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Original Article

Leukocyte cell-derived chemotaxin 2 (LECT2) regulates liver ischemia–reperfusion injury

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ABSTRACT

Background and aim: Hepatic ischemia–reperfusion injury (IRI) is a significant challenge in liver transplantation, trauma, hypovolemic shock, and hepatectomy, with limited effective interventions available. This study aimed to investigate the role of leukocyte cell-derived chemotaxin 2 (LECT2) in hepatic IRI and assess the therapeutic potential of Lect2-short hairpin RNA (shRNA) delivered through adeno-associated virus (AAV) vectors.

Materials and methods: This study analyzed human liver and serum samples from five patients undergoing the Pringle maneuver. *Lect2*-knockout and C57BL/6J mice were used. Hepatic IRI was induced by clamping the hepatic pedicle. Treatments included recombinant human LECT2 (rLECT2) and AAV-Lect2-shRNA. LECT2 expression levels and serum biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and blood urea nitrogen (BUN) were measured. Histological analysis of liver necrosis and quantitative reverse-transcription polymerase chain reaction were performed.

Results: Serum and liver LECT2 levels were elevated during hepatic IRI. Serum LECT2 protein and mRNA levels increased post reperfusion. *Lect2*-knockout mice had reduced weight loss; hepatic necrosis; and serum ALT, AST, creatinine, and BUN levels. rLECT2 treatment exacerbated weight loss, hepatic necrosis, and serum biomarkers (ALT, AST, creatinine, and BUN). AAV-Lect2-shRNA treatment significantly reduced weight loss, hepatic necrosis, and serum biomarkers (ALT, AST, creatinine, and BUN), indicating therapeutic potential.

Conclusions: Elevated LECT2 levels during hepatic IRI increased liver damage. Genetic knockout or shRNA-mediated knockdown of *Lect2* reduced liver damage, indicating its therapeutic potential. AAV-mediated Lect2-shRNA delivery mitigated hepatic IRI, offering a potential new treatment strategy to enhance clinical outcomes for patients undergoing liver-related surgeries or trauma.

1. Introduction

Hepatic ischemia–reperfusion injury (IRI) remains a major complication in liver transplantation, trauma, hypovolemic shock, and hepatectomy, significantly contributing to postoperative morbidity and mortality.[1–3] Vascular control techniques, such as the Pringle maneuver, contribute to reduce intraoperative blood loss but often lead to hepatic IRI, thereby affecting liver function and distant organs, such as the kidneys, intestines, and lungs.[2,4–8] The mechanisms underlying hepatic IRI are complex and not fully understood, indicating the critical need for effective clinical interventions. Current strategies to mitigate hepatic IRI include avoiding inflow occlusion, implementing ischemic preconditioning, and administering pharmacological agents. Recently, gene therapy has emerged as a promising therapeutic approach. In hepatic IRI, therapeutic genes are delivered via viral vectors, such as

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recombinant viruses.[9,10] For instance, high doses of mitochondrial superoxide dismutase delivered via viral vectors in rat models have effectively prevented liver IRI by inhibiting the activation of nuclear factor kappa B and activator protein-1 (AP-1).[11] This highlights the potential of gene therapy in addressing the challenges of hepatic IRI.[9–11]

Endothelial cells play a crucial role in IRI by secreting signaling molecules that trigger inflammatory cell infiltration and cytokine production. Liver sinusoidal endothelial cells (LSECs) are vulnerable to IRI, resulting in plasma membrane disruption, nuclear vacuolation, and morphological alterations. During ischemia, LSECs and Kupffer cells swell, leading to reduced bioavailability of nitric oxide and increased production of endothelin and thromboxane A2, which narrows sinusoids and impairs microcirculation. Reperfusion exacerbates this condition by inducing the release of reactive oxygen species (ROS) and pro-inflammatory cytokines, which facilitate neutrophil infiltration, platelet aggregation, and Kupffer cell and hepatic stellate cell activation. This cascade of events leads to compromised microcirculation, persistent vasoconstriction, oxidative stress, and ultimately hepatocyte death, positioning endothelial cell damage as the critical initial event in hepatic IRI.[12–14]

Leukocyte cell-derived chemotaxin 2 (LECT2) is a 16 kDa secreted protein that acts as a neutrophil chemotactic factor.[15] It participates in various physiological and pathological processes, including sepsis, diabetes, liver cancer, non-alcoholic fatty liver disease, and hematopoietic stem cell homeostasis.[16–18] However, its regulatory functions in vascular endothelial cells and IRI are unclear. Previously, we identified LECT2 as a functional ligand of the orphan receptor Tie1 in vascular endothelial cells, revealing the novel regulatory LECT2/Tie1 signaling pathway.[19] This pathway regulates liver injury repair by modulating hepatic vascular function and remodeling, indicating a potential strategy for treating liver fibrosis by precisely normalizing different vascular sites.[19–21] This study investigated the role of LECT2 in hepatic IRI, demonstrating its upregulation during liver IRI and exploring the therapeutic potential of adeno-associated virus (AAV)-Lect2-small hairpin RNA (shRNA) in treating hepatic IRI.

