

# SERP1 reduces inchoate acute hepatic injury through regulation of endoplasmic reticulum stress via the GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling pathway

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Received August 24, 2021; Accepted December 10, 2021

DOI: 10.3892/mmr.2022.12709

**Abstract.** The liver is a crucial digestive organ of humans and in charge of detoxification. Acute hepatic injury is an aggressive type of hepatic disease and its harmful effect cannot be ignored. The present study examined the role and mechanism of stress-associated endoplasmic reticulum protein 1 (SERP1) in acute hepatic injury. Mice were injected intraperitoneally with D-galactosamine/lipopolysaccharide (LPS) and rat hepatocytes were induced by LPS to establish an acute hepatic injury model. Tissue lesions were observed by H&E staining, and biomarkers of hepatic injury in the serum were examined. Western blotting, immunohistochemistry and reverse transcription-quantitative PCR were performed to assess SERP1 expression in tissues and hepatocytes. A SERP1 overexpression plasmid was constructed to evaluate the role of SERP1 in inflammation, apoptosis, endoplasmic reticulum stress (ERS) and the GSK3 $\beta$ / $\beta$ -catenin/T-cell factor (TCF)/lymphoid enhancing factor (LEF) signaling pathway. In addition, a GSK3 $\beta$  overexpression plasmid was constructed to investigate the role of GSK3 $\beta$ / $\beta$ -catenin signal activation. Additionally, the present study investigated whether SERP1 regulated the endoplasmic reticulum via this pathway. In the present study, reliable animal and cellular hepatic injury models were established and verified. SERP1 overexpression reduced the expression of inflammatory factors, apoptosis-related proteins and ERS-related proteins, as well as the expression of proteins related to GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling pathways. A GSK3 $\beta$  overexpression plasmid was constructed and it was revealed that GSK3 $\beta$  overexpression could reverse the effects of SERP1 overexpression in

forementioned aspects. This suggested that the activation of the GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling pathway may be required for the regulation of SERP1. In conclusion, SERP1 regulated ERS via the GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling pathway, thereby reducing inchoate acute hepatic injury.

## Introduction

The liver is a crucial digestive organ of humans and in charge of detoxification. Acute hepatic injury is an aggressive type of hepatic disease (1), and its harmful effect cannot be ignored. Acute hepatic injury refers to acute damage or necrosis of hepatocytes (2), causing abnormal hepatic function. Patients with acute hepatic injury exhibit a series of symptoms of acute hepatitis, such as yellowing of the skin and sclera, loss of appetite, and pain in the hepatic area. Generally, drug-induced hepatic injury is the most widespread cause (3,4). In addition, alcohol (5), hepatitis B (6) and appendicitis are also principal factors. Endoplasmic reticulum stress (ERS) is a signal response pathway of the cellular self-protection mechanism, which participates in the physiological and pathological processes of numerous diseases (7-11). Without exception, ERS is also involved in the occurrence and development of a variety of hepatic injuries (12). By studying the effect of drugs on hepatic injury in mice, the protective effect of drugs against ERS-induced acute hepatic injury has been revealed (13). Through the establishment of alcoholic hepatic injury in mice, it has been demonstrated that alcohol may induce ERS and cause hepatic damage (14). The aforementioned mechanisms suggest that hepatic damage is relevant to ERS.

Stress-associated endoplasmic reticulum protein 1 (SERP1) is involved in the regulation of ERS (15). SERP1 stimulates the generation of  $\beta$ -amyloid in cells which undergo ERS, as is observed in diabetes (16). Additionally, increased ERS in the pancreas and other organs of mice lacking SERP1 indicated that SERP1 can alleviate ERS (17). The levels of ERS increases during early acute hepatic injury, and it can activate GSK3 $\beta$  signaling (18,19). GSK3 $\beta$ , a serine/threonine kinase, was originally reported to be a key enzyme in the process of glycogen synthesis. It acts on numerous signal proteins and transcription factors, and regulates cell survival, differentiation, proliferation and apoptosis. Furthermore, using a D-galactosamine

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**Key words:** hepatic injury, stress-associated endoplasmic reticulum protein 1, endoplasmic reticulum stress, GSK3 $\beta$ , D-galactosamine/lipopolysaccharide

(D-GalN)/lipopolysaccharide (LPS)-induced acute hepatic failure mouse model, it has been revealed that ERS is activated in these mice. Inhibition of activation may have a protective effect on hepatic injury, and the mechanism of action involves reducing inflammation, expression of apoptosis factors and signal transduction (20).

In the present study, D-GalN/LPS induction was used to construct a model of early acute hepatic injury. Furthermore, a series of assays was performed to explore whether the overexpression of SERP1 could reduce ERS by inhibiting the activity of GSK3 $\beta$  signaling, thereby reducing hepatocyte apoptosis.

## Materials and methods

**Cell culture and mouse model establishment.** Normal rat hepatocytes (BRL) were purchased from the American Type Culture Collection. Cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin/streptomycin (Thermo Fisher Scientific, Inc.) and maintained in an incubator with 5% CO<sub>2</sub> at 37°C. LPS (1 mg/l) was used to induce the formation of damaged hepatocytes. Sodium phenylbutyrate (4-PBA; Sigma-Aldrich), an ERS inhibitor, was dissolved using sterile PBS.

The present study was performed following the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Huaihe Hospital of Henan University Animal Ethics Committee (approval no. HHIACUC-201956; Kaifeng, China). A total of 16 male wild-type (C57BL/6) mice (age, 6-8 weeks; weight, 20-22 g; Guangdong Medical Laboratory Animal Center) were used in the present study. Half of the mice were injected intraperitoneally with D-GalN (400 mg/kg) and LPS (0.02 mg/kg; Sigma-Aldrich). The control mice were injected with the same amount of normal saline. Throughout the experiment, mice were kept at a room temperature of 25°C with 12 h light/12 h dark cycle and a relative humidity of 55%. They had free access to water and food. For blood collection, the mice were anesthetized using sodium pentobarbital (80 mg/kg) and the abdominal cavity was then opened through an incision along the midline of the abdomen to expose the abdominal aorta. A total of 0.6 ml of blood was collected using a syringe. Subsequently, the mice were euthanized by cervical dislocation. All efforts were made to minimize animal suffering.

