RESEARCH ARTICLE

IL-11 Attenuates Liver Ischemia/Reperfusion Injury (IRI) through STAT3 Signaling Pathway in Mice

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Abstract

Background
The protective role of IL-11, an IL-6 family cytokine, has been implicated in ischemia/reperfusion injury (IRI) in the heart and kidney, but its role has not been elucidated in liver IRI. This study was designed to evaluate the effects of IL-11 and its mechanism of action on liver IRI in a mouse model.

Methods
A partial (70%) warm liver IRI was induced by interrupting the artery/portal vein blood supply to the left/middle liver lobes. IL-11 mRNA expression of ischemic liver after reperfusion was analyzed. Signal transducer and activator of transcription 3 (STAT3) was analyzed following IL-11 treatment in vivo and in vitro. Next, IL-11 was injected intraperitoneally (ip) 1 hour before ischemia. Liver injury was assessed based on serum alanine aminotransferase levels and histopathology. Apoptosis and inflammation were also determined in the ischemic liver. To analyze the role of STAT3 in IL-11 treatment, STAT3 siRNA or non-specific (NS) siRNA was used in vitro and in vivo.

Results
IL-11 mRNA expression was significantly increased after reperfusion in the ischemic liver. STAT3, as a target of IL-11, was activated in hepatocytes after IL-11 treatment in vivo and in vitro. Next, effects of IL-11/STAT3 signaling pathway were assessed in liver IRI, which showed IL-11 treatment significantly attenuated liver IRI, as evidenced by reduced hepatocellular function and hepatocellular necrosis/apoptosis. In addition, IL-11 treatment significantly inhibited the gene expressions of pro-inflammatory cytokines (TNF-α and IL-10) and chemokines (IP-10 and MCP-1). To determine the role of STAT3 in the hepatoprotective
effects of IL-11, STAT3 siRNA or NS siRNA was used prior to IL-11 treatment. The results showed STAT3 knockdown abrogated the protective effects of IL-11 in vitro and in vivo.

Conclusions
This work provides first-time evidence for the protective effect of IL-11 treatment on hepatocyte in liver IRI, through the activation of the STAT3 pathway.

Introduction
Ischemia-reperfusion injury (IRI) is a key contributing factor in liver dysfunction and failure after hepatic trauma, resection, liver transplantation, and circulatory shock [1–4]. An effective method for preventing or minimizing liver IRI is urgently needed in liver surgery. The factors/pathways have been involved in the hepatic IRI process include anaerobic metabolism, mitochondria damage, oxidative stress, endoplasmic reticulum stress, intracellular calcium overload, Kupffer cell (KC) activation, neutrophil infiltrations, and production of cytokines and chemokines [1–3]. The adverse factors mentioned above finally lead to cell death/apoptosis, which indicates that cell death/apoptosis is a significant and perhaps principal contributor to liver IRI [5–7]. Thus, understanding the sequence of events central to the cell death/apoptosis mechanism may potentially lead to treatments for liver IRI.

IL-11 is a hematopoietic IL-6 family cytokine with multifunctional effects. Indeed, IL-11 has thrombopoietic activity, and recombinant human IL-11 has been used for thrombocytopenia in clinical settings [8]. Different from other IL-6 family cytokines, IL-11 holds anti-inflammatory function against chronic inflammatory diseases, lipopolysaccharide-induced sepsis, etc [9–11]. Kimura’s group reported that IL-11 played a cardioprotective role, and conferred resistance to heart IRI in a mouse model by enabling significant anti-necrotic/apoptotic effects [12]. In addition, IL-11 pretreatment also reduces IR-induced cell death/apoptosis by up-regulating Bcl-2 [13]. More importantly, IL-11 shares some similar effects to other IL-6 family members. It has been reported that IL-11 binding with gp130 receptor induces activation of STAT3, which is involved in many physiological and pathological processes [14]. Inhibitors of STAT3 phosphorylation or dominant-negative STAT3 mutants facilitate the expression of pro-apoptosis factors, suggesting that STAT3 plays a critical role in regulating cell proliferation and anti-apoptosis [15]. Furthermore, STAT3 knockout mice exhibit complete embryonic lethality [16]. Conditional ablation of STAT3 in myocardial cells leads to higher susceptibility to drug-induced heart failure [17]. To the best of our knowledge, there has been no report on IL-11 preconditioning before liver IRI. In the present study, we tested the hypothesis that exogenous IL-11 attenuates liver IRI by STAT3-mediated anti-necrotic/apoptotic effects.