2. Materials and methods

2.1. Ethical approval

The study protocols concerning human subjects are consistent with the principles of the Declaration of Helsinki and were approved by the Clinical Research Ethics Committee of The First People's Hospital of Zhaoqing, Zhaoqing, Guangdong, China (No. B2024-07-12). Written informed consent was obtained from all study participants. Animal research protocols adhered to the U.S. Public Health Service Policy on Laboratory Animal Use and were approved by the Ethics Committee on Animal Care and Use at Southern Medical University, Guangzhou, Guangdong, China (No. NFYY-2018-031).

2.2. Patients and clinical specimens

Liver tissues and serum samples were obtained from five patients who had undergone the Pringle maneuver at the Department of Hepato-Biliary-Pancreatic and Hernia Surgery, The First People's Hospital of Zhaoqing. Hepatectomies were performed for liver tumors, such as hepatic hemangioma and hepatocellular carcinoma. Patient information is presented in Supplemental Table 1.

2.3. Mice

Lect2-knockout (KO) mice (*Lect2*$^{-/-}$) were generated following the CRISPR/Cas9 strategy, whose genotype was previously identified.[19] In brief, extracted genomic DNA was subjected to polymerase chain reaction (PCR) amplification using specific primers (forward, 5′-GTGAGACTTAGATGTGGGAAGTTCCTG-3′; reverse, 5′-CACCCT-GAGGTATTCAGGCCATTAT-3′). C57BL/6J mice were sourced from the Guangdong Medical Laboratory Animal Centre in Guangzhou, China. All experiments were conducted on male mice. The mice were housed under specific pathogen-free conditions with a standard 12-h light–dark cycle and had unlimited access to food and water. All mice examined in this study were healthy and had normal immune function.

To construct the hepatic IRI model, male mice aged 6–8 weeks were fasted for 12 h and anesthetized with isoflurane (#R510-22-10, RWD Life science Co. Ltd, Shenzhen, China). A midline abdominal incision (3–5 cm) was made, and the intestines were gently moved aside to expose the portal vein. The hepatic portal was separated from the surrounding tissues, and a microvascular clamp (#W40140, Shanghai medical instrument Co. Ltd, Shanghai, China) was used to block the portal triad structures, i.e., hepatic artery, portal vein, and bile duct, of the left and median lobes. Successful clamping was confirmed by the lobes turning white. After 60 min of ischemia, reperfusion was initiated by removing the clamp, which resulted in an immediate color change in the median and left lateral lobes. The incision was closed using a two-layer suture technique, and disinfectant was applied to the surgical area to reduce the risk of infection. Then, the mice were placed in heated, clean cages. Samples were collected at reperfusion times of 0.5 h, 6 h, 24 h, and 48 h. Recombinant human LECT2 (rLECT2) and AAV-Lect2-shRNA were prepared and characterized as before.[19] Mice were treated with rLECT2 (10 mg/kg, intravenously; #ab188467, Abcam, Cambridge, MA, USA) or AAV-Lect2-shRNA (1×10^{11} vg/mouse, intravenously; #46081404, Hanbio Biotechnology Co. Ltd, Shanghai, China) (2 weeks before performing hepatic IRI). The serum samples and liver tissues were harvested at different time points for subsequent analyses. The sequence for shRNA targeting Lect2 is as follows: 5′-GCTAACATATGTGCCAGCAAATCTT-3′, and the control, 5′-TTCTCGAACGTGTCACGT-3′.

2.4. Histological analysis of hepatic necrosis using hematoxylin and eosin (HE) staining

Liver tissues were collected at reperfusion times of 0.5, 6, 24, and 48 h and then fixed in 10% neutral-buffered formalin for 24 h. The tissues were processed, embedded in paraffin, and sectioned into 2.5-μm-thick slices. Sections were stained with HE following standard protocols. The stained sections were examined under a light microscope (Olympus, Central Valley, PA, USA) to assess liver morphology and identify necrotic areas. Necrosis was quantified using image analysis software ImageJ (General Public License); the percentage of the necrotic area relative to the total liver tissue area was calculated based on histological features, such as loss of cellular architecture, increased eosinophilia, and cell debris.