**Cell transfection.** BRL cells (5x10<sup>5</sup> cells/well) were seeded into 6-well plates until they reached 70-80% confluence. The coding sequences of SERP1 (Gene ID: 80881, Accession Number: NM\_030835.2) and GSK3 $\beta$  (Gene ID: 84027, NM\_032080.1) were cloned into pcDNA3.1 (Hunan YouBio Co., Ltd.). pcDNA3.1-SERP1, pcDNA3.1-GSK3 $\beta$  and empty vector as a negative control (NC) were transfected into BRL cells at 37°C for 6 h using Lipofectamine<sup>®</sup> 3000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Cells were induced using LPS as requested (1 mg/l) after 48 h of transfection.

**H&E staining.** The hepatic tissue was obtained after lavage, fixed in 4% paraformaldehyde at room temperature overnight, dehydrated and embedded in paraffin. Subsequently, it was

cut into 4  $\mu$ m sections and stained with H&E for 10 min at room temperature. Following color separation, rinsing and dehydration, the tissue was pressed flat and fixed with a new cover glass. After drying, physiological changes of the tissue were observed under a light microscope (magnification, x200; Olympus Corporation).

**Immunohistochemistry.** Tissues were pretreated as aforementioned for H&E staining. Subsequently, paraffin sections were washed with xylene, rehydrated with descending alcohol and antigen retrieval was performed at 95°C for 20 min. Then, 5% goat serum (Beijing Solarbio Technology Co. Ltd.) was used for blocking for 10 min at room temperature and sections were then incubated with the SERP1 primary antibody (1:200; cat. no. DF9873; Affinity Biosciences) at 4°C overnight. After washing with PBS, biotin-labeled goat anti-rabbit secondary antibody (1:2,000; cat. no. 31820; Thermo Fisher Scientific, Inc.) was added. Streptavidin Biotin-peroxidase Complex (Beyotime Institute of Biotechnology) was diluted and added after incubation at 37°C for 0.5 h. Subsequently, the sections were developed using 3,3'-diaminobenzidine staining solution, counterstained with hematoxylin for 25 sec at room temperature, dehydrated and mounted. The differences were observed under a light microscope (magnification, x400; Olympus Corporation).

**Detection of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).** The mouse abdominal aorta blood was drawn and the upper serum was separated by centrifugation (1,000 x g) for 10 min at 4°C. The sample was added to the well and mixed with the matrix solution at 37°C, and the color developing agent was added. The stop solution was added after 20 min in a 37°C water bath. After 15 min at room temperature, the microplate reader was used to detect the optical density (OD) value at 510 nm. ALT and AST levels were calculated using the standard curve.

**Western blotting.** Protein was extracted from BRL cells and homogenized in RIPA lysis buffer (Beyotime Institute of Biotechnology). A BCA protein assay kit (Beyotime Institute of Biotechnology) was used for protein quantification. The proteins (20  $\mu$ g/lane), which were separated using an 10% SDS-polyacrylamide gel, were transferred to a PVDF membrane. The membranes were first incubated in 5% skimmed milk for 1 h at room temperature. Blots were incubated with primary antibodies [glucose-regulated protein 78 (GRP78; product code ab108615; 1:1,000), glucose-regulated protein 94 (GRP94; product code ab238126; 1:1,000), SERP1 (product code ab254839; 1:1,000), NLR family pyrin domain containing 3 (NLRP3; product code ab263899; 1:1,000), apoptosis-associated speck-like protein containing a CARD (ASC; product code ab180799; 1:1,000), GSK3 $\beta$  (cat. no. ab227208; 1:1,000), phosphorylated (p-)GSK3 $\beta$  (product code ab75745; 1:1,000),  $\beta$ -catenin (product code ab32572; 1:5,000), Bcl-2 (product code ab196495; 1:1,000), Bad (product code ab32445; 1:1,000), Bax (product code ab182733; 1:2,000), all from Abcam; cleaved-caspase3 (cat. no. AC033; 1:1,000) and caspase3 (cat. no. AF1213; 1:1,000), caspase-1 (cat. no. AF1681; 1:1,000), CHOP (AF6684; 1:1,000) and GAPDH (AF1186; 1:2,000) from Beyotime Institute of Biotechnology] at 4°C

overnight after washing with Tris-buffered saline with 0.01% Tween-20. Subsequently, they were washed again, and strips were incubated with HRP-conjugated anti-rabbit antibody (cat. no. 31460; 1:50,000; Thermo Fisher Scientific, Inc.) at room temperature for 1.5 h. ECL (Shanghai Yeasen Technology Co. Ltd.) was added. GAPDH was used as a control and Image Lab software (v4.0; Bio-Rad Laboratories, Inc.) was used to analyze data.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was isolated from BRL cells using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and cDNA was primed using a Reverse Transcriptase kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RT-qPCR was performed using a QuantiTect SYBR Green PCR Kit (Qiagen, Inc.). The conditions of PCR amplification were as follows: 95°C for 3 min, followed by 40 cycles at 95°C for 10 sec and 60°C for 30 sec. The  $2^{-\Delta\Delta C_q}$  method (21) was applied for data analysis with normalization to GAPDH. The sequences of the primers were as follows: SERP1 forward, 5'-CGGGCGGAGTCCATACTTC-3' and reverse, 5'CCGGGAGGAAAGAGTCCAAC-3'; GSK3 $\beta$  forward, 5'-CACTGTGTA GCCGTCTCCTG-3' and reverse, 5'-GAAGAGGGCAGG TGTGTCTC-3'; and GAPDH forward, 5'-TCTCTGCTCCTC CCTGTTCT-3' and reverse, 5'-TACGGCCAAATCCGTTCA CA-3'.