Materials and Methods
Animals
Male C57BL/6 mice were purchased from the Laboratory Animal Resources Center of Nanjing Medical University (NMU). The animals were fed a laboratory diet with water and food and kept under constant environmental conditions with 12h light–dark cycles. Procedures were carried out in accordance with the Guidelines for Laboratory Animal Care. The animal protocol had been approved by the Institutional Animal Care & Use Committee (IACUC) of Nanjing Medical University (Protocol Number NMU08-092).
Surgical procedure and IL-11 treatment

The present study used a well-established mouse model of partial (70%) warm hepatic IRI [18]. Anesthesia was induced by 10% chloral hydrate (0.3g/kg, intraperitoneally). Mice were injected with heparin (100U/kg), and an atraumatic clip was used to interrupt the artery/portal vein blood supply to the left/middle liver lobes. After 90 minutes of ischemia, the clip was removed, and the mice were sacrificed (cervical dislocation) at required times after reperfusion. Some mice received a single injection of recombinant human IL-11 (500μg/kg, ip) (PeproTech, Rocky Hill, NJ) or medium (PBS) 1 hour prior to ischemia. PBS injection was used as a control. Sham-operated controls underwent the same procedure but without vascular occlusion. To access effects of STAT3 on IL-11 treatment, STAT3 siRNA or NS siRNA (2mg/kg) was given intravenously 4 hours prior to ischemia [19]. Reports have previously documented the efficacy of this siRNA approach in the liver, with >40% of intravenously infused siRNA accumulating in the ischemic mouse livers [20].

Serum biochemical examination

Blood samples collected 6h after reperfusion was centrifuged to obtain serum. The serum level of alanine aminotransferase (sALT) or supernatant level of lactate dehydrogenase (LDH) was measured to assess the extent of hepatocyte damage using an automated chemical analyzer (Olympus Automated Chemistry Analyzer AU5400, Tokyo, Japan).

Histopathologic study

Liver specimens were fixed with 10% neutral formaldehyde and then embedded in paraffin. The specimens were sectioned at 4μm and stained with hematoxylin and eosin. The sections were used in histopathologic analysis by light microscopy. Sections were scored from 0 to 4 for sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchymal, as described by Suzuki et al [21].

Caspase-3 activity assay

Caspase-3 activity was assayed in liver tissues 6h after reperfusion. Frozen samples of ischemic tissues were homogenized with a Polytron homogenizer and centrifuged at 16,000g for 20 minutes. Activity was measured with an assay kit (Calbiochem) according to the manufacturer’s instructions.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining

Paraffin sections (4μm in thickness) were deparaffinized in toluene and then dehydrated in a graded series of ethanol solutions. Sections were stained by TUNEL using a commercially available kit (in situ cell death detection kit, Roche-Boehringer Mannheim, Germany).

Western blot analysis

Proteins were extracted from liver tissues subjected to ischemia or from cell lysates, and their concentrations were determined by the Bradford assay (Bio-Rad, CA). About 30 μg protein was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Sunshine Biotechnology, China). These membranes were blocked in skim milk powder (5% wt/vol) with phosphate buffered saline containing 0.1% Tween 20 (PBS-T) at 4°C overnight. Membranes were then incubated with primary antibodies for cleaved Caspase-3, P-STAT3, Bcl-2, Bax, β-actin (Cell Signaling Technology, Danvers, MA), and...
STAT3 (Santa Cruz Biotechnology, Santa Cruz, CA). Following three washes with PBS-T, the membranes were incubated for 1h at room temperature with peroxidase-conjugated secondary antibody (Cell Signaling Technology, Danvers, MA). The final results were obtained by exposure to autoradiographic film (Kodak XAR film), and then visualized via a chemiluminescent detection system (ECL Substrate Western blot detection system, Pierce, IL).

