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KLF15 reduces the level of apoptosis in mouse liver induced by sepsis by inhibiting p38MAPK/ERK1/2 signaling pathway

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Abstract. – OBJECTIVE: Sepsis-induced acute liver injury (ALI) involves multiple systems in the body. The disease is acute and critical, with various symptoms, including extensive necrosis of liver cells. There is currently no effective treatment to deal with ALI in a timely manner. This study verified the therapeutic effect of Krüppel-like factor 15 (KLF15) on ALI by studying its anti-apoptotic effect on the liver.

MATERIALS AND METHODS: We induced ALI in mice with lipopolysaccharide (LPS)/D-galactosamine (D-GalN). Recombinant mouse KLF15 was used to treat mice to examine the protective effects of KLF15 on mouse liver and the effects of apoptosis-related molecules. In addition, we cultured Kupffer cells and determined the anti-inflammatory and anti-apoptotic effects of KLF15 and its mechanism by overexpressing KLF15.

RESULTS: Exogenous KLF15 effectively reduced the levels of TIBL, ALT, AST, and inflammatory factors (COX-2, MCP-1, IL-1β, and TNF-α) in mouse serum. The results of HE staining also demonstrate that KLF15 improves the morphology of liver tissue. In addition, the expression of KLF15 in LPS-induced Kupffer cells was significantly reduced and KLF15 increased the viability of Kupffer cells and decreased the level of inflammation in Kupffer cells. In both *in vivo* and *in vitro* experiments, KLF15 reduced the level of apoptosis in hepatocytes or Kupffer cells and inhibited the activity of the p38MAPK/ERK1/2 signaling pathway.

CONCLUSIONS: KLF15 reduces the apoptosis and inflammation levels of liver and Kupffer cells by inhibiting the p38MAPK/ERK1/2 signaling pathway and alleviates LPS/D-GalN-induced ALI.

Key Words:
Acute liver injury, Sepsis, Apoptosis, MAPK/ERK1/2 signaling pathway.

Introduction

Acute liver injury (ALI) refers to a syndrome in which a large number of hepatocyte necrosis or abnormal liver function suddenly occurs, which can affect multiple organs. The main causes of the disease include viral infection, drug toxic reaction, liver fat lesion, and autoimmune diseases, etc[1]. The disease develops rapidly and can lead to death caused by complications such as multiple organ failure. The treatment of liver failure has always been a difficult problem for clinicians[2]. In recent years, increasing research has begun to focus on the application of new treatments in the treatment of liver diseases.

Normal cell apoptosis and production maintain a dynamic balance, but under pathological conditions, this balance is broken, presenting liver injury, and thereby promoting apoptosis of liver cells. Sustained apoptosis can lead to acute injury, such as fulminant hepatitis, reperfusion injury, and some chronic damage (such as alcoholic liver disease, cholestatic liver disease, and viral hepatitis). Therefore, inhibition of apoptosis in the process of liver injury is the key to alleviating ALI[3]. Krüppel-like factor like factor 15 (KLF15) is a member of the zinc finger protein family. Zinc finger proteins are transcriptional regulators with finger-like domains that are structurally stable by binding to zinc ions. The domain of zinc finger proteins can bind to DNA, proteins, and even mRNA. Upon binding to the DNA binding site sequence of the promoter, KLF15 regulates transcription of downstream factors. After binding to a protein (e.g., a co-activator), KLF15 can act together with a co-activator to regulate gene transcription[4]. KLF15 also has a post-transcrip-

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tional effect upon binding to mRNA. In recent years, it has been found that KLF15 can regulate cell differentiation, proliferation, apoptosis, and fibrosis through transcriptional regulation or post-transcriptional regulation[5]. The role of KLF15 in ALI is still lacking. Therefore, we used lipopolysaccharide (LPS)/D-galactosamine (D-GaIN) to induce ALI in mice to detect the effect of recombinant mouse KLF15 on mouse ALI. In addition, we cultured mouse Kupffer cells to detect the effect of KLF15 on the liver and mechanisms.

Materials and Methods

Animals and Grouping

This investigation was approved by the Animal Ethics Committee of Liaocheng People's Hospital Animal Center. The mice were all 8-week old C57/BL6 male mice and we selected the littermates as controls. Mice were housed in specific-pathogen free (SPF) barriers and given SPF graded mice chow and drinking water. LPS and D-GalN were used to make mouse ALI model. We used sterile saline solution to prepare the LPS/D-GalN solution and construct mouse ALI model by intraperitoneal injection. After intraperitoneal injection of LPS/D-GalN solution (LPS, 100 μg/kg; D-GalN, 400 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) for 24 h, we anesthetized the mice and took serum and liver tissue. In the mice of the treatment group, we gave subcutaneous injection of recombinant KLF15 (50 μg/kg; Lianmai, Beijing, China) while modeling.

Cell Culture and Treatment

We cultured Kupffer cells [American Type Culture Collection (ATCC) Manassas, VA, USA)] using Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12) medium (Gibco, Rockville, MD, USA). Fetal bovine serum (FBS; Gibco, Rockville, MD, USA) was added to the basal medium and configured to concentration of 10%. All cell experiments were performed in a clean bench. After the cell growth density reached 70%, we treated the cells with drugs or lentiviruses. LPS (Sigma-Aldrich, St. Louis, MO, USA) was used to induce Kupffer cell damage.