**ELISA.** Expression levels of inflammatory factors: TNF- $\alpha$  (product no. PT516; Beyotime Institute of Biotechnology), IL-18 (cat. no. E-EL-R0567c; Wuhan Elabscience Co. Ltd.), IL-6 (product no. PI328; Beyotime Institute of Biotechnology) and IL-1 $\beta$  (product no. PI303; Beyotime Institute of Biotechnology) in BRL cells were detected using ELISA kits according to the manufacturer's protocol. A microplate reader was used to detect the OD value at 450 nm.

**TUNEL assay.** Cells (1x10<sup>4</sup>/well) were seeded in a 24-well plate and cultured to occupy 80% of the well in an incubator with 5% CO<sub>2</sub> at 37°C. Subsequently, cells were fixed with 4% paraformaldehyde for 1 h at 4°C, stained using the TUNEL test kit for 1 h at 37°C (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. DAPI (2  $\mu$ g/ml) was used for nuclear staining for 5 min at room temperature. Antifade mounting medium (Beyotime Institute of Biotechnology) was added on the slide and cells were observed from five field of view under a fluorescence microscope (magnification, x200; Olympus Corporation).

**Luciferase assay.** BRL cells (5x10<sup>5</sup>/well) were seeded into 6-well plates and co-transfected with 1  $\mu$ g reporter gene TOP Flash and FOP Flash (Beyotime Institute of Biotechnology), and overexpression vector. After transfection with Lipofectamine<sup>®</sup> 3000 reagent (Thermo Fisher Scientific, Inc.) for 18 h, cells were lysed in passive phenylbenzothiazole buffer and sample lysates were measured using a Mithras LB 940 Multimode Microplate Reader (Berthold Technologies GmbH & Co. KG). The activity was measured using a dual-luciferase reporter gene assay kit (Beyotime Institute of Biotechnology) and normalized to Renilla luciferase. The results are presented as a normalized TOP Flash/FOP Flash value.

**Statistical analysis.** Data are presented as the mean  $\pm$  SD and were analyzed using GraphPad Prism 8.0 software (GraphPad Software, Inc.). Statistical significance was determined by one-way ANOVA with Tukey's post hoc test for multiple groups and unpaired Student's t-test for two groups. Each cell experiment was performed at least three times. P<0.05 was considered to indicate a statistically significant difference.

## Results

**SERP1 expression in acute hepatic injury tissues and cells.** The mice were injected intraperitoneally with D-GalN/LPS. H&E staining was performed to examine pathological changes of the tissues of mice in the model group. The hepatic structure of the control group was normal and the structure of the LPS/D-GalN group exhibited a mass of vacuoles. The hepatic cells were disintegrated, with diffuse necrosis, and normal structure was lost, accompanied by inflammatory cell infiltration (Fig. 1A). ALT and AST are both biomarkers of acute hepatic injury in the serum (22). The value of the LPS/D-GalN group was significantly increased compared with that of the control group (Fig. 1B). The expression levels of ERS-related proteins were determined using western blotting. The expression levels of GRP78, GRP94 and CHOP were all significantly increased (Fig. 1C).

The expression levels of SERP1 in acute hepatic injury tissues were subsequently assessed using immunohistochemistry and western blotting. Compared with that of the control, the brownish yellow color of the cytoplasm in the LPS/D-GalN group was less intense (Fig. 1D). The SERP1 expression in the LPS/D-GalN group was significantly decreased according to the results of western blotting (Fig. 1E). In addition, SERP1 expression in acute hepatic injury cells was determined by western blotting and RT-qPCR. In these two assays, the expression levels in the induced group were significantly decreased (Fig. 1F).

**Effect of SERP1 overexpression on the expression of inflammatory factors and cell apoptosis.** The overexpression plasmid of SERP1 was constructed, and overexpression was confirmed using RT-qPCR and western blotting. SERP1 expression in the overexpression-SERP1 (OE-SERP1) group was higher than that in the NC group (Fig. 2A and B). The expression levels of inflammatory factors (TNF- $\alpha$ , IL-18, IL-6 and IL-1 $\beta$ ) were subsequently determined by ELISAs. It was observed from the histograms that the levels of inflammatory factors of hepatocytes in the LPS-induced group were increased; however, when SERP1 was overexpressed, the levels of inflammatory factors were significantly decreased. The effect of its overexpression on inflammatory factors was similar to the effect of 4-PBA, which is an ERS inhibitor (Fig. 2C). In addition, NLRP3 inflammasome (NLRP3, ASC and caspase-1) expression was determined by western blotting. The expression levels were increased when cells were induced by LPS, and SERP1 overexpression contributed to the decreased inflammasome expression. Although the inhibitory effect on inflammasome expression was not as distinct as that of 4-PBA, it was still notable (Fig. 2D).

A TUNEL assay was performed to assess cell apoptosis. Normal cells will not be stained because there is no DNA break. It was observed from the images that the cytoplasm of