Quantitative real-time PCR

Quantitative real-time PCR was performed using the DNA Engine with a Chromo 4 Detector (MJ Research, Waltham, MA). In a final reaction volume of 25μl, the following were added: 1×SuperMix (Platinum SYBR Green qPCR Kit; Invitrogen), cDNA, and 2.5μM of each primer. The amplification conditions were as follows: 50°C (2 min), 95°C (5 min), followed by 50 cycles at 95°C (15 sec) and 60°C (30 sec). The expression of the target genes (IL11, TNF-α, IL-6, IP-10 and MCP-1) (Invitrogen, Shanghai, China) was calculated based on the ratio of the gene of interest to the housekeeping gene HPRT. Primer sets (sense sequence and antisense sequence, respectively) for the following genes were: HPRT forward, 5'-TCA ACG GGG GAC ATA AAA GT-3', reverse, 5'-TGC ATT GTT TTA CCA GTG TCA A'; IL-11 forward: 5'-CTG CCC ACC TGG GCC ATG AG-3'; IL-11 reverse: 5'-CCA GGC GAG ACA TCA AGA A-3'; TNF-α forward, 5'-GCC TCT TCT CAT TCC TGC TTG T-3', reverse, 5'-TTG AGA TCC ATG CCG TTG-3'; IL-6 forward, 5'-GCT ACC AAA CTG GAT ATA ATC AGG A-3', reverse, 5'-CCA GGT AGC TAT GGT ACT CCA GAA-3'; IP-10 forward, 5'-GCT GCC GTC ATT TTC TGC-3', reverse, 5'-TCT CAC TGG CCC GTC ATC-3'; MCP-1 forward, GGT GAT AAC CGC CCT AGC-3', reverse, 5'-TGT GTC GGC TGG ATA GGC-3'.

Cell culture and treatment

Mouse hepatocytes were isolated using a two-step in situ collagenase perfusion procedure [18]. Livers from the C57BL/6 mice were perfused in situ through the portal vein with ethylene glycol tetraacetic acid (EGTA) buffer (0.5mM EGTA, 137mM NaCl, 4.7mM KCl, 1.2mM KH2PO4, 0.65mM MgSO4, and 10.07mM HEPES at pH 7.4) at a flow rate of 5ml/min for 10 min, followed by collagenase buffer (67mM NaCl, 6.7mM KCl, 4.76mM CaCl2, 0.035% collagenase type II, and 10.07mM HEPES at pH 7.6) at a flow rate of 5ml/min for 15 min. After centrifugation, the hepatocytes were collected and seeded in DMEM containing 10% FBS, 100units/ml penicillin, and 100μg/ml streptomycin. Cells were preincubated with IL-11 (1μg/ml for 1h), then H2O2 (200μM for 24h) to induce cell death.

Knockdown of STAT3 expression using STAT3 siRNA transfection

Hepatocytes were grown and transiently transfected with STAT3 siRNA or NS siRNA using Transfection Reagent LipofectamineTM RNAiMAX (Invitrogen, CA, USA) according to the manufacturer’s instructions. In brief, cells were seeded at 1 x 10⁶ per well in 1.5ml of OPTI-medium (Invitrogen, CA, USA) in a 6-well plate. After 20h, the cells were transfected with 20nmol/ml STAT3 siRNA or NS siRNA. About 6h after transfection, the medium was changed to a regular medium, and the cells were treated as described above after 24h.

Statistical analysis

The data are presented as the mean ± SEM from at least three independent experiments. One-way analysis of variance test [—] was used in comparisons of three groups. Student’s t-test [II] was used for comparison of two groups. All P values were two-sided, and P<0.05 was considered to be statistically significant.
Results

IL-11 is elevated in IR-stressed liver

To determine the effects of IL-11 on liver IRI, we first analyzed the gene expression of IL-11 in ischemic livers after various reperfusion time points. As shown in Fig 1, the expression of IL-11 was increased at 0h post reperfusion and reached its peak 3h post reperfusion. The data indicate that IL-11 was present in the liver 3h after reperfusion.

IL-11 activates STAT3 in liver and hepatocytes

IL-11, an IL-6 family cytokine, was supposed to activate STAT3. Here we determined whether administration of IL-11 stimulates STAT3 in liver by western blotting with antibody (Fig 2A). Phosphorylation of STAT3 was rapidly induced and recovered nearly to baseline after 2 hours. To further ascertain that phosphorylation of STAT3 occurred in hepatocytes, we analyzed the effects of IL-11 on STAT3 activity of hepatocytes in vitro. Fig 2B shows phosphorylation of STAT3 was also significantly elevated after IL-11 treatment, indicating that IL-11 administration may activate STAT3 within parenchymal cells in the liver.