2.5. Quantitative reverse-transcription PCR (qRT-PCR)

Total RNAs from human patient and mouse livers were extracted and reverse transcribed. The resulting cDNAs were used for PCR with the SYBR-Green Master PCR Mix (Accurate Biology, Changsha, China). All PCR reactions were conducted in triplicate using the QuantStudio 3 real time-PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Data for each sample were normalized to endogenous GAPDH controls. The primers for real time-PCR were

as follows: human LECT2: forward, 5′-GCTGGTCTGATTTCTACCGCA-3′; reverse, 5′-CCAGCAGAGCACAAGATGTC-3′; mouse Lect2: forward, 5′-GGACGTGTGACAGCTATGGC-3′; reverse, 5′-TCCCAGTGAATGGTGCATACA-3′; mouse IL-6: forward, 5′-GCTACCAAACTGGATATAATCAGGA-3′; reverse, 5′-CCAGGTAGCTATGGTACTCCAGAA-3′; mouse tumor necrosis factor-alpha (TNF-α): forward, 5′-AGCCCACGTAGCAAACCACCAA-3′; reverse, 5′-ACACCCATTCCCTTCACAGAGCAAT-3′; mouse interleukin-1beta (IL-1β): forward, 5′-TGACCTGGGCTGTCCAGATG-3′; reverse, 5′-CTGTCCATTGAGGTGGAGAG-3′; human GAPDH: forward, 5′-ACATGTTCCAATATGATTCCACC-3′; reverse, 5′-TACTCCTTGGAGGCCATGTG-3′; and mouse GAPDH: forward, 5′-AGAAGGTGGTGAAGCAGGCATC-3′; reverse, 5′-CGAAGGTGGAAGAGTGGGAGTTG-3′.

2.6. Measurement of serum biomarkers

Serum LECT2 levels were measured using enzyme-linked immunosorbent assay. The assay kits for human LECT2 (Cat. No. SEF541Hu) and mouse LECT2 (Cat. No. SEF541Mu) were purchased from Wuhan USCN Life Science Inc. (Wuhan, China). A fully automated biochemical analyzer (BS-240VET, Shenzhen Mindray Medical Equipment Co., Ltd., Shenzhen, China) was used to calculate the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (Cr), and blood urea nitrogen (BUN) in mice.

2.7. Reactive oxygen species (ROS) detection

ROS were detected using an ROS assay kit (Beyotime Biotechnology, Shanghai, China). The assay was performed according to the manufacturer's instructions.

2.8. Statistical analysis

The results are expressed as mean ± standard deviation. Data analysis was conducted using Student's *t*-test. Statistical evaluations and graph generation were performed using GraphPad Prism Software (San Diego, CA, USA). A *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. The LECT2 expression was elevated in clinical hepatic IRI

Temporary clamping of the hepatic pedicle using the Pringle maneuver is a common strategy to reduce bleeding during hepatectomy, and it can lead to hepatic IRI injury. Serum and liver tissue samples were collected from patients preoperatively, post ischemia, post reperfusion, and postoperatively, and LECT2 expression levels were measured (Fig. 1A). Serum LECT2 protein levels (Fig. 1B) and LECT2 mRNA levels in liver tissues (Fig. 1C) increased during ischemia and reperfusion.

3.2. The LECT2 expression was elevated in mouse hepatic IRI model

Using a mouse hepatic IRI model, liver ischemia was induced by clamping the hepatic pedicle for 1 h, followed by reperfusion. LECT2 expression was measured in serum and liver tissue samples collected at various time points (Fig. 1D). Results indicated that serum LECT2 protein levels increased at 0.5 h post reperfusion (Fig. 1E), and high LECT2 mRNA levels in liver tissues sustained from 0.5 to 24 h post reperfusion, decreasing at 48 h (Fig. 1F).

3.3. Hepatic IRI was reduced in *Lect2*$^{-/-}$ mice

Lect2-KO mice were used to determine whether Lect2 plays a critical role in hepatic IRI.[19] Compared with wild-type controls, *Lect2*$^{-/-}$ mice exhibited less weight loss post reperfusion at 24 and 48 h (Fig. 2A), reduced hepatic necrosis areas (Fig. 2B and C), and lower serum ALT and AST levels (Fig. 2D and E), indicating milder liver damage. In addition, *Lect2*$^{-/-}$ mice showed lower serum Cr and BUN levels, suggesting less renal injury (Fig. 2F and G).

3.4. rLECT2 exacerbated hepatic IRI

To further confirm the role of LECT2 in hepatic IRI, mice were treated with rLECT2 (Fig. 3A). rLECT2 led to greater weight loss (Fig. 3B), increased hepatic necrosis areas (Fig. 3C and D), and high serum ALT and AST levels (Fig. 3E and F), indicating exacerbated liver damage. Moreover, rLECT2-treated mice had higher serum Cr and BUN levels, indicating aggravated renal injury (Fig. 3G and H).

3.5. AAV-Lect2-shRNA as a therapeutic strategy for hepatic IRI

The therapeutic potential of targeting LECT2 for hepatic IRI was explored using AAV as a delivery vector. AAV-Lect2-shRNA was constructed to knock down Lect2 expression *in vivo* (Fig. 4A). Results demonstrated that AAV-Lect2-shRNA significantly reduced weight loss (Fig. 4B), hepatic necrosis areas (Fig. 4C and D), and serum ALT and AST levels (Fig. 4E and F). Additionally, AAV-Lect2-shRNA-treated mice exhibited lower serum Cr and BUN levels, indicating reduced renal injury (Fig. 4G and H). Oxidative stress-induced ROS production and inflammatory factor release are primary contributors to IRI. AAV-Lect2-shRNA treatment was observed to significantly lower ROS levels and reduce the expression of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α (Fig. 4I–L).