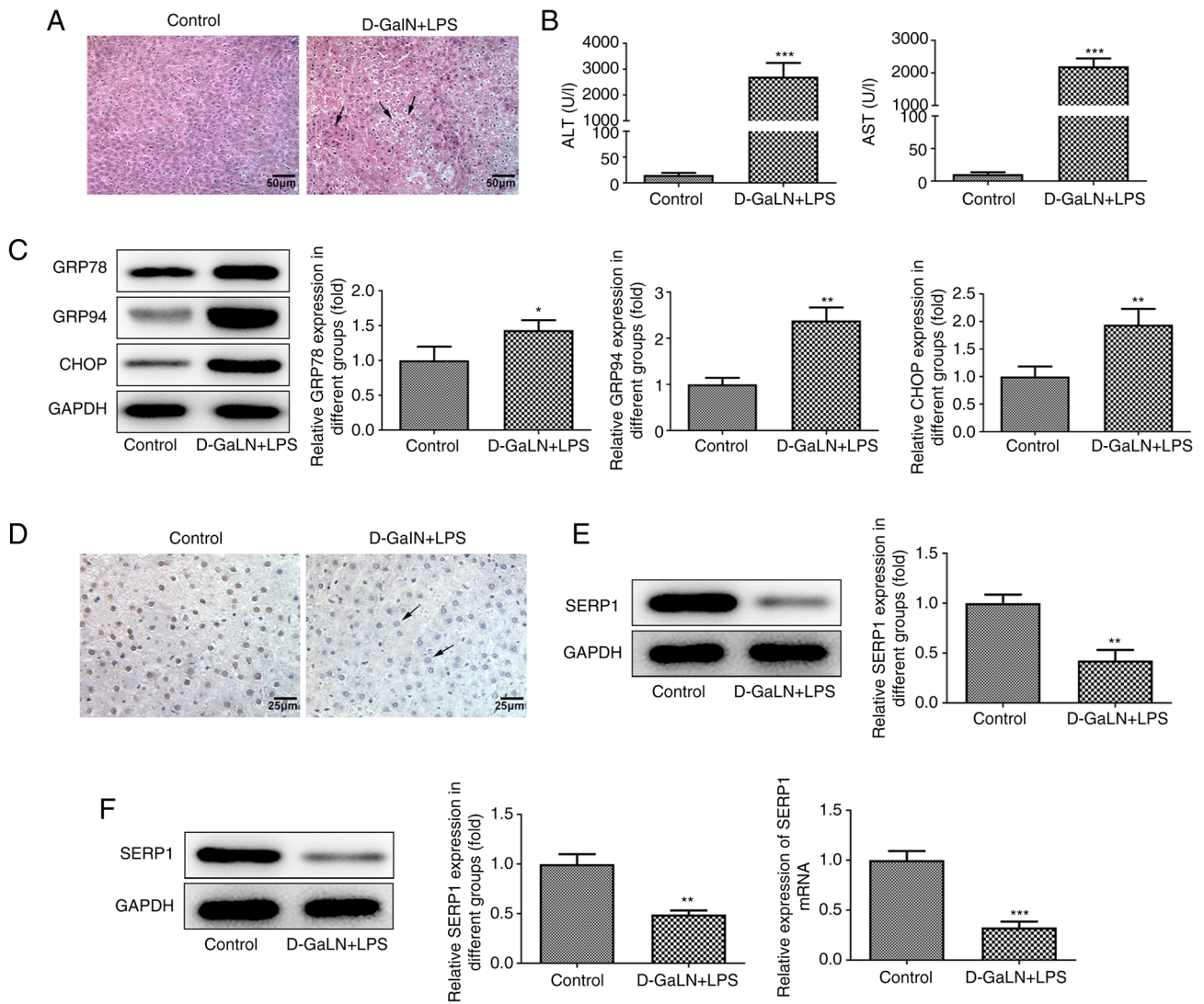


Figure 1. Establishment of an inchoate acute hepatic injury model and detection of ERS-related proteins. (A) Pathological changes of tissues of mice in the model group detected by H&E staining. (B) Levels of alanine aminotransferase and aspartate aminotransferase in the serum. (C) ERS-related protein expression detected by western blotting. SERP1 expression in acute hepatic injury tissues detected by (D) immunohistochemistry and (E) western blotting. (F) SERP1 expression in acute hepatic injury cells detected by western blotting and reverse transcription-quantitative PCR. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. the control;  $n \geq 3$ . ERS, endoplasmic reticulum stress; SERP1, stress-associated endoplasmic reticulum protein 1; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LPS, lipopolysaccharide; D-GalN, D-galactosamine; GRP78, glucose-regulated protein 78; GRP94, glucose-regulated protein 94.

LPS-induced cells was stained, but not that of the control group. Reduced staining was observed following SERP1 overexpression, which was consistent with the results observed for the 4-PBA group (Fig. 3A). The expression levels of apoptosis-related proteins were detected by western blotting. The expression levels of Bad, Bax and cleaved caspase-3 were increased when cells were induced by LPS and decreased when SERP1 was overexpressed. The opposite trend was observed for Bcl-2 (Fig. 3B).

**Effect of SERP1 overexpression on the expression levels of ERS-related proteins and GSK3 $\beta$ / $\beta$ -catenin/T-cell factor (TCF)/lymphoid enhancing factor (LEF) signaling in hepatocytes.** Western blotting was performed to assess the expression levels of GRP78, GRP94 and CHOP in hepatocytes. The expression levels were all increased when cells were induced by LPS and decreased following SERP1 overexpression or addition of 4-PBA (Fig. 4A). This suggested that SERP1 overexpression alleviated ERS caused by LPS. To investigate the effect

of SERP1 overexpression on signaling, a TOP Flash/FOP Flash fluorescent gene reporter assay was used to detect TCF/LEF activity. TOP Flash/FOP Flash luciferase activity ratios in the control, LPS, LPS with NC, LPS with OE-SERP1 and LPS with 4-PBA groups were investigated. FOP Flash was used to correct for  $\beta$ -catenin-independent expression and transfection efficiency. In the LPS combined with OE-SERP1 group, the TOP Flash/FOP Flash luciferase activity ratio was decreased compared with that of the LPS + NC group (Fig. 4B). Additionally, GSK3 $\beta$ / $\beta$ -catenin expression was detected by western blotting. The expression levels of  $\beta$ -catenin and GSK3 $\beta$  in the LPS group were increased, and this was reversed by SERP1 overexpression. Conversely, the levels of p-GSK3 $\beta$  in different groups revealed the opposite trend (Fig. 4C).