Lenti-KLF15 Transfection

Before transfection, we replaced the original cell culture medium in the culture dish with fresh complete medium. We added 500 μL of serum-free medium to each of the two Eppendorf (EP; Hamburg, Germany) tubes and added lentivirus and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), respectively. After blending EP tubes, we incubated them for 5 min at room temperature. Then, we mixed the liquids in the two EP tubes and incubated for another 20 min. Then, we added the mixture to the cell culture dish. After 6 h, we took out the culture dish and replaced the fresh complete medium. After 24 h, we took out the culture dish and tested the transfection efficiency.

Western Blot Analysis

We extracted total protein from mouse liver tissue or Kupffer cells using radioimmunoprecipitation assay (RIPA) lysate (Invitrogen, Carlsbad, CA, USA) and stored them in a -80°C freezer. We configured 10% electrophoresis gel and added an equal amount of protein to each well of the gel. After electrophoresis of the protein, we transferred the protein to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) by transfer. At the end of the transfer, we placed the PVDF membrane in the Lichen red dye solution (Invitrogen, Carlsbad, CA, USA) and observed red bands. After washing the PVDF membrane, we blocked the non-specific antigen with 5% skim milk and incubated the PVDF membrane with primary antibody dilution (KLF15, 1:3000, Rabbit, Abcam, Cambridge, MA, USA; Bcl-2, 1:1000, Rabbit, Abcam, Cambridge, MA, USA; Bax, 1:3000, Rabbit, Abcam, Cambridge, MA, USA; caspase-3, 1:5000, Rabbit, Abcam, Cambridge, MA, USA; caspase-9, 1:1000, Rabbit, Abcam, Cambridge, MA, USA; p38MAPK, 1:1000, Rabbit, Abcam, Cambridge, MA, USA; p-p38MAPK, 1:3000, Rabbit, Abcam, Cambridge, MA, USA; ERK1/2, 1:3000, Rabbit, Abcam, Cambridge, MA, USA; p-ERK1/2, 1:1000, Rabbit, Abcam, Cambridge, MA, USA; β-actin, 1:3000, Rabbit, Abcam, Cambridge, MA, USA) at 4°C overnight. After washing the PVDF membrane, we incubated the PVDF membrane with secondary antibody dilution (Goat anti-rabbit, 1:3000, Abcam, Cambridge, MA, USA) for 2 h. Finally, we observed the protein bands by exposure using chemiluminescent solution.

RNA Isolation and Quantitative Real Time-Polymerase Chain Reaction (RT-PCR)

We extracted total RNA from liver tissue and Kupffer cells using TRIzol reagent (Invitrogen,

Carlsbad, CA, USA) and stored the RNA solution in a -80°C freezer. We reversed mRNA to complementary deoxyribose nucleic acid (cDNA) by reverse transcription. The SYBR Green kit (Invitrogen, Carlsbad, CA, USA) was used to amplify cDNA. The primers were constructed by Shanghai Jierui Biotechnology Co., Ltd. (Shanghai, China). The primer sequence included KLF15 sense, CGGTCTAAAATCGAGTCTACG, KLF15 anti-sense, GGCATTCGAATATATGCGCA; COX-2 sense, ACGATCAGTCGTACTGAGTC, COX-2 anti-sense, GGCATTAGCGATCGAAATTGC; MCP-1 sense, ACGATCGTCGATCGGATACGT, MCP-1 anti-sense, GCCGAGCTAGTGTGTACACCG; Bcl-2 sense, AAGGCTAGCTACTGCGTCAGT, Bcl-2 anti-sense, GGATCGTAAGTCAGTGTCACAGT; Bax sense, CTACGAGCGTCATTAAGCCGAT, Bax anti-sense, AGCTACGTAGTCATGACGCAAT; caspase-3 sense, GCATCGTACGCTGAGTCA, caspase-3 anti-sense, CGATAGTACCAGTGCTACA; caspase-9 sense, CAGTACGCTACAGTCATGAA, caspase-9 anti-sense, GGATATTTTAGCGCATCAT.

Enzyme Linked Immunosorbent Assay (ELISA)

We extracted the supernatant of mouse serum and Kupffer cells and centrifuged them to remove impurities. COX-2, MCP-1, IL-1β, and TNF-α ELISA kits (Lianke, Hangzhou, China) were used to detect the expression levels of inflammatory factors in serum and supernatant.

Detection of Total Bilirubin (TBIL), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Activity

After obtaining mouse blood, we left it at room temperature for 20 min. Then, we obtained the supernatant by centrifugation, which is serum. Serum was stored in a -80°C freezer. TBIL, ALT, and AST kits (Lianke, Hangzhou, China) were used to detect TIBL, ALT, and AST levels in serum.