4. Discussion

This study demonstrates that LECT2 expression is upregulated during hepatic IRI and that recombinant LECT2 exacerbates it, whereas *Lect2* KO mice ameliorate liver damage. Moreover, AAV-Lect2-shRNA has shown potential as a therapeutic strategy for hepatic IRI.

IRI is common in various traumatic and surgical events and has long been considered a critical cause of clinical sequelae.[22] IRI is a complex cascade initiated by ischemic events, resulting in oxidative stress and excessive ROS production during ischemia and causing cellular organelle damage and cell death.[23,24] Upon reperfusion, cellular damage and death are exacerbated by the restoration of oxygen delivery and shear stress, resulting in an overwhelming inflammatory response that significantly disrupts endothelial and epithelial barriers, causing IRI. Severe IRI and the ensuing inflammatory response can cause acute respiratory distress syndrome or systemic inflammatory response syndrome, which is central to the pathogenesis of multiple organ failure, with mortality rates reaching 70%.[25–28] Currently, treatment options for IRI are mainly palliative, addressing patient discomfort but not improving the underlying condition.[29] AAVs have been used as safe and effective drug delivery vectors in clinical settings,[30,31] and this study demonstrated that AAV-Lect2-shRNA significantly alleviates hepatic IRI, demonstrating its clinical potential. In the IRI model, *Lect2* KO mice exhibited a significant reduction in body weight 24–48 h after reperfusion. In contrast, mice with *Lect2* knockdown induced by AAV-Lect2-shRNA experienced significant weight loss at 6 h; however, this effect was not observed at 24–48 h. This discrepancy may be attributed to compensatory mechanisms caused by the