**GSK3 $\beta$ / $\beta$ -catenin signaling activation reverses the effect of SERP1 overexpression on ERS and cell apoptosis.** The GSK3 $\beta$  overexpression plasmid was constructed and transfected into

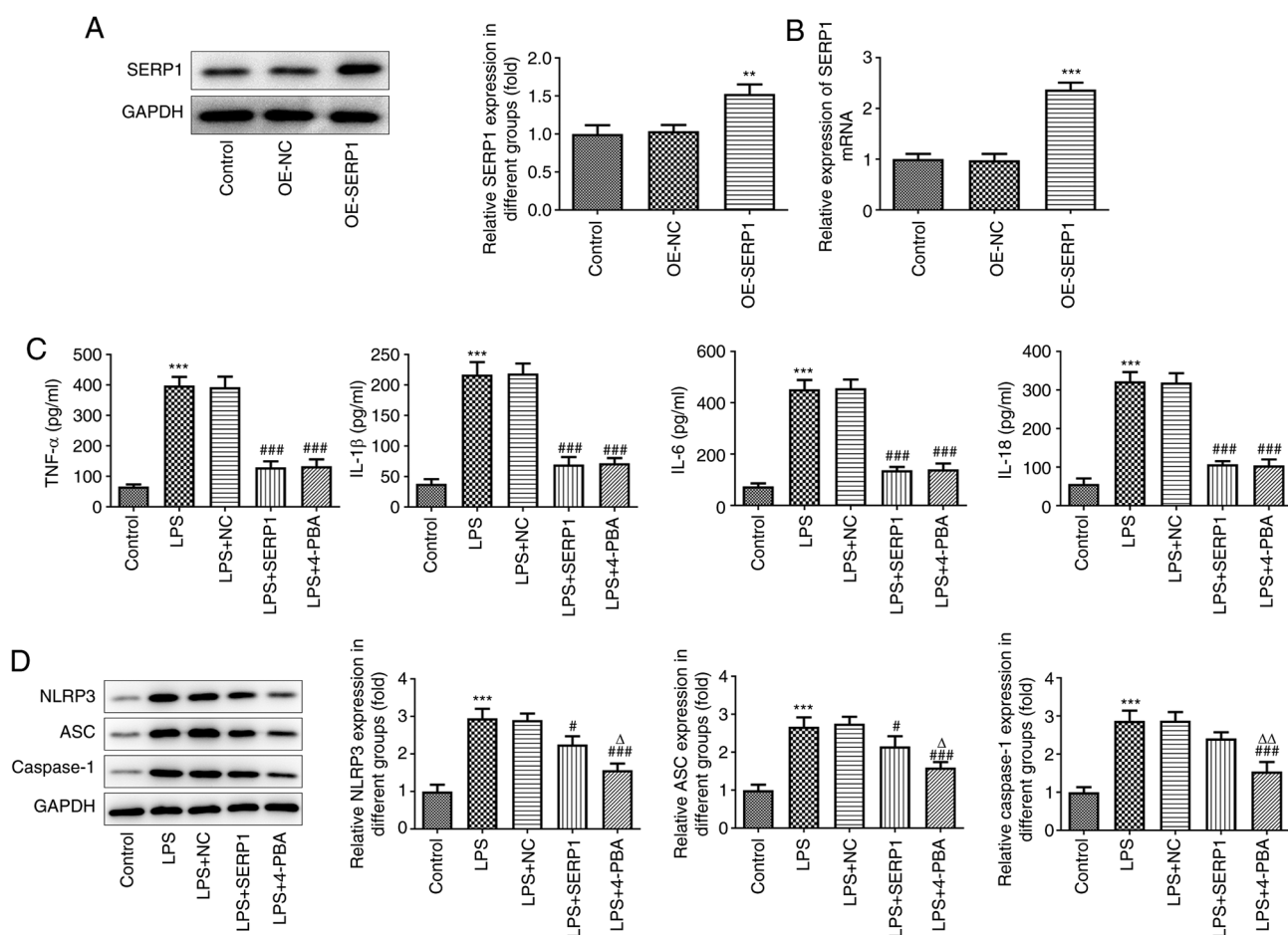


Figure 2. Effect of SERP1 overexpression on the expression of inflammatory factors in hepatocytes. Expression levels of SERP1 following transfection with SERP1 plasmid detected by (A) western blotting and (B) reverse transcription-quantitative PCR. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. OE-NC. (C) Expression levels of inflammatory factors (TNF- $\alpha$ , IL-18, IL-6 and IL-1 $\beta$ ) detected by ELISA. (D) NLRP3 inflammasome (NLRP3, ASC and caspase-1) expression detected by western blotting. \*\*\* $P < 0.001$  vs. the control; # $P < 0.05$ , ### $P < 0.001$  vs. LPS + NC;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$  vs. LPS + SERP1;  $n \geq 3$ . SERP1, stress-associated endoplasmic reticulum protein 1; OE, overexpression; NC, negative control; NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; LPS, lipopolysaccharide.

hepatocytes, and the overexpression was verified by RT-qPCR and western blotting. The results demonstrated that GSK3 $\beta$  was successfully overexpressed (Fig. 5A). Additionally, GSK3 $\beta$ / $\beta$ -catenin expression was detected by western blotting and TCF/LEF activity was detected using a luciferase assay. The results of the TOP Flash/FOP Flash luciferase assay were reversed following GSK3 $\beta$  overexpression compared with the results following SERP1 overexpression (Fig. 5B). Additionally, based on the results regarding GSK3 $\beta$  and  $\beta$ -catenin expression, GSK3 $\beta$  overexpression reversed the inhibitory effect of SERP1 overexpression (Fig. 5C). These results indicated that GSK3 $\beta$ / $\beta$ -catenin signaling activation reversed the role of SERP1 overexpression in GSK3 $\beta$ / $\beta$ -catenin signaling expression. In addition to the aforementioned experiments, western blotting was performed to detect the expression levels of proteins related to ERS and apoptosis, and a TUNEL assay was employed to detect cell apoptosis. It was revealed that GSK3 $\beta$  overexpression reversed the effect of SERP1 overexpression (Fig. 5D-F).

