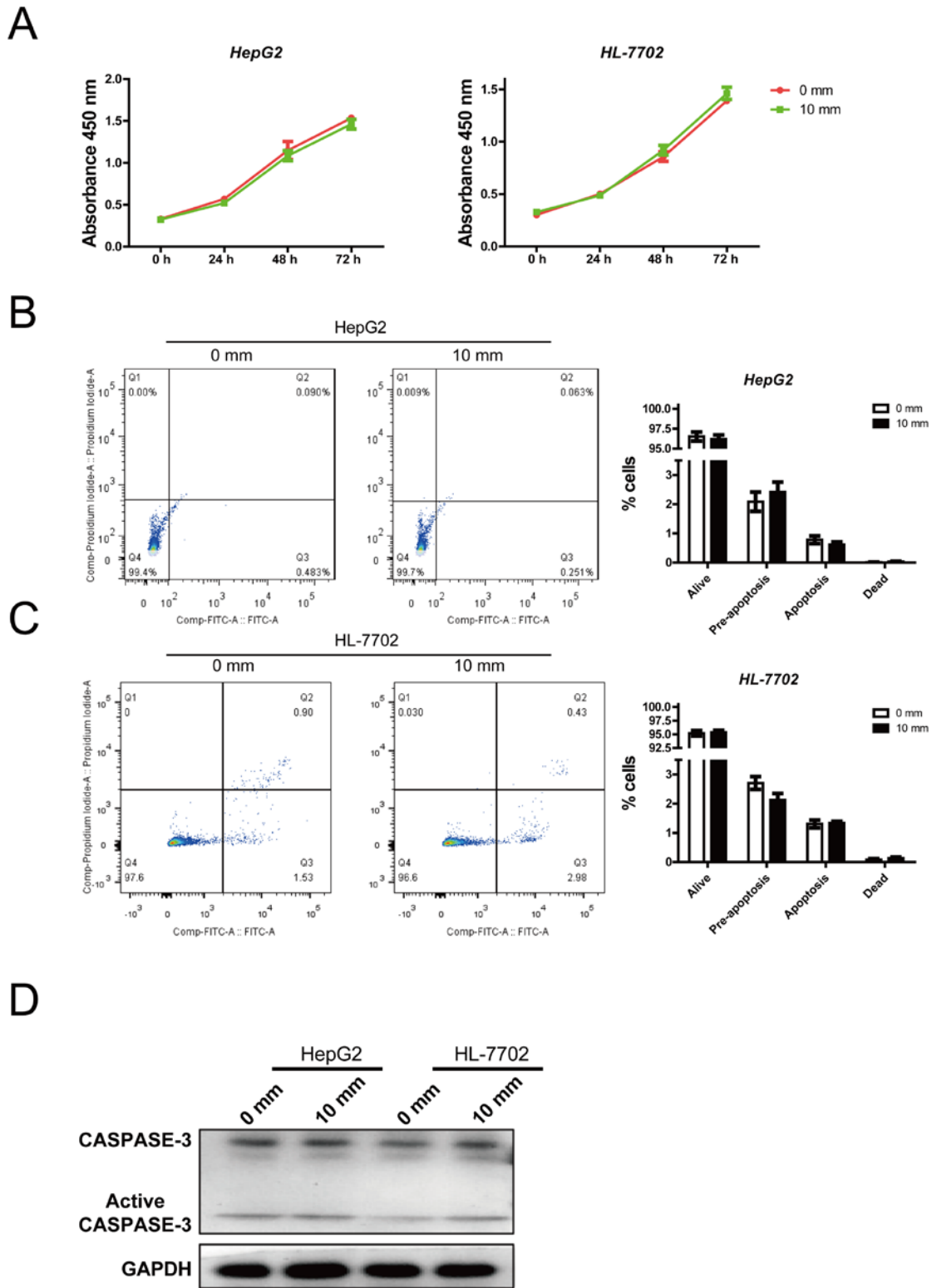


Figure 2. Increasing pressure does not affect cell viability and apoptosis. (A) Cell Counting Kit-8 analysis of the viability of HepG2 and HL-7702 cells treated with different pressures. Flow cytometric analysis of (B) HepG2 and (C) HL-7702 cells treated with different pressures (0 and 10 mmHg). (D) Western blot analysis of a protein related to apoptosis in HepG2 and HL-7702 cells treated with different pressures (0 and 10 mmHg). mm, mmHg.