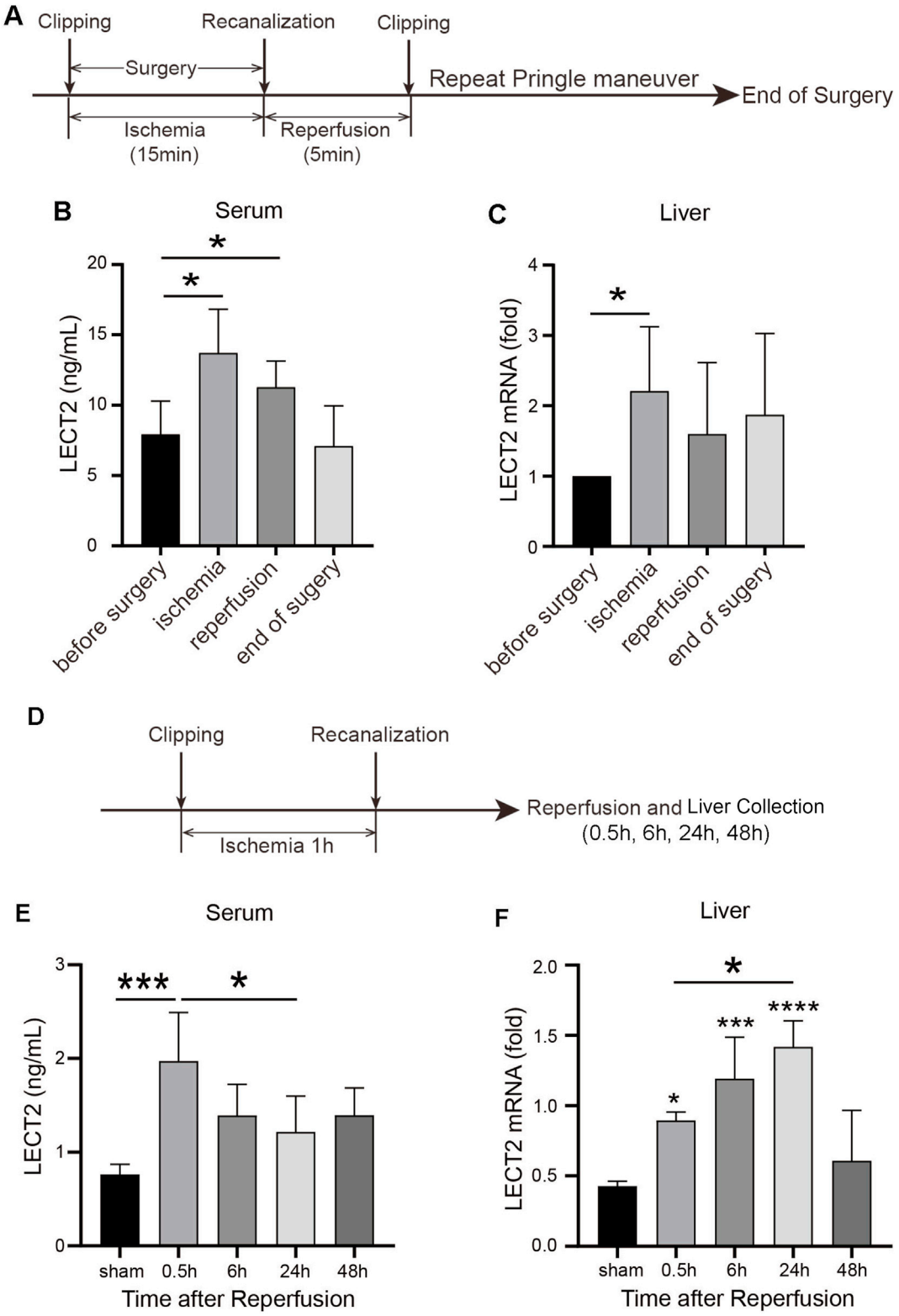

Fig. 1. Expression level of leukocyte cell-derived chemotaxin 2 (LECT2) was upregulated in patients who had undergone the Pringle maneuver and in mice with hepatic ischemia–reperfusion injury. (A) Schematic diagram of the surgical process for the Pringle maneuver. **(B)** Serum LECT2 concentration in patients who had undergone the Pringle maneuver. **(C)** mRNA level of LECT2 in the liver of patients who had undergone the Pringle maneuver. Number of patients = 5. **(D)** Schematic diagram of the experimental design. C57BL/6J mouse livers were clamped at the hepatic pedicle for 1 h, followed by reperfusion. LECT2 expression was measured in serum and liver tissue samples collected at various time points. **(E)** Serum concentration of LECT2. **(F)** Hepatic mRNA level of LECT2. $n = 6$ for each group. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