Hematoxylin and Eosin (HE) Staining

After obtaining the liver of the mouse, we took the liver lobe at the same location and made paraffin sections. After dewaxing and hydration, we placed the paraffin sections in hematoxylin soak for 3 min. After rinsing the paraffin sections with water, we placed the paraffin sections in hydrochloric acid for 3 s and then immediately rinsed them in water. The hematoxylin staining results were observed under a microscope. If the hematoxylin staining results are good, the eosin staining is continued. If the over-differentiation results in hematoxylin staining too shallow, hematoxylin staining is resumed. Then, we dehydrated the paraffin sections and soaked them in xylene for 10 min. Finally, we sealed the paraffin section and observed the results under a microscope.

Cell Counting Kit-8 (CCK8) Assay

We added 100 μL of the cell suspension to a 96-well plate and placed it in an incubator for 24 h. We then added different concentrations of recombinant mouse KLF15 (10, 25, 50, 75, 100 μM) to the plates. After continuing to culture for 24 h, we added 10 μL of CCK-8 reagent (Dojindo Molecular Technologies, Kumamoto, Japan) to each well and continued incubation for 2 h. Finally, we measured the absorbance at 450 nm of each well using a microplate reader (Molecular Devices, San Jose, CA, USA).

Immunofluorescence (IF) Staining

All cells were cultured on cell slides. After the cells were fixed at room temperature for 30 min using 4% paraformaldehyde, we washed the cells twice with phosphate-buffered saline (PBS), and then we treated the cells with 0.1% Triton for 10 min. After washing the cells with PBS, we diluted the primary antibody (Bcl-2, 1:500, rabbit, Abcam, Cambridge, MA, USA; Bax, 1:500, rabbit, Abcam, Cambridge, MA, USA) in 5% BSA-PBS and incubated the cells at 4°C overnight. We then incubated the cells with the fluorescently labelled secondary antibody (FITC- Goat anti-rabbit, 1:500, Abcam, Cambridge, MA, USA) using the same method. We blocked the cell slides on a glass slide using a closure containing 4',6-diamidino-2-phenylindole (DAPI). Finally, we observed the staining results using a fluorescence microscope (Leica-DM2500, Wetzlar, Germany).

Annexin V-FITC Detection

After collecting the cells, we took approximately 10^6 cells by cell counting and washed the cells with cold PBS. Then, we suspended the cells in 200 μL of binding buffer. After adding 10 μL of Annexin V-FITC (Keygen, Nanjing, China), we incubated the cells for 15 min in the dark. Finally, we added 300 μl of Binding buffer and 5 μL of propidium iodide (PI) and detected the proportion of apoptotic cells using flow cytometry.

Statistical Analysis

All experimental results are expressed in terms of the mean ± standard deviation. Differences between two groups were analysed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). A statistical difference between the two groups was considered at $p<0.05$. Statistical Product and Service Solutions (SPSS) 21.0 statistical software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 software (La Jolla, CA, USA) were used for data analysis and processing. All experiments were repeated more than three times.

Results

Exogenous KLF15 Attenuates LPS/D-GalN-Induced Mouse ALI

To demonstrate the protective effect of KLF15 on the liver, we induced mouse ALI using LPS/D-GaIN and treated mice with subcutaneous injection of recombinant KLF15. The results showed that the levels of TIBL, ALT, and AST in the serum of mice in the ALI group were significantly increased, indicating that LPS/D-GaIN successfully induced ALI in mice. The serum levels of TIBL, ALT, and AST in mice administered with KLF15 were lower than those in the ALI group (Figure 1A-1C). The expression of inflammatory