An increase in pressure activates the p53/Pten pathway. To further investigate the mechanisms underlying the effects of pressure exertion on glycogen synthesis, genome-wide gene expression alterations in HepG2 cells treated with different pressures for 24 h were analyzed using an RNA-seq assay. The gene expression at 10 mmHg was different from that at

0 mmHg; of the 13,456 mapped genes in HepG2 cells, 2,253 were differentially expressed, including 949 downregulated and 1,304 upregulated genes (Fig. 4A). To identify the effects on cell signaling, the data were further analyzed with GSEA. The expression levels of genes associated with the regulation of glycogen metabolism were not significantly altered (Fig. 4B),

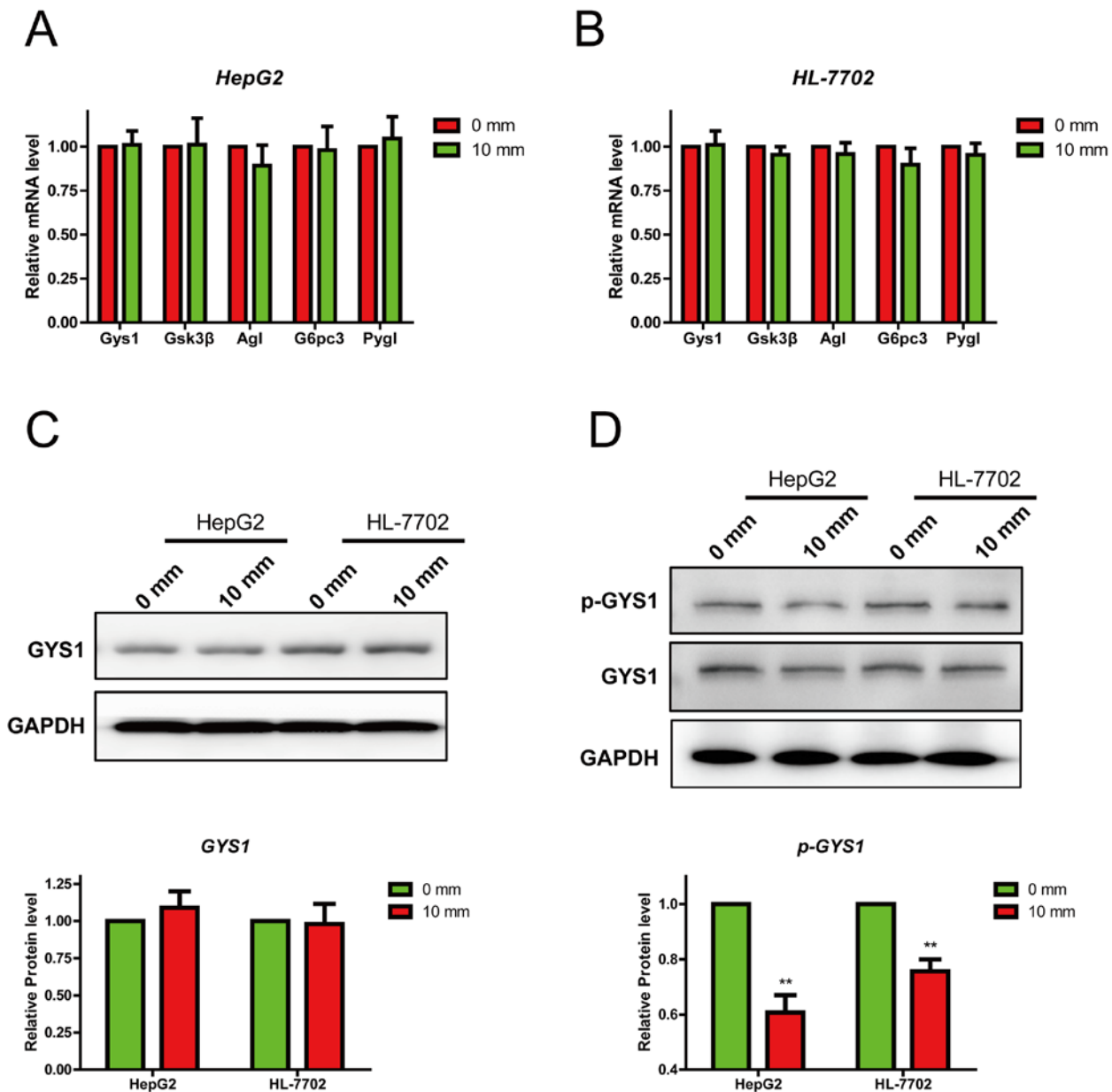


Figure 3. Increasing pressures suppress glycogen synthesis through inhibiting phosphorylation of GYS1. RT-qPCR analysis of the mRNA expression levels of genes related to glycogen synthesis and glycogenolysis in (A) HepG2 and (B) HL-7702 cells treated with different pressures (0 and 10 mmHg). (C) Western blot analysis of total GYS1 in HepG2 and HL-770 cells treated with different pressures (0 and 10 mmHg). GAPDH was used as a loading control. RT-qPCR data are displayed relative to controls as the means ± standard error of the mean (n=3). Data are presented as the mean ± standard error of the mean, n=3. **P<0.01 vs. control. Agl, amylo- α -1, 6-glucosidase, 4- α -glucanotransferase; G6pc3, glucose-6-phosphatase catalytic subunit 3; Gys1, glycogen synthase 1; Pygl, glycogen phosphorylase L; mm, mmHg; RT-qPCR, reverse transcription-quantitative PCR.

consistent with the aforementioned results (Fig. 3A and B). Notably, genes associated with the regulation of apoptosis were significantly upregulated (Fig. 4C). The expression of p53 and Pten were also markedly increased (Fig. 4D). These data indicated that increased pressure inhibited hepatocellular glycogen synthesis by activating the p53/Pten pathway.

An increase in pressure suppresses glycogen synthesis through activation of the p53/Pten pathway. It has been reported that Pten and p53 regulate glycogen synthesis by interacting with the Akt/GSK3 β signaling pathway (27). Therefore, the expression levels of p53, Pten and their

downstream gene Akt were detected in HepG2 and HL-7702 cells, which were previously incubated under 0 and 10 mmHg pressure for 24 h. When pressure was increased, the mRNA expression levels of p53 and Pten were also increased; however, Akt expression was not significantly altered (Fig. 5A and B). A similar effect was observed in the resultant protein expression levels (Fig. 5C and D). Notably, p53 regulates Akt activity by inhibiting its phosphorylation at Ser473; in this study, p-Akt was significantly reduced in response to 10 mmHg pressure (Fig. 5D). These data confirmed that pressure may influence glycogen synthesis by regulating the p53/Pten pathway.