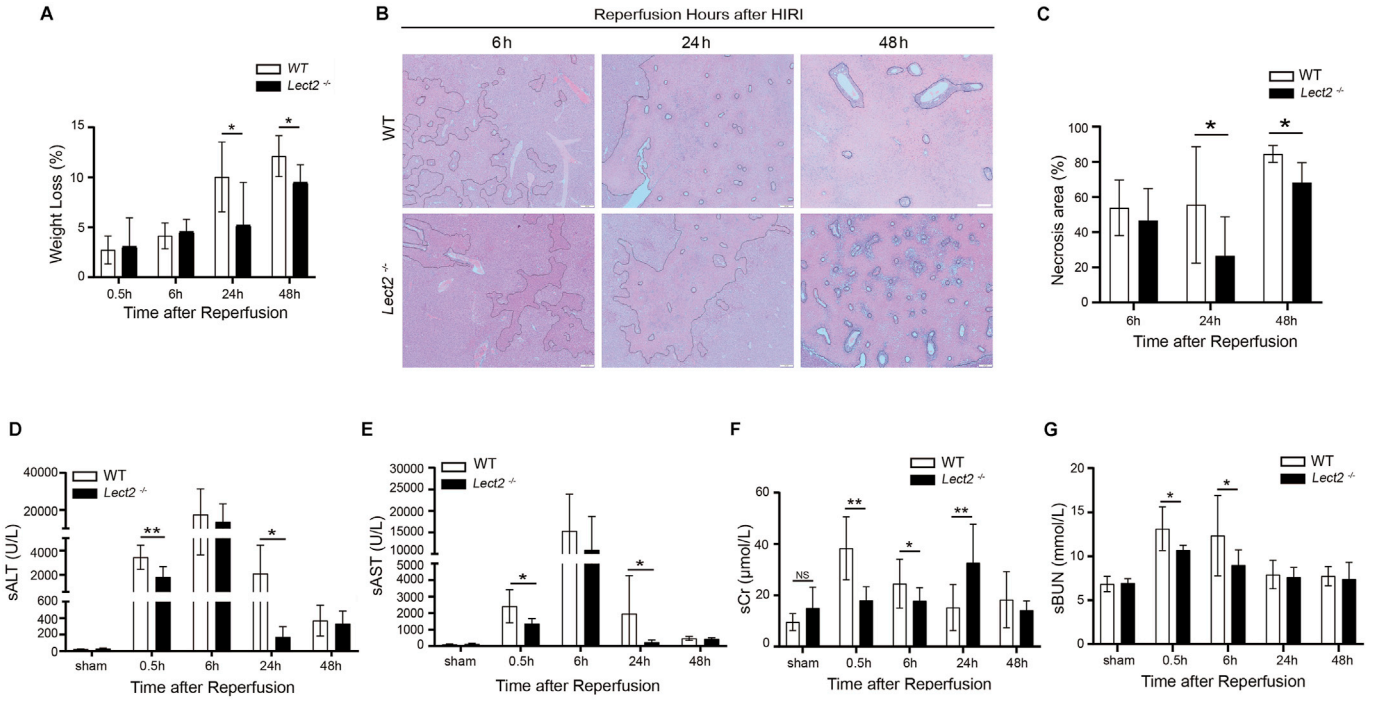

Fig. 2. Hepatic IRI (HIRI) was reduced in *Lect2*$^{-/-}$ mice. The livers of *Lect2*$^{-/-}$ mice and their littermate controls were clamped at the hepatic pedicle for 1 h, followed by reperfusion. Serum and liver tissue samples were collected at various time points. **(A)** The mouse body weight was measured at the indicated time. **(B)** HE staining in livers showed necrosis. Scale bar, 100 μm. **(C)** Statistics of the liver necrosis areas. **(D–G)** Serum levels of alanine aminotransferase (ALT) (D), aspartate aminotransferase (AST) (E), creatinine (Cr) (F), and blood urea nitrogen (BUN) (G) were measured. $n = 6$ for each group. $^{*}P < 0.05$, $^{**}P < 0.01$. Abbreviations: HE, hematoxylin and eosin; IRI, ischemia–reperfusion injury; Lect2, leukocyte cell-derived chemotaxin 2; WT, wild-type.

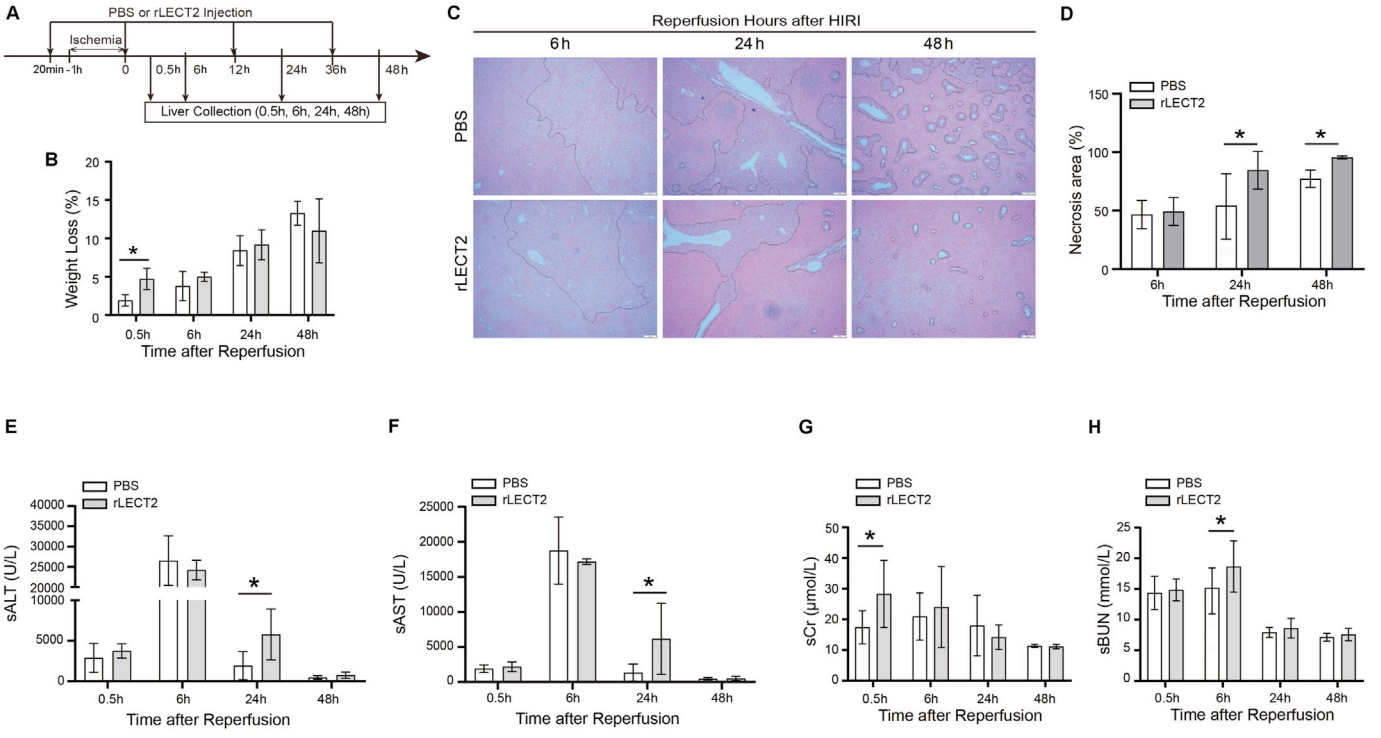

Fig. 3. Recombinant human leukocyte cell-derived chemotaxin 2 (rLECT2) exacerbated hepatic IRI (HIRI). (A) Schematic diagram of the experimental design. C57BL/6J mouse livers were clamped at the hepatic pedicle for 1 h, followed by reperfusion. rLECT2 was intravenously injected. Serum and liver tissue samples were collected at various time points. **(B)** The mouse body weight was measured at the indicated time. **(C)** HE staining of the liver tissues showed necrosis. Scale bar, 100 μm. **(D)** Statistics of the liver necrosis areas. **(E–H)** Serum levels of alanine aminotransferase (ALT) (E), aspartate aminotransferase (AST) (F), creatinine (Cr) (G), and blood urea nitrogen (BUN) (H) were measured. $n = 6$ for each group. $^{*}P < 0.05$. Abbreviations: HE, hematoxylin and eosin; IRI, ischemia–reperfusion injury.