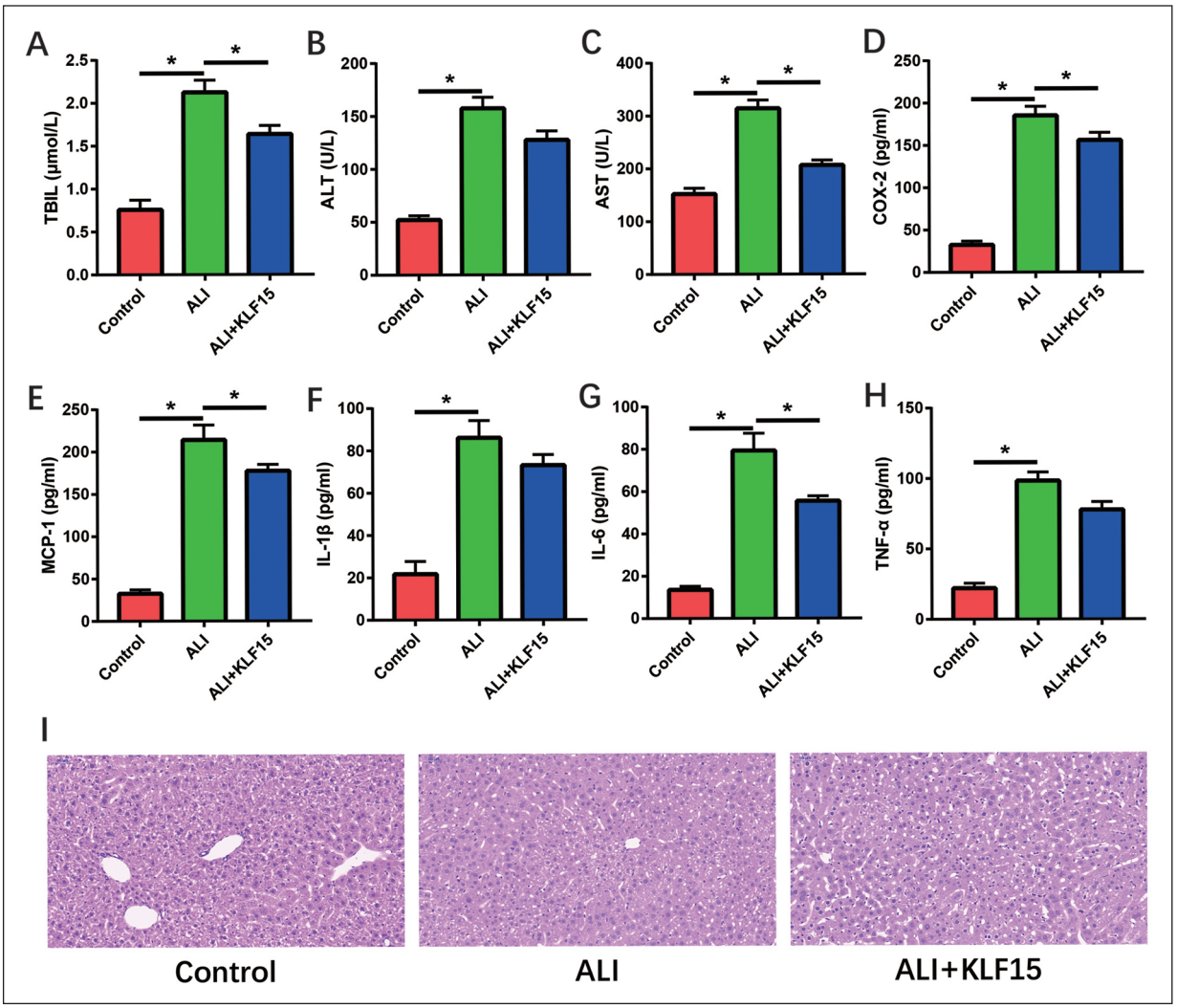

Figure 1. Exogenous KLF15 attenuates LPS/D-GaIN-induced mouse ALI. **A-C,** Expression of TBIL, ALT and AST in serum of mice. **D-H,** ELISA results of COX-2, MCP-1, IL-1β, IL-6, and TNF-α. **I,** HE staining of mice liver tissue (magnification: 400×). ("*" means $p<0.05$ between two groups).

factors in mouse serum was detected by ELISA (Figure 1D-1H) and the results showed that KLF15 effectively reduced the expression of COX-2, MCP-1, IL-1β, and TNF-α in mouse serum. The results of HE staining (Figure 1I) showed that the liver tissue of the ALI group was disordered and inflammatory cell infiltration, while the liver tissue morphology of the ALI+KLF15 group was better than that of the ALI group.

LPS-Induced Kupffer Cells Expressed Lower KLF15 and KLF15 Reduced Inflammation Levels in Kupffer Cells

We used LPS to induce Kupffer cell damage and found that the expression of KLF15 in Kupffer cells gradually decreased with increasing LPS concentration (Figure 2A, 2B). The results of the CCK-8 (Figure 2C) showed that the activity of Kupffer cells gradually increased with the increase of the concentration of recombinant mouse KLF15. In addition, the results of ELISA (Figure 2D, 2E) showed that KLF15 can reduce the expression of COX-2 and MCP-1 in Kupffer cells in a dose-dependent manner. We used Lenti-KLF15 to increase the expression of endogenous KLF15 in Kupffer cells and verified transfection efficiency by Western blot (Figure 2F) and RT-PCR (Figure 2G). The results of ELISA (Figure 2H) and RT-PCR (Figure 2I) showed that Lenti-KLF15 can significantly reduce the LPS-induced inflammatory response.

KLF15 Reduces Apoptosis in Liver and Kupffer Cells

We took mouse liver and extracted protein and total RNA to detect the expression of apoptosis-related indicators. Western blot (Figure 3A) and RT-PCR (Figure 3B) results showed that exogenous KLF15 increased the expression of Bcl-2 in the liver of mice, decreased the expression of Bax, caspase-3, and caspase-9 and increased Bcl-2/Bax (Figure 3C). In cell experiments, Lenti-KLF15 also reduced the level of apoptosis in Kupffer cells, showing similar results to animal experiments (Figure 3D-3F). The results of flow cytometry (Figure 3G) also showed that KLF15 can reduce the proportion of apoptotic cells in Kupffer cells.

KLF15 Inhibits the Activity of the p38MAPK/ERK1/2 Signaling Pathway In Vivo and In Vitro

To determine the mechanism of KLF15's anti-apoptotic effect on the liver, we examined the expression of p38MAPK/ERK1/2 signaling pathway in mouse liver tissue. Western blot (Figure 4A) results showed that there was no significant change in the expression of p38MAPK and ERK1/2, but KLF15 decreased their phosphorylation, showing an increase in p-p38MAPK/p38MAPK (Figure 4B) and (p-ERK1/2)/(ERK1/2) (Figure 4C). Similar results also occurred in cell experiments (Figure 4D-4F).