IL-11 attenuates liver IRI

Next, we analyzed effects of IL-11 administration in liver IRI. Mouse livers were subjected to 90 min of warm ischemia 6h after reperfusion. sALT levels in each group were analyzed (Fig 3A). sALT levels were markedly increased in the IR group compared with that of the sham group (33.33±5.49 and 10610.00±1393.00, respectively; *P<0.01). By contrast, when mice were pretreated with IL-11, sALT levels (3832.00±834.90, *P<0.01) were significantly decreased compared with those in the IR control. Liver serum enzyme data were in line with liver pathological analysis (Fig 3B and 3C). The histological parameters in the sham (0.25±0.25), IR (3.20±0.37), and IL-11 administration (1.8±0.37) groups were observed according to Suzuki et al [26].

These data indicate that IL-11 treatment significantly attenuates IR-induced liver injury.
IL-11 exhibits anti-apoptotic functions and reduces apoptosis

Apoptosis/necrosis is a central mechanism of cell death in liver IRI. In this study, hepatocellular apoptosis was analyzed in ischemic livers by TUNEL staining 6 hours after reperfusion. Our results showed TUNEL-positive cells were significantly lower in liver sections of the IL-11 treatment group compared with those in IR control group (Fig 4A and 4B). TUNEL-positive cells in the total hepatocytes of the three groups were (0.60±0.25)%, (10.20±1.28)%, and (4.00±0.63)%, respectively, indicating that hepatocellular apoptosis was significantly reduced by IL-11 administration. In addition, anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax were analyzed in ischemic liver, which showed that IL-11 treatment effectively upregulated Bcl-2 and inhibited Bax expression (Fig 4C). Caspase-cascade system, especially the component Caspase-3, plays a critical role during liver IRI. Caspase-3 affects apoptosis by component cleaved Caspase-3. Next, we assessed the expression of cleaved Caspase-3 by immune analysis, suggesting that IL-11 administration effectively inhibited expression of cleaved Caspase-3 during liver IRI (Fig 4C). Apoptotic active caspase-3 directly caused hepatocellular apoptosis and reflected the progress of apoptosis in the ischemic liver. Along with the TUNEL assay and expression of cleaved Caspases-3, Fig 4D shows that the activity of caspase-3 was significantly repressed after IL-11 preconditioning in ischemic liver tissue compared with the IR group (1.38±0.08 and 4.44±0.289, respectively; P<0.001).

IL-11 regulates inflammatory program and MPO activity in IR-stressed liver

A complex inflammatory program involving cytokines and chemokines is engaged in liver IRI. Inflammatory cytokines (TNF-α and IL-6) and chemokines (IP-10 and MCP-1) displayed
proinflammatory and proapoptotic roles in ischemic liver post-reperfusion. To further assess the hepatoprotective effects of IL-11 treatment, mRNA expressions of TNF-α, IL-6, IP-10 and MCP-1 were determined in ischemic liver after 6h of reperfusion by qRT-PCR. Fig 5A shows a significantly lower level of TNF-α (1.13±0.13 and 0.31±0.04, respectively; \( P < 0.001 \)), IL-6 (6.73±1.12 and 2.43±0.33, respectively; \( P < 0.001 \)), IP-10 (9.69±1.63 and 2.93±0.39, respectively; \( P < 0.001 \)) and MCP-1 (4.08±0.70 and 1.22±0.27, respectively; \( P < 0.001 \)) in the IL-11 administration group compared with that in the IR group. These data indicated that IL-11 effectively inhibited the expression of inflammatory cytokines in ischemic liver after reperfusion. In addition, serum TNF-α was further analyzed by ELISA, which showed IL-11 treatment significantly decreased TNF-α secretion after liver IRI (Fig 5B). The protein level was consistent with gene expression. The MPO activity, reflecting neutrophil activity and infiltration, was reduced after reperfusion in IL-11 treated liver compared with controls (5.40±0.81U/g and 2.40±0.25U/g, respectively; \( P < 0.001 \)) (Fig 5C).
Activation of STAT3 is essential for IL-11-mediated protective role

To evaluate the effect of STAT3 activation in an IL-11-mediated protective role, primary hepatocytes were transiently transfected with STAT3 siRNA or NS siRNA. Next, STAT3 gene expression was assessed after IL-11 treatment by qRT-PCR, which showed STAT3 gene expression was significantly repressed compared with the NS siRNA control (Fig 6A). These data indicated that STAT3 expression was successfully knocked down in hepatocytes. Then, these transfected cells were treated with H2O2 to induce cell death. The released LDH level was checked in the supernatant after H2O2 treatment for 24h. Fig 6B shows that IL-11 treatment remarkably inhibited LDH release of hepatocytes after H2O2 treatment (14.00±4.32 and 14.80±1.99, respectively; P<0.001). However, STAT3 knockdown almost restored the decreased LDH release after IL-11 treatment (14.80±1.99 and 45.80±7.14, respectively; P<0.001). These data indicated STAT3 activation is necessary for IL-11-mediated protective role.
Knockdown of STAT3 restores liver IRI in IL-11-treated mice

To further assess the effect of STAT3 activation in IL-11-mediated hepato-protective role during liver IRI, mice were injected intravenously with STAT3 siRNA or NS siRNA prior to ischemia. As shown in Fig 7A, IL-11 treatment significantly increased expression of P-STAT3, which was reversed in STAT3 siRNA group. Fig 7B shows STAT3 siRNA treatment effectively neutralized the reduced sALT levels after IL-11 treatment (P<0.001) (3795±879U/L versus 11460±1941U/L, respectively). These data correlated with Suzuki’s histological grading of liver IRI (Fig 7C and 7D). Indeed, IL-11 resulted in minimal liver sinusoidal congestion, vacuolization without edema, or necrosis [Fig 7C (c); score = 1.80±0.37]. In contrast, livers in untreated or STAT3 siRNA-treated mice displayed moderate to severe edema and extensive hepatocellular necrosis [Fig 7C (b and d), Fig 7D (panels b and d); score = 3.60±0.25 and 3.40±0.40, respectively]. In addition, cleaved Caspase-3 was further analyzed in ischemic liver, which

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Fig 5. IL-11 treatment inhibited pro-inflammatory responses induced by IR. (A) Cytokine gene (TNF-α, IL-6, IP-10 and MCP-1) expressions were analyzed in ischemic livers by RT-PCR analysis. Expressions of cytokine gene were normalized with that of HPRT. (B) TNF-α secretion was examined in serum by ELISA. (C) MPO activity in ischemic liver. Data are expressed as mean±SD (n = 6/group), *P<0.05, **P<0.001.

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showed IL-11 treatment partially inhibited IR-triggered cleaved Caspase-3 upregulation (Fig 7A). These data indicated knockdown of STAT3 recreates liver IRI in IL-11-treated mice.

Discussion

IL-11 has displayed protective roles during ischemia-reperfusion injury (IRI) in the heart, kidney and intestine, but a similar role has not been elucidated in liver IRI [12, 13, 22, 23]. In this study, we have for the first time demonstrated that IL-11 pretreatment is a promising method for preventing or minimizing liver IRI. Indeed, IL-11 treatment improved liver function, attenuated histology damage, impaired proinflammatory cytokine/chemokine programs, and reduced hepatocellular death/apoptosis in IR-stressed livers. However, the IL-11 mediated protective role was partially impaired by STAT3 siRNA in vivo and in vitro. These findings demonstrate that IL-11 treatment attenuates liver IRI through activating the STAT3 signaling pathway.

IL-11 is a hematopoietic IL-6 family cytokine first identified from marrow-derived stromal cells. It is a key regulator of hematopoesis and promotes megakaryocyte maturation [24]. IL-11 as well as its receptors is expressed in many tissues and cell types, including macrophages, hepatocytes, etc. in liver tissues [25]. Du’s group for the first time reported the protective effects of IL-11 in models of cytoablative chemoradiotherapy in 1994. Then, some researchers have demonstrated that IL-11 play protective roles in various pathophysiologic states [25]. Different from other IL-6 family members, IL-11 administration reduces inflammatory responses in chronic inflammatory diseases, lipopolysaccharide-induced sepsis, macrophages inflammation,
nephrotoxic nephritis and T-cell mediated liver injury [9–11]. The present study also demonstrated that IL-11 treatment effectively inhibited inflammatory responses of ischemic liver, as evidenced by reducing pro-inflammatory cytokines (TNF-α and IL-6) and chemokines (IP-10 and MCP-1), and repressing MPO activity. In addition to its anti-inflammatory properties, IL-11 treatment has been shown to attenuate necrotic and apoptotic cell death in many organs including the heart, intestines and endothelial cells [25]. In fact, apoptosis/necrosis is a key mechanism of cell death in liver IRI, which directly indicates the extent of liver damage. In our
study, hepatocellular death/necrosis as well as apoptosis was also observed in ischemic liver. Our data showed that IL-11 attenuated hepatocellular death/necrosis (ALT, Suzuki’s score) as well as apoptosis (TUNEL staining, cleaved-Caspase 3 fragmentation) in ischemic liver after IRI. In addition, we also analyzed effects of IL-11 on hepatocellular death/necrosis by LDH release level in vitro, which was consistent with in vivo results. Thus, we conclude that IL-11 administration provides liver protection against IRI by reducing inflammation and necrosis/apoptosis.

The molecular mechanisms of IL-11 mediated signaling pathway for an anti-inflammatory and anti-necrosis/apoptosis might involve the activation of multiple intercellular pathways. After IL-11 binds to the IL-11 receptor, the ligand-receptor complex interacts with a common receptor subunit, glycoprotein 130 (gp130), leading to gp130-associated kinase-mediated tyrosine phosphorylation [26]. In vascular endothelial and intestinal epithelial cells, IL-11 protects against oxidant induced necrosis and apoptosis via mechanisms involving ERK, MAPK, AKT and/or induction of HSP25 [22, 27, 28]. In renal IRI, IL-11 performs renal protection by direct induction of sphingosine kinase-1 (SK-1) via nuclear translation of HIF-1α. In cardiac myocytes, IL-11 treatment attenuates injury and fibrosis via Janus Kinase-Signal Transducer and Activation of Transducer 3 (JAK-STAT3) pathway activation [12, 23, 29]. Kawakami T et al have demonstrated rhIL-11 confers significant protection against CCl4-induced hepatic injury by virtue of its liver-specific HO-1 induction [30]. In addition, a previous study demonstrated STAT3 activation after Ad-HO-1 treatment improved the hepatocellular function in a mouse model of segmental liver warm IRI [31]. In the present study, IL-11 treatment rapidly activated STAT3 in hepatocytes in vivo and in vitro, and reduced liver injury after reperfusion. However, IL-11 administration shows weak hepatocellular protective effects in STAT3siRNA transfected hepatocytes or STAT3siRNA transfected mice. Therefore, hepatocyte is an important target in the action of IL-11, and IL-11-mediated protection of liver IRI is partially dependent on STAT3 activation of hepatocytes. Whether HO-1 is involved in this protective procedure will be further investigated in the future.

Evidence exists that the STAT3 signaling pathway transduces stress-activating extracellular chemical signals into cellular responses for a number of pathophysiological processes, such as immunity, inflammation and apoptosis, and is involved in liver IRI. The function of activated STAT3 is controversial; some studies have associated it with survival [31–33], while others have related it to cell death [34]. Previous studies have confirmed that STAT3 alterations affect Bcl-2 and Bax protein expression and induce inflammation and apoptosis in many types of tumor cells [35–37]. In mycosis fungoides tumor cells, some apoptosis-related genes, such as Bcl-2 and Bax, have been identified as STAT3 target genes [38]. In our study, STAT3 activation reduced hepatocellular necrosis/apoptosis and liver injury induced by IR after IL-11 treatment, but STAT3 knockdown restored the hepatocellular necrosis/apoptosis and liver injury in IL-11-treated mice. These data indicate that IL-11 treatment reduces hepatocellular necrosis/apoptosis by STAT3 activation. Lee et al demonstrated that co-activated NF-κB and STAT3 modulate Bax/Bcl-xL expression and promote cell survival in head and neck squamous cell carcinoma [38]. In primary cortical neurons and murine models of stroke, the activation of STAT3 pathway by secretoneurin has been found to exert neuroprotective effects and induce neuronal plasticity after hypoxia and ischemic insult [39]. Using a mouse model of myocardial infarction, Obana M et al demonstrated that IL-11 exerted protective effects against myocardial ischemic injury through IL-11R-mediated STAT3 activation, antiapoptotic signaling and proangiogenic activity [23]. In addition, STAT3 has been demonstrated to have an anti-inflammatory function in many pathophysiological processes [23]. Inflammatory response plays a pathogenic role in liver I/R injury, especially innate immune responses involved in cytokines and chemokines, including TNF-α, IL-6, IP-10, MCP-1 and so on [40–43]. Ke B et al
demonstrated that STAT3 activation repressed TLR4-driven inflammation by activating PI3K/Akt signaling during liver IRI [23]. Consistent with the above data, our results also showed that IL-11-induced STAT3 signaling inhibited pro-inflammatory cytokines and chemokines.

In conclusion, our findings demonstrate for the first time IL-11-mediated STAT3 attenuates IR-triggered liver injury. IL-11-mediated STAT3 signaling not only reduces hepatocellular apoptosis, but also inhibits inflammatory responses. Thus, our study provides a rationale for novel therapeutic approaches to the management of liver injury triggered by IR.

Author Contributions
Conceived and designed the experiments: MZ BL QC FL. Performed the experiments: MZ BL QC ZW ZX WL. Analyzed the data: MZ BL XY. Contributed reagents/materials/analysis tools: MZ BL QC XY. Wrote the paper: MZ BL QC FL. Primary responsibility for final content: FL.

References
1. Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol. 2002; 284: G15–G26. PMID:12488232
2. Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology. 2000; 32: 69–173. PMID:10975759
3. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. Am J Surg. 2001; 181: 160–166. PMID:11425059
4. Que X, Debonera F, Xie J, Furth EE, Aldeguer X, Gelman AE, et al. Pattern of ischemia reperfusion injury in a mouse orthotopic liver transplant model. J Surg Res. 2004; 116: 262–268. PMID:15013365
5. Shah KA, Shurey S, Green CJ. Characterization of apoptosis in intestinal ischaemia–reperfusion injury—A light and electron microscopic study. Int J Exp Pathol. 1997; 78: 355–63. PMID:9462333
6. Noda T, Iwakiri R, Fujimoto K, Matsuo S, Aw TY. Programmed cell death induced by ischemia–reperfusion in rat intestinal mucosa. Am J Surg. 1998; 274: G270–G276. PMID:9486179
7. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995; 267: 1456–1462. PMID:7878464
8. Gordon MS, McCaskill-Stevens WJ, Battioto LA, Loewy J, Loesch D, Breeden E, et al. A phase I trial of recombinant human interleukin-11 (neumega rhIL-11 growth factor) in women with breast cancer receiving chemotherapy. Blood. 1995; 87: 3615–3624.
9. Sands BE, Winston BD, Salzberg B, Safdi M, Barish C, Wruble L, et al. Randomized, controlled trial of recombinant human interleukin-11 in patients with active Crohn’s disease. Aliment Pharmacol Ther. 2002; 16:399–406. PMID:11876692
10. Trepicchio WL, Bozza M, Pedneau GT, Dorner AJ. Recombinant human IL-11 attenuates the inflammatory response through downregulation of proinflammatory cytokine release and nitric oxide production. J Immunol. 1996; 157: 3627–3634. PMID:8871663
11. Trepicchio WL, Dorner AJ. The therapeutic utility of Interleukin-11 in the treatment of inflammatory disease. Expert Opin Investig Drugs. 1998; 7: 1501–1504. PMID:15992047
12. Kimura R, Maeda M, Arita A, Oshima Y, Obana M, Ito T, et al. Identification of cardiac myocytes as the target of interleukin 11, a cardioprotective cytokine. Cytokine. 2007; 38: 107–115. PMID:17629706
13. Du X, Liu Q, Yang Z, Orazi A, Rescorla FJ, Grosfeld JL, et al. Protective effects of interleukin-11 in a murine model of ischemic bowel necrosis. Am J Physiol. 1997; 272: G545–G552. PMID:9124579
14. Yin T, Yasukawa K, Taga T, Kishimoto T, Yang YC. Identification of a 130-kilodalton tyrosine-phosphorylated protein induced by interleukin-11 as JAK2 tyrosine kinase, which associates with gp130 signal transducer. Exp Hematol. 1994; 22: 467–472. PMID:8174677
15. Timofeeva OA, Tarasova NI, Zhang X, Chasovskikh S, Cheema AK, Wang H, et al. STAT3 suppresses transcription of proapoptotic genes in cancer cells with the involvement of its N-terminal domain. Proc Natl Acad Sci USA. 2013; 110: 1267–1272. doi:10.1073