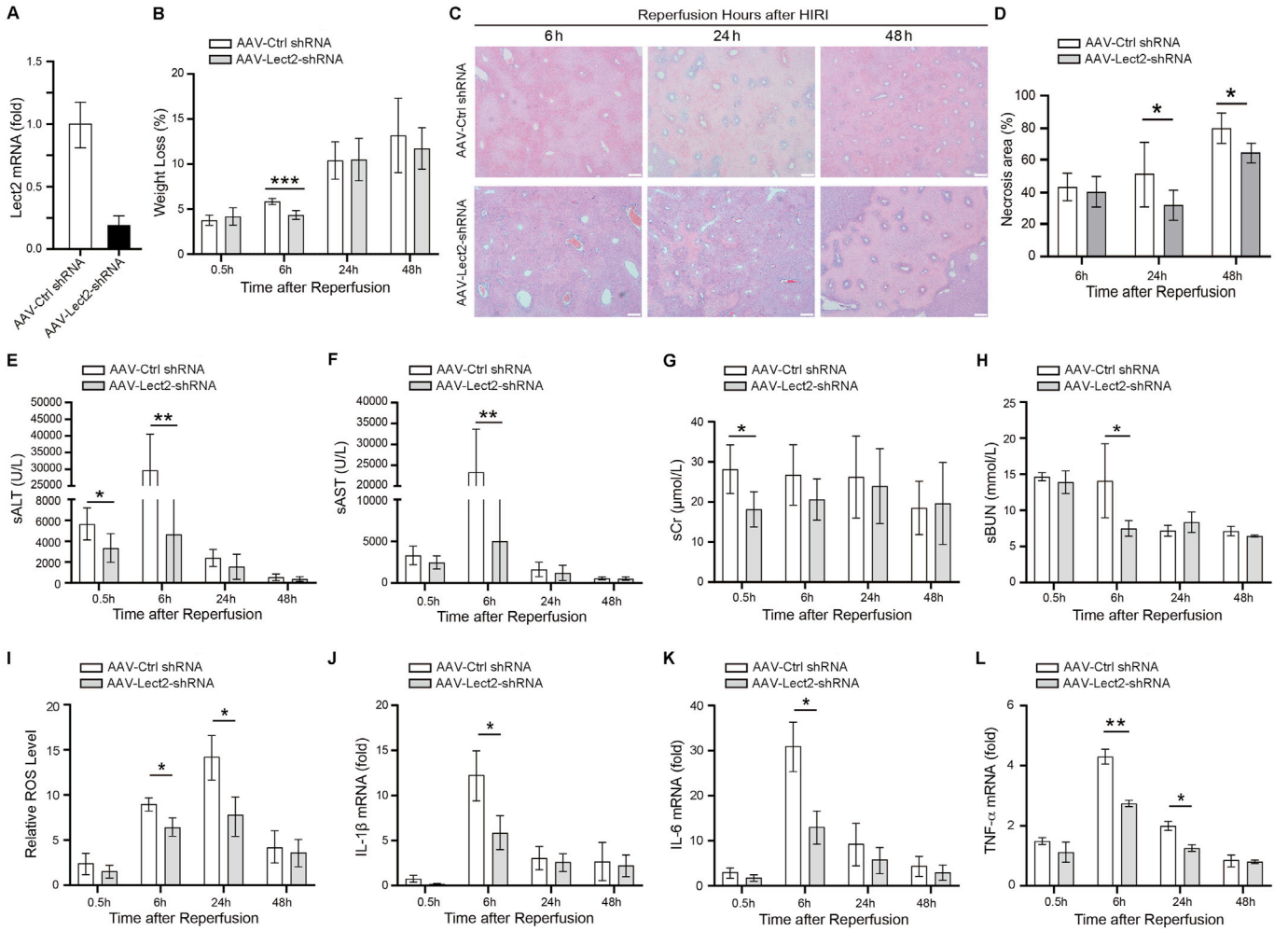

Fig. 4. AAV-Lect2-shRNA decreased hepatic IRI (HIRI). C57BL/6J mouse livers were clamped at the hepatic pedicle for 1 h, followed by reperfusion. AAV-Lect2-shRNA was intravenously injected. Serum and liver tissue samples were collected at various time points. **(A)** The knockdown effect of AAV-Lect2-shRNA. **(B)** The mouse body weight was measured at the indicated time. **(C)** HE staining of liver tissues showed necrosis. Scale bar, 100 μm. **(D)** Statistics of liver necrosis areas. **(E–H)** Serum levels of alanine aminotransferase (ALT) (E), aspartate aminotransferase (AST) (F), creatinine (Cr) (G), and blood urea nitrogen (BUN) (H) were measured. **(I–L)** The levels of ROS (I), IL-1β (J), IL-6 (K), and TNF-α (L) in liver tissues were measured. $n = 6$ for each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Abbreviations: AAV, adeno-associated virus; HE, hematoxylin and eosin; IL, interleukin; IRI, ischemia–reperfusion injury; Lect2, leukocyte cell-derived chemotaxin 2; ROS, reactive oxygen species; shRNA, short hairpin RNA; TNF-α, tumor necrosis factor-alpha.

continuous absence of Lect2 in *Lect2* KO mice, potentially delaying the manifestation of IRI-induced weight changes. In addition to LECT2 knockdown, the use of a neutralizing antibody targeting LECT2 presents a promising therapeutic strategy for IRI, with potentially greater clinical applicability.

LECT2, a multifunctional protein, interacts with various receptors. For instance, LECT2 interacts with CD209a to regulate bone marrow stem cell activity and macrophage function and with c-MET to regulate hepatocyte malignancy.[18] In the present study, LECT2 was identified as a ligand for the vascular endothelial cell receptor Tie1, regulating hepatic vascular remodeling and fibrosis progression.[19–21] Microvascular dysfunctions, including imbalances in vasodilation and contraction, increased vascular permeability, endothelial cell inflammation, and activation of the coagulation and complement systems, are crucial clinical manifestations of IRI. Endothelial cells can induce various inflammatory cell infiltrations and cytokine production through multiple signaling factors, playing a critical regulatory role in IRI.[12,13] In this study, serum LECT2 protein levels increased during the Pringle maneuver, indicating that LECT2 might serve as a potential biomarker for IRI and modulate IRI by targeting LSECs. However, given the small sample size, further validation with a larger cohort is necessary to confirm these findings.