Discussion

The KLFs family is a large class of basic transcription factors in eukaryotes. They are involved in early embryonic growth and development, cell differentiation, tissue, and organ formation, protooncogene mutations and angiogenesis, and other physiological and pathological processes, and are an extremely important basic regulator[6]. At present, scientists have found a total of 17 KLF factors in mammals. They were named KLF 1 to 17 in the order of discovery. These KLF factors are widely expressed in various cell types of organisms and play different important roles, including multi-differentiation of stem cells, myelosuppression, cancer development, tissue remodelling, vascular regeneration, cell phenotype switching and so on[7]. KLF15 has an important influence on liver metabolism. Deletion of the KLF15 gene can improve insulin resistance in mice under the influence of high-fat diet (HFD) but does not affect endoplasmic reticulum stress and hepatic inflammatory response with insulin resistance. Knockout of mouse KLF15 gene can improve liver endoplasmic reticulum stress markers in HFD mice without affecting endoplasmic reticulum stress and hepatic inflammatory response with insulin resistance. Therefore, gene intervention targeting the KLF15 gene can improve HFD-induced insulin resistance. In addition, after endoplasmic reticulum stress is activated, the liver stress response of $KLF15^{-/-}$ mice is significantly reduced when hepatic steatosis and insulin resistance are induced. However, inhibition of the KLF15 gene promotes the expression of C-Jun N-terminal kinase (JNK) phosphorylation. Thus, the deletion of KLF15 gene may lead to insulin resistance and steatosis in obese patients with HFD and uncoupling of endoplasmic reticulum stress and inflammatory response. In $KLF15^{-/-}$ mice, enhanced fatty acid oxidation inhibits the mTORC1 signaling pathway, thereby inhibiting hepatic