## Discussion

The liver serves a central role in immune homeostasis and metabolism. The liver is responsible for numerous functions,

such as blood coagulation, energy production, hormone balance and detoxification (23). Additionally, it contains numerous macrophages, which can detect, capture and eliminate bacteria and viruses that enter the human body. At the same time, the biotransformation process of the human body is mainly carried out in the liver, and thus, it is vulnerable to numerous factors (24). Alcoholism, excessive drug use and viral infections can all cause acute hepatic damage (25,26). D-GalN can cause hepatic degeneration and necrosis through competitive depletion of uridine phosphate. As a uridine phosphate-interfering agent, D-GalN is converted into uridine galactose diphosphate in the body, which affects the dysfunction of hepatocyte synthesis, causing the liver to produce an increased amount of reactive oxygen species, and thus causing hepatic damage in the body (27). LPS is one of the main components of the cell wall of gram-negative bacteria and is also called endotoxin. It can produce numerous inflammatory factors. When LPS and D-GalN are used in combination, D-GalN can strengthen the hepatic toxicity caused by LPS. The combined use is widely used as a method of modeling acute hepatic injury (28).

In the present study, following intraperitoneal injection of LPS/D-GalN in mice, by examining pathological sections obtained from the mice, it was revealed that hepatocytes were

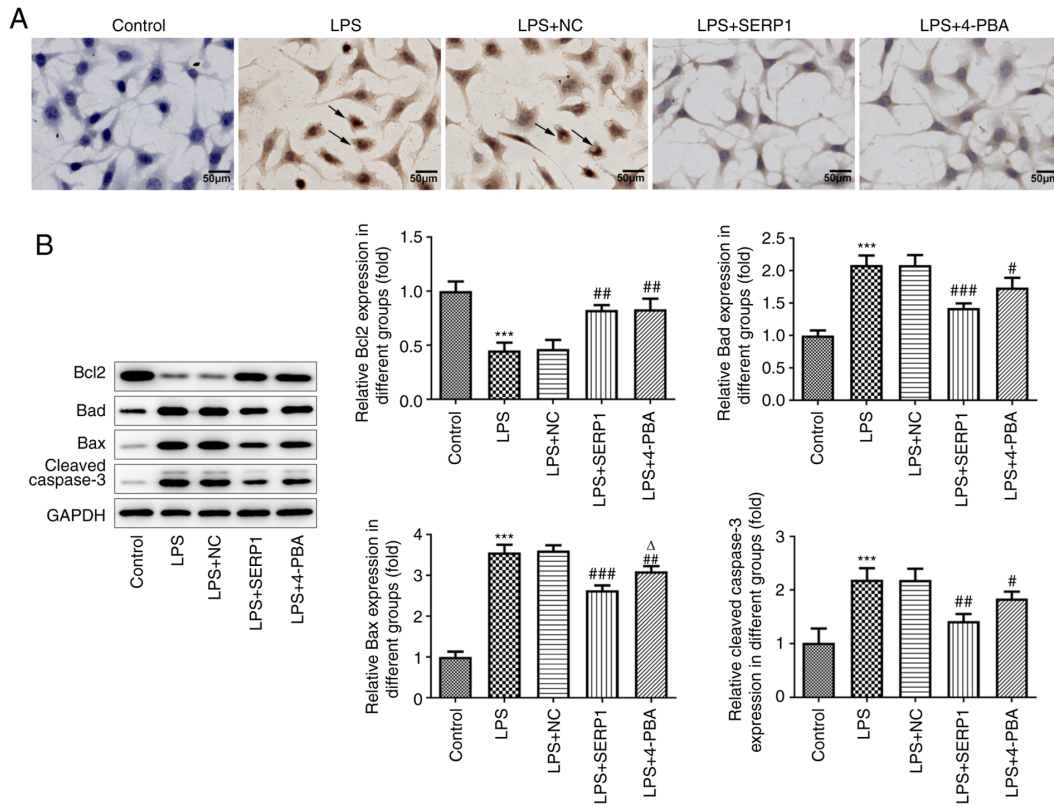


Figure 3. Effect of SERP1 overexpression on apoptosis of hepatocytes. (A) A TUNEL assay was employed to detect cell apoptosis. (B) Apoptosis-related protein expression was detected by western blotting. \*\*\* $P < 0.001$  vs. the control; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. LPS + NC; Δ $P < 0.05$  vs. LPS + SERP1;  $n \geq 3$ . SERP1, stress-associated endoplasmic reticulum protein 1; LPS, lipopolysaccharide; NC, negative control.