Our previous study identified that LECT2 binds to Tie1, leading to a transition from Tie1/Tie2 heterodimers to Tie2/Tie2 homodimers. This binding results in Tie1 dephosphorylation and Tie2 phosphorylation, subsequently activating the MAPK signaling pathway.[19] MAPK, a pivotal extracellular signal-regulated kinase, plays a critical role in cellular responses to oxidative stress, pro-inflammatory cytokines, UV radiation, and heat shock. MAPK activation further stimulates the production of TNF-α and IL-6 and increases ROS levels.[32,33] Therefore, LECT2 may modulate hepatic IRI via the Tie1/Tie2/MAPK pathway. However, further experimental studies are warranted to clarify the specific mechanisms involved.

This study had several limitations. First, clinical validation was conducted on a small cohort of patients, limiting the generalizability of the results. Larger studies are warranted to confirm these results. Second, although mouse models were instrumental in investigating the role of LECT2 in hepatic IRI, they may not fully replicate human conditions, necessitating further studies in human participants. In addition, the precise mechanisms by which LECT2

regulates hepatic IRI remain unclear, and more studies are needed to elucidate the underlying molecular pathways. Finally, AAV-Lect2-shRNA shows promise as a therapeutic approach, but the long-term safety and efficacy of this gene therapy in clinical settings remain to be determined through comprehensive trials.

5. Conclusions

This study demonstrated that high LECT2 levels during hepatic IRI exacerbate tissue necrosis and increase levels of serum biomarkers, indicating severe liver damage. Conversely, genetic knockout or shRNA-mediated knockdown of LECT2 reduced tissue damage and improved outcomes, indicating the potential of LECT2 as a therapeutic target. The use of AAV vectors in LECT2-shRNA delivery can aid in the mitigation of hepatic IRI, offering a novel treatment strategy to enhance clinical outcomes for patients undergoing liver-related surgeries or trauma.

Data availability statement

Data are contained within the article or supplementary material. The data presented in this study are available on request from the corresponding author Wei-Jie Zhou.

Authors' contributions

Meng-Qi Dong and Yuan Xie contributed equally to this work. **Meng-Qi Dong:** Investigation, Data curation. **Yuan Xie:** Resources, Investigation. **Zhi-Liang Tang:** Resources. **Xue-Wen Zhao:** Resources. **Fu-Zhen Lin:** Resources. **Guang-Yu Zhang:** Resources. **Zhi-Hao Huang:** Investigation, Data curation. **Zhi-Min Liu:** Investigation. **Yuan Lin:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Feng-Yong Liu:** Writing – review & editing, Supervision, Investigation. **Wei-Jie Zhou:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that there is no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.livres.2024.09.004.

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