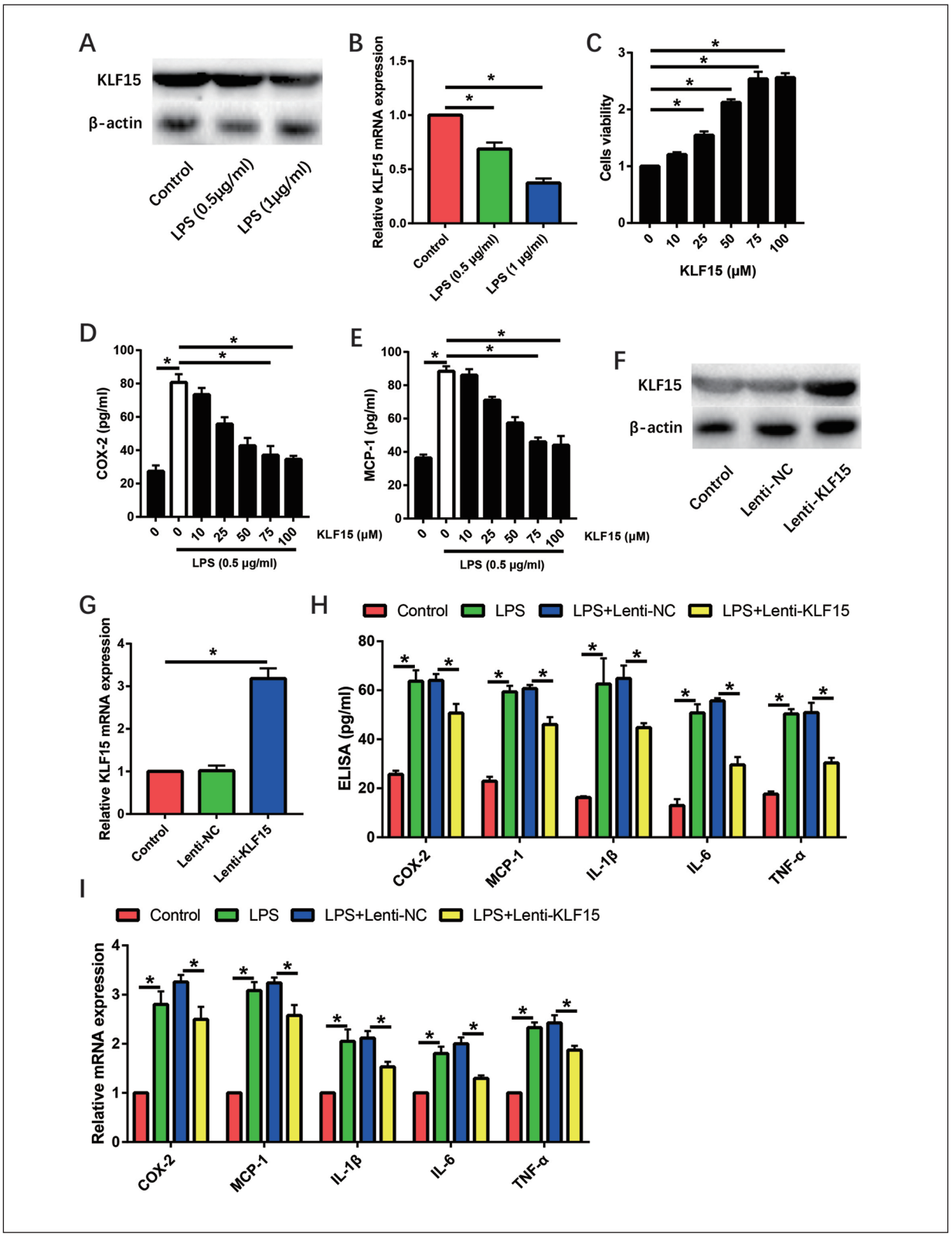

Figure 2. LPS-induced Kupffer cells expressed lower KLF15 and KLF15 reduced inflammation levels in Kupffer cells. **A, B,** Western blot and RT-PCR results of KLF15. **C,** CCK-8 assay of KLF15. **D, E,** ELISA results of COX-2 and MCP-1. **F, G,** Western blot and RT-PCR results of KLF15. **H, I,** ELISA and RT-PCR results of COX-2, MCP-1, IL-1β, IL-6, and TNF-α. ("*" means $p<0.05$ between two groups).

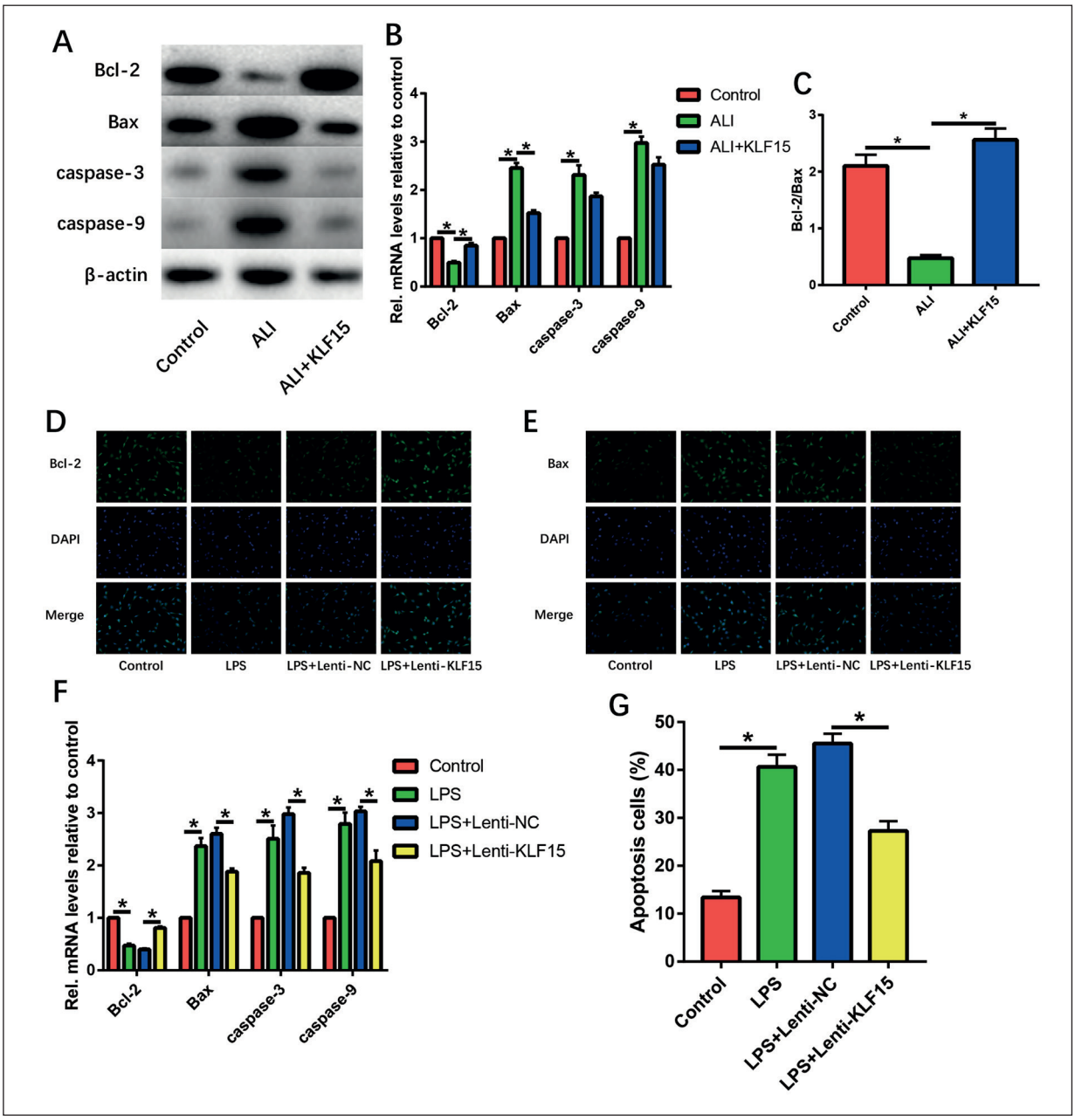

Figure 3. KLF15 reduces apoptosis in liver and Kupffer cells. **A, B,** Western blot and RT-PCR results of Bcl-2, Bax, caspase-3, and caspase-9. **C,** Ratio of expression of Bcl-2 and Bax. **D, E,** IF staining results of Bcl-2 and Bax (magnification: 400×). **F,** RT-PCR results of Bcl-2, Bax, caspase-3, and caspase-9. **G,** Apoptosis cells rate in Kupffer cells. ("*" means $p<0.05$ between two groups).

steatosis[8]. Therefore, the KLF15 gene is a key factor regulating liver metabolism, and interference with the expression of the KLF15 gene may result in changes in HFD-induced liver lesions. We treated mice with recombinant mouse KLF15 and found that KLF15 effectively ameliorated liver injury in mice, manifested by a decrease in TIBL, ALT, and AST, a decrease in serum levels of inflammation, and an improvement in mouse hepatocyte morphology, indicating KLF15 has a protective effect on the liver. In addition, expression of KLF15 was inversely correlated with the concentration of LPS in Kupffer cells treated with LPS.