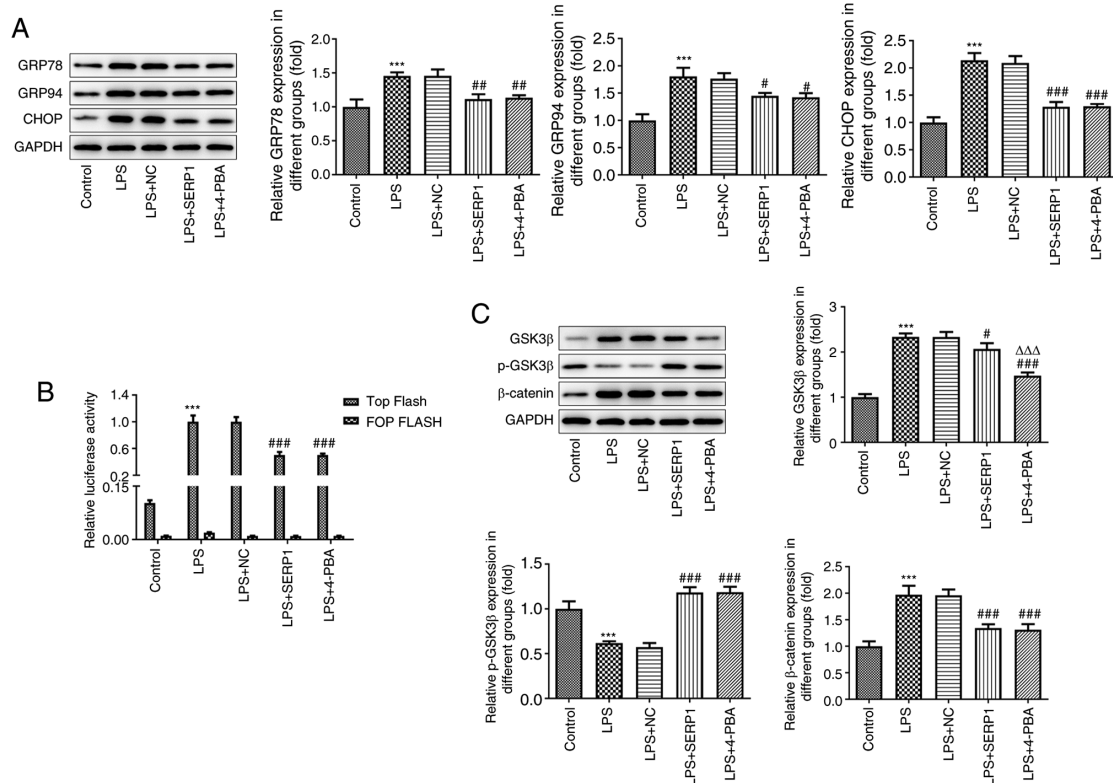


Figure 4. Effect of SERP1 overexpression on endoplasmic reticulum stress-related protein expression and GSK3 $\beta$ /β-catenin/TCF/LEF signaling in hepatocytes. (A) Expression levels of GRP78, GRP94 and CHOP in hepatocytes detected by western blotting. (B) TOP Flash/FOP Flash fluorescent gene reporter assay to detect TCF/LEF activity. (C) GSK3 $\beta$ /β-catenin expression was detected by western blotting. \*\*\* $P < 0.001$  vs. the control; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. LPS + NC; ΔΔΔ $P < 0.001$  vs. LPS + SERP1;  $n \geq 3$ . SERP1, stress-associated endoplasmic reticulum protein 1; GRP78, glucose-regulated protein 78; GRP94, glucose-regulated protein 94; TCF, T-cell factor; LEF, lymphoid enhancing factor; LPS, lipopolysaccharide; NC, negative control.