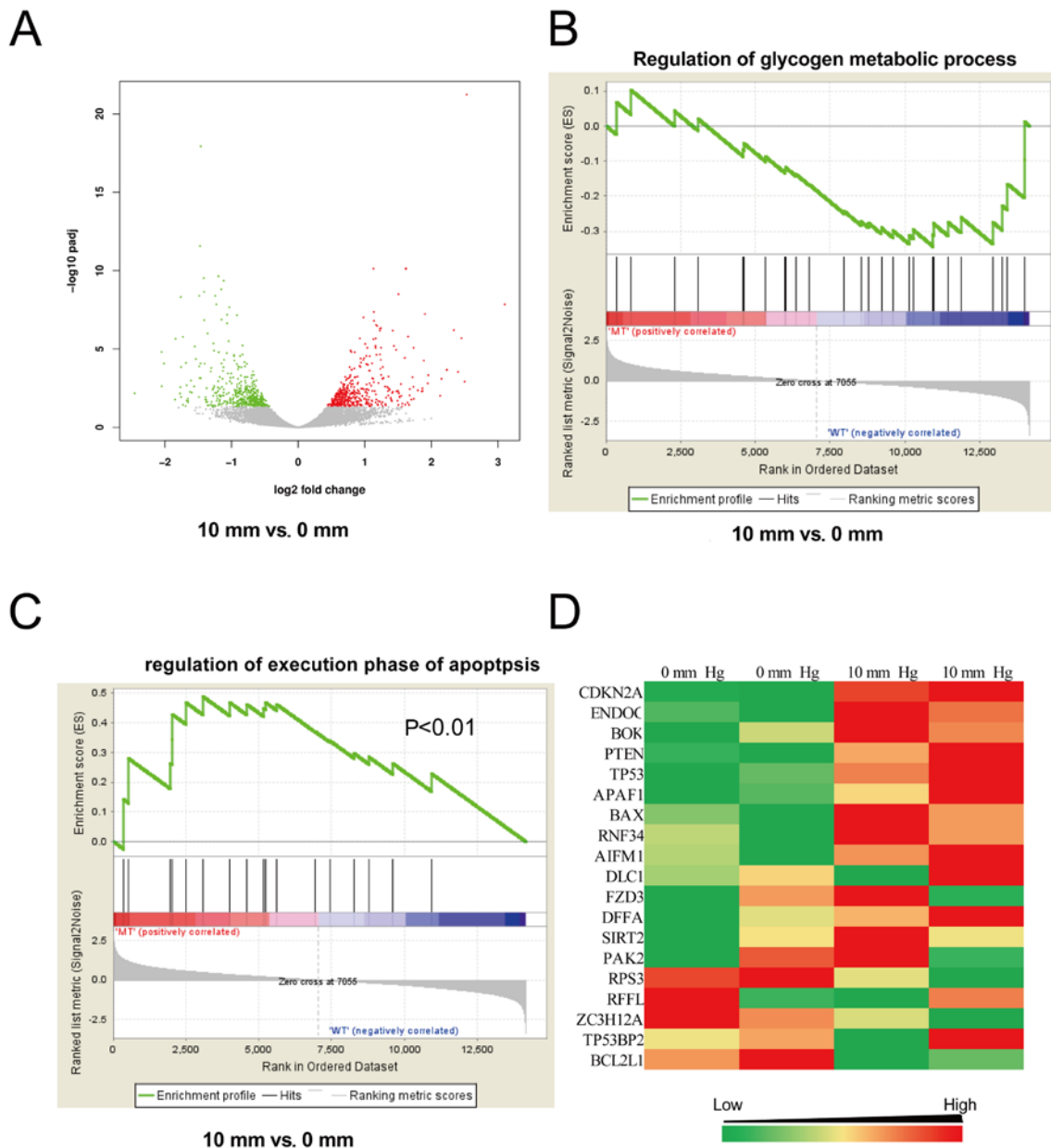


Figure 4. Increasing pressures activate the p53 pathway. (A) Volcano plot of gene expression analysis. Plots of log P-value versus log₂ fold change for HepG2 cells treated with different pressures (0 and 10 mmHg) are shown. The x-axis indicates fold change; the y-axis indicates the $-\log_{10} \text{padj}$. Red and green genes indicate significantly upregulated and downregulated genes, respectively. (B) GSEA of genes related to glycogen metabolic processes in HepG2 cells treated with different pressures (0 and 10 mmHg). (C) GSEA of genes related to the execution phase of apoptosis in HepG2 cells treated with different pressures (0 and 10 mmHg). (D) Heat map analysis of the genes related to the execution phase of apoptosis in HepG2 cells treated with different pressures (0 and 10 mmHg). mm, mmHg; GSEA, gene set enrichment analysis; padj, adjusted P-value.

Discussion

The regulation of hepatocellular glycogen synthesis and glycolysis is one of the principle methods in the maintenance of blood glucose concentration. The effects of portal hypertension on hepatocellular glycogen metabolism remain unclear. The present study demonstrated that an increase in extracellular pressure significantly inhibited hepatocellular glycogen synthesis and GYS1 activity. Further experiments demonstrated that these effects were closely associated with the p53/Pten pathway. This pathway inhibits Akt signaling and glycogen synthesis (Fig. 5E). Therefore, the present study revealed an association between mechanical pressure and hepatocyte metabolism.

Mechanical factors, including pressure, influence the extracellular microenvironment. It has been reported that such factors regulate cellular proliferation and differentiation. Numerous studies have demonstrated that mechanical force, such as pressure and stretch, is an important regulator in cell metabolism (28-30). The present study suggested that portal hypertension significantly inhibited the synthesis of hepatocellular glycogen. It has been reported that the right atrial pressure of patients with portal hypertension has an interquartile range of 12.0-15.5 mmHg (11,31). The gradient pressures detected in this study were <15.5 mmHg; therefore, it was hypothesized that this study could partially reflect the pressure environment *in vivo*. Additionally, it

The liver is the primary organ involved in the regulation of blood glucose, and glycogen synthesis and glycogenolysis are key regulatory processes. Previous studies have demonstrated that glycogen synthesis is regulated by chemical signals, including hormones and small molecules (35,36). In the present study, it was established that pressure served an important regulatory role in glycogen synthesis. Furthermore, previous studies have confirmed that glycogen synthesis is tightly regulated by intracellular and extracellular microenvironmental signals (37,38). The Akt/GSK3 β pathway is a critical component of hepatocellular glycogen synthesis; notably, insulin promotes the synthesis of glycogen by activating this pathway (37). The present study revealed that extracellular pressure inhibited this pathway, and regulated the expression of associated genes. In addition to glycogen synthesis and glycogenolysis, the liver also facilitates gluconeogenesis and lipid metabolism, and previous studies have demonstrated that the Akt1/GSK3 β pathway is also critically involved in gluconeogenesis and lipid metabolism (39,40). These data suggested that portal hypertension may also serve a principal role in gluconeogenesis and lipid metabolism.

The p53 pathway is frequently activated in response to external stimuli, and chemotherapy-induced apoptosis is caused by p53 activation (41). The present study revealed that extracellular pressure induced upregulation of p53 mRNA expression, inducing the expression of its downstream gene, Pten. These results suggested that activation of the p53 pathway may be one of the ways in which cells respond to external stimuli. The p53 pathway regulates the metabolism of cellular glucose and lipids (42,43); these studies further demonstrated that when cells are exposed to external stimuli at a low-intensity, p53 may be upregulated, and subsequently inhibit glucose metabolism. Therefore, the p53 pathway may serve a prominent role in the metabolic response to external stimuli.

Despite these promising results, several questions remain unanswered. Firstly, it is intriguing that the glycogen concentration in the 15 mmHg pressure group was not considerably decreased compared with in the 10 mmHg group. The reason for this could be that 15 mmHg pressure may reduce glycogen concentration compared to 10 mmHg; however, the difference may be too small and therefore could not be detected. Secondly, this study indicated that p53 is a critical gene that functions as a sensor that may respond to mechanical pressure. However, further work is required to reveal the direct relationship between pressure and p53. In addition, this study demonstrated the effects of pressure on HepG2 and HL-7702 cell lines; further research should be undertaken to reveal the physiological role of pressure in primary hepatocytes. Genes associated with the regulation of apoptosis were significantly upregulated; however, cell apoptosis was not affected by mechanical pressure treatment for 24 h. One reason for this may be that the alterations in the expression of apoptosis-associated genes are insufficient to influence cell apoptosis in a short period of time. Finally, the results revealed that pressure did not affect cell viability and apoptosis *in vitro*. A further study focusing on the effects of pressure on cell viability and apoptosis, as well as glycogen metabolism, *in vivo* is therefore suggested.

To the best of our knowledge, the present study is the first to demonstrate that extracellular pressure significantly inhibited hepatocellular glycogen synthesis. It was further

revealed that the p53 pathway was involved in the regulation of hepatocellular glycogen synthesis. These results not only suggested a regulatory effect for mechanical pressure in hepatocellular carbohydrate metabolism, but also implied its role in hepatocellular lipid. Given the current challenges of portal hypertension treatment, the present study, and the associated questions raised, demonstrated the requirement for further investigation into the role of pressure in hepatocytes, and the potential associated molecular mechanisms.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LZ and JC were involved in study conception and design, manuscript revision, funding support and study supervision. JS performed the experiments, data analysis and manuscript writing. YS performed data analysis and manuscript writing. SS and XL performed statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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