A major regulator of apoptosis is the member of the Bcl-2 protein family. Bcl-2 is a potent inhibitor of mammalian cell apoptosis by inhibiting apoptosis to prolong cell survival. Bcl-2 is the

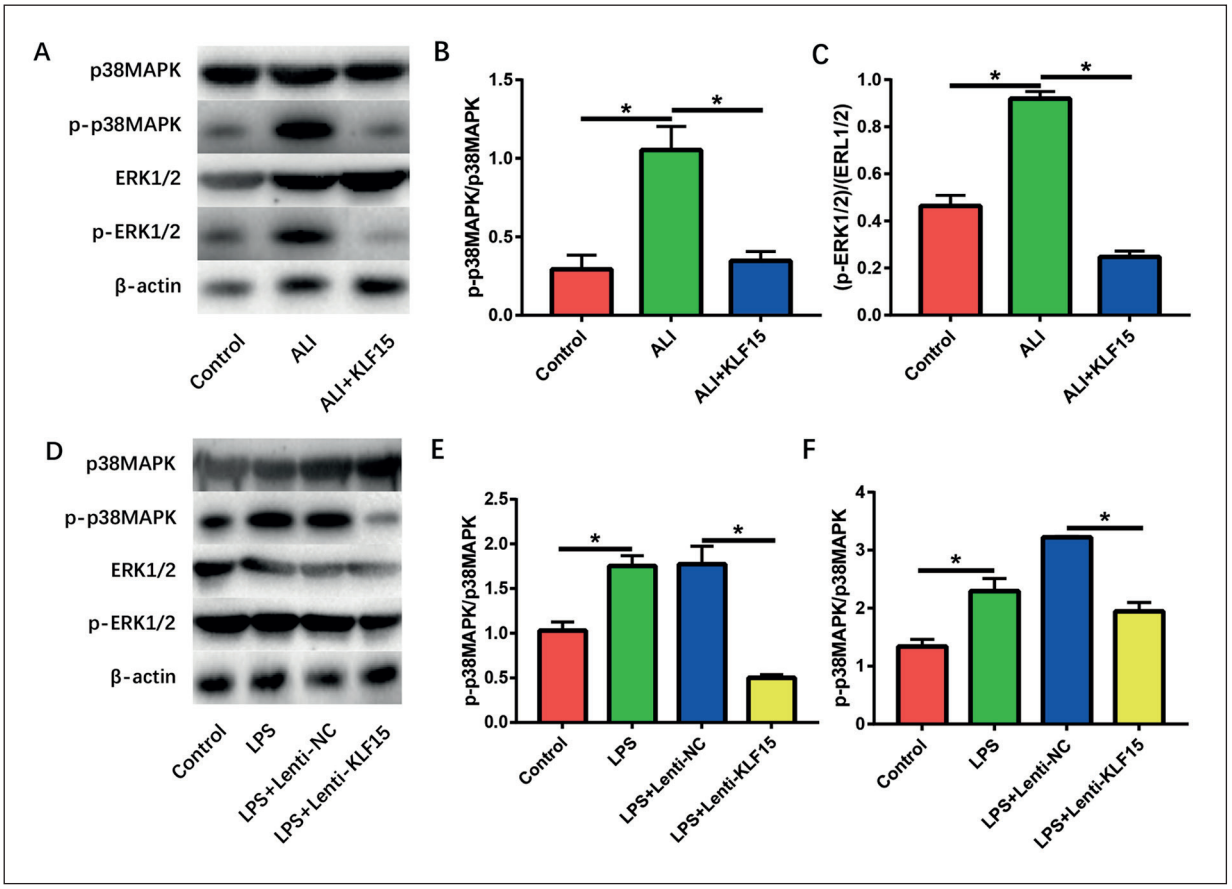

Figure 4. KLF15 inhibits the activity of the p38MAPK/ERK1/2 signaling pathway *in vivo* and *in vitro*. **A-C,** Western blot results of p38MAPK, p-p38MAPK, ERK1/2, and p-ERK1/2 in mice liver tissue. **D-F,** Western blot results of p38MAPK, p-p38MAPK, ERK1/2, and p-ERK1/2 in Kupffer cells. ("*" means $p<0.05$ between two groups).

first gene identified to have an inhibitory effect on apoptosis. High expression of Bcl-2 can inhibit apoptosis induced by various apoptosis-inducing factors[9]. Hardy et al[10] found that the inhibition of apoptotic gene Bcl-2 and the promotion of apoptotic gene Bax played an important role in the process of apoptosis in the experimental model of rat liver cirrhosis induced by CCl_4. The expression of Bax and Bcl-2 mRNA was detected by RT-PCR at 6, 12, 24, and 72 h. The mRNA level of Bax was significantly higher than that of the normal group at the same time, and it was negatively correlated with the mRNA level of Bcl-2. The equilibrium state between the pro-apoptotic gene Bax and the apoptosis-inhibiting gene Bcl-2 is closely related to hepatocyte apoptosis. Lee et al[11] found that the expression levels of D-type virus antigen, Bax, and hepatocyte apoptosis were related to the degree of liver tissue inflammation and pathological damage. D-type virus infection can induce hepatocytes to express Bax and enhance hepatocyte apoptosis. Liang et al[12] on the expression of Bax and Bcl-2 in rat liver tissues by isoniazid showed that normal rat liver tissues showed positive Bax staining and little or low expression in central vein and surrounding hepatocytes. After administration, the rat liver tissue Bax is centered on the central vein and is strongly positive in the liver cytoplasm near it and is also strongly positive and even diffusely distributed in most of the denatured hepatocyte cytoplasm. Bcl-2 was weakly positive in the normal rat hepatocyte cytoplasm and central vein wall, but positive in the hepatocyte cytoplasm, hepatic sinusoid and portal area of the drug-administered group. In the comparison of different drug-administered groups, the absorbance value and area of Bcl-2 in liver tissue were the highest after 7 days of administration. In our research, KLF15 improved the apoptosis of hepatocytes induced by sepsis *in vivo* and *in vitro*, as shown by the elevation of Bcl-2/Bax and caspase-3 and caspase-9.

The p38MAPK/ERK1/2 signaling pathway plays an important role in apoptosis[13]. p38MAPK can be activated by phosphorylation to regulate gene transcription, induce the expression of specific genes, which causes cell proliferation, differentiation, and synthesis of various cytokines[14]. p38MAPK mainly induces Bcl-2 and caspase-3 mediated apoptosis pathways, which cause a series of biological effects. Activation of p38MAPK by up-regulating certain transcription factor genes (e.g., activation transcription factors, growth arrest and DNA damage genes, interferon regulatory factor-3, NF-κB, and heat shock transcription factors) and caspase family members (caspase-3, 6, 7) expression and biological activity affects various physiological activities of cells[15]. When the transcription factor is activated, the phosphorylated transcription factor binds to the cis-acting element and then expresses a large number of apoptosis-related gene. Inducible NO synthase, selectin, tumor necrosis factor, interleukin, cytokine MAPK, transcription factor ATF2, ERK1/2, and other gene expression products will affect cell proliferation, differentiation, apoptosis, and cytokine synthesis. When ALI occurs, the signaling molecules brought by ALI bind to tyrosine kinases and G-protein coupled receptors on the cell membrane and transduce the stimulatory signal into the cytoplasm. Then, stimulation signal acts on the p38MAPK in the cytosol. When p38MAPK is activated by phosphorylation, it is transferred to the nucleus and activates different transcription factors to finally activate the corresponding gene, producing physiological effects[16]. HIF (hypoxia-inducible-factor) -1α plays a self-balancing role during hypoxia and can mediate the body's inflammatory response[17]. The p38MAPK/ERK1/2 signaling pathway can promote the expression of HIF-1α. When ALI occurs, massive necrosis of liver cells induces hypoxic environment. The p38MAPK/ERK1/2 signaling pathway activates HIF-1α and also generates a large number of pro-inflammatory factors and causes apoptosis[18]. Therefore, p38MAPK/ERK1/2 signaling pathway may be one of the pathogenesis of ALI. Verma et al[19] used isoniazid to damage Hep 3B cells and found that isoniazid promoted apoptosis through oxidative stress and promoted Nrf2 translocation to the liver nucleus by increasing p-ERK protein expression, thus destroying liver cells, indicating that ERK signaling pathway may be involved in isoniazid-induced Hep 3B cell damage. Liu et al[20] studied the protective effect mechanism of Hippophae rhamnoides polysaccharides (HRP) on LPS/D-GalN-induced ALI mice and found that transaminase and inflammatory factors (TNF-α and IL-1β) were reduced, and the expression levels of p-ERK, p-JNK, p-p38MAPK, and NF-κB were decreased, suggesting that HRP reduces the inflammation and necrosis of hepatic cell induced by LPS/D-GalN by inhibiting the p38MAPK/ERK1/2 signaling pathway. In our study, KLF15 potently inhibited phosphorylation of p38MAPK and ERK1/2 in Kupffer cells and inhibited the activity of the p38MAPK/ERK1/2 signaling pathway. This explains the superior anti-apoptotic effect of KLF15.

Therefore, KLF15 has a significant protective effect on the liver and can provide help for clinical treatment of ALI.

Conclusions

KLF15 has a good anti-apoptotic effect on the liver. Exogenous KLF15 can alleviate LPS/D-GaIN-induced ALI. In addition, KLF15 can attenuate ALI by inhibiting the level of apoptosis in the liver via the inhibition of the p38MAPK/ERK1/2 signaling pathway and the reduction of inflammation caused by sepsis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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