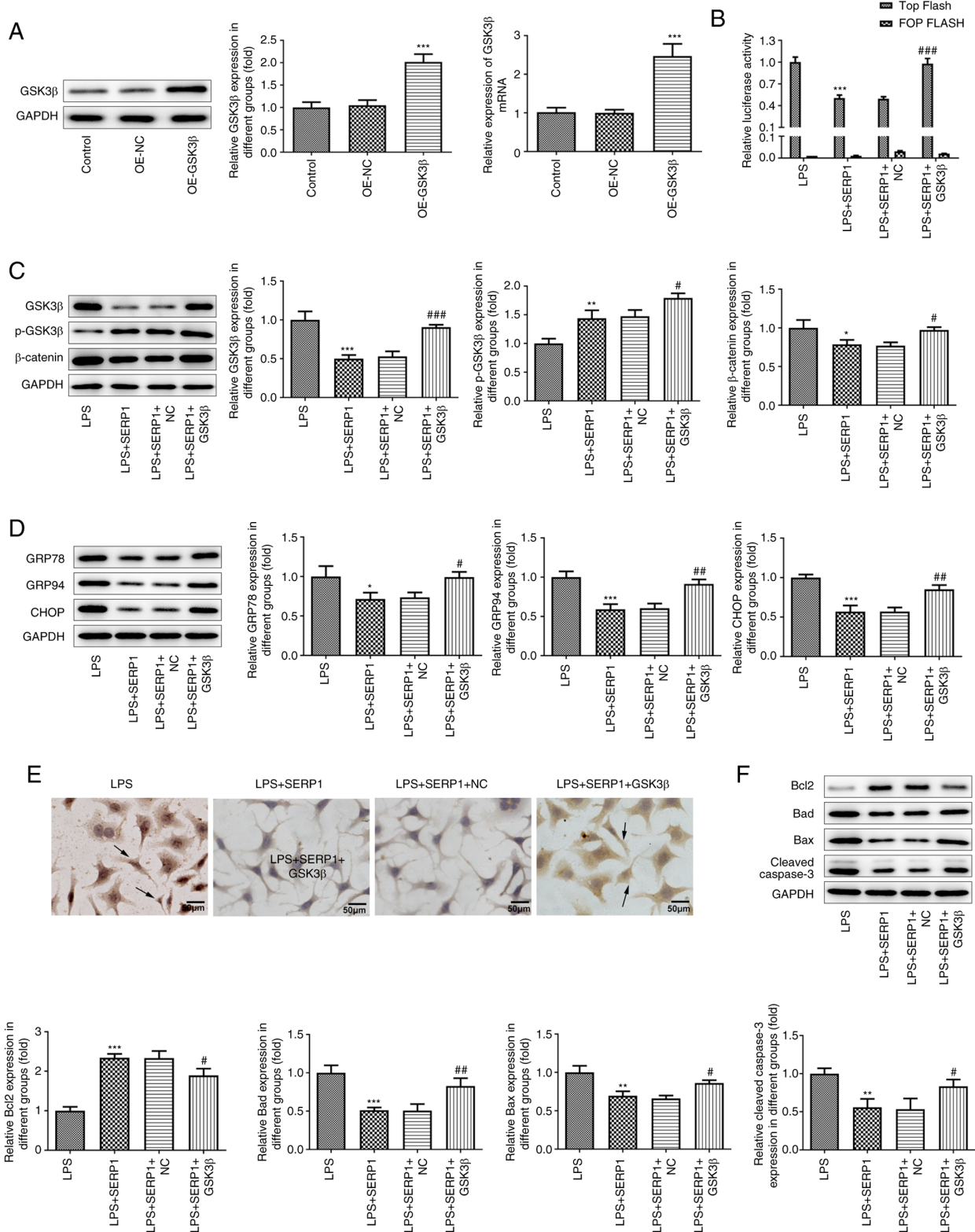


Figure 5. GSK3β/β-catenin signaling activation reverses the effect of SERP1 overexpression on ERS and cell apoptosis. (A) Overexpression was verified by reverse transcription-quantitative PCR and western blotting. \*\*\*P<0.001 vs. OE-NC; n≥3. (B) TCF/LEF activity was detected using a luciferase assay. (C) GSK3β/β-catenin expression was detected by western blotting. (D) Western blotting was applied to detect ERS-related protein expression. (E) A TUNEL assay was employed to detect cell apoptosis. (F) Apoptosis-related protein expression was detected by western blotting. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. LPS; #P<0.05, ##P<0.01, ###P<0.001 vs. LPS + SERP1 + NC; n≥3. SERP1, stress-associated endoplasmic reticulum protein 1; ERS, endoplasmic reticulum stress; OE, overexpression; TCF, T-cell factor; LEF, lymphoid enhancing factor; LPS, lipopolysaccharide; NC, negative control.

disintegrated and necrotic, and inflammatory cell infiltration was observed, which is consistent with the pathological manifestations of acute hepatic injury. The activities of ALT and AST

in the serum, which are related to liver function, were significantly increased compared with those of the control group. Glucose-regulated proteins GRP78 and GRP94, which are

endoplasmic reticulum chaperones, are expressed after being induced by ERS (29). In addition, induction of CCAAT/enhancer binding protein-homologous protein CHOP expression is one of the main mechanisms of ERS (30). Therefore, their expression can be detected as a marker of ERS.

SERP1 is a protein-coding gene. When the SERP1 protein was discovered, histological analysis of rabbits infected with the myxoma virus demonstrated that the absence of the protein was associated with increased inflammation (31). Furthermore, mice with SERP1 gene deletion exhibit growth retardation, increased mortality and impaired glucose tolerance (17). The results of the aforementioned study indicated that subtle changes of SERP1 serve a vital role in regulating ERS. The results of these two studies based on animal models revealed that SERP1 is obligatory for the normal growth of rabbits and mice. Additionally, a recent study revealed that microRNA-1-3p improves cell permeability and causes membrane damage by targeting SERP1, leading to endothelial cell dysfunction, which means that SERP1 serves an essential role in sepsis-induced lung injury (32). The present study detected SERP1 expression in acute liver injury tissues and cells, and the results revealed low expression in tissues as well as cells. On this basis, a SERP1-overexpression plasmid was established, and the effect of its overexpression was further studied.

ERS is associated with inflammation and cell apoptosis. In terms of inflammation, quercetin protects the intestinal barrier destruction and inflammation in acute necrotizing pancreatitis by inhibiting Toll-like receptor 4/MYD88 innate immune signal transduction adaptor/p38 MAPK and ERS activation (33). Additionally, the NLRP3 inflammasome is a complex composed of a variety of proteins, which activates the secretion of the pro-inflammatory cytokine IL-1 $\beta$  in a caspase-1-dependent manner, thereby regulating inflammation (34). The reduced expression levels of inflammatory factors and inflammasomes in the present study indicated the role of SERP1 upregulation in inflammation. In terms of apoptosis, in the case of persistently high ERS, activation of the unfolded protein response can trigger cell death, which has already been demonstrated by numerous studies (34-36). ERS induced by cyclic mechanical stretching can give rise to caspase-12 lysis and myoblast apoptosis (37). Additionally, in cardiomyopathy (38), nephropathy (39) and osteosarcoma (40), ERS has been found to be induced and results in cell apoptosis. In the present study, the reduced expression levels of apoptosis-related proteins demonstrated that SERP1 overexpression reduced hepatocyte apoptosis. This finding extends the potential role of SERP1 in alleviating liver disease through regulating ERS.

Based on the confirmed role of SERP1 overexpression, the present study explored its mechanism. In the research of cancer and neuropsychiatric diseases, GSK3 $\beta$  has been selected as a therapeutic target and has attracted increasing attention. Astragaloside IV protects nerve cells from ERS by inactivating GSK3 $\beta$  (41). Additionally, GSK3 $\beta$  regulated by ERS participates in the pathogenesis of Alzheimer's disease (42). TCF/LEF is a type of transcription factor with a dual regulatory role in the nucleus, and when combined with  $\beta$ -catenin, it promotes the transcription of downstream target genes. Subsequently, the activity of TCF/LEF and GSK3 $\beta$ / $\beta$ -catenin expression was detected in the present study, and the

effect of SERP1 overexpression was similar to that of the ERS inhibitor. By constructing a GSK3 $\beta$  overexpression plasmid, it was revealed that GSK3 $\beta$  overexpression reversed the effect of SERP1 overexpression. This demonstrated that SERP1 acts on ERS via the GSK3 $\beta$  signaling pathway. In addition, the present study demonstrated that when ERS was inhibited, TCF/LEF activity was also inhibited, however, the specific mechanism of their mutual influence has not been revealed yet.

The present study revealed that SERP1 overexpression alleviated ERS via the GSK3 $\beta$ / $\beta$ -catenin/TCF/LEF signaling pathway and reduced hepatocyte apoptosis. This enables the public to have a more comprehensive understanding of the mechanism of hepatic injury and provides a novel direction for its therapeutic targets.

### Acknowledgements

Not applicable.

### Funding

No funding was received.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

JC designed and performed experiments and made considerable contributions to manuscript writing. ZS and LZ performed the experiments and analyzed the data. HX performed the experiments and provided critical opinions on the manuscript. JC and HX confirm the authenticity of data. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

This research followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The present study was approved by the Animal Ethics Committee of Huaihe Hospital of Henan University (approval no. HHIACUC-201956; Kaifeng, China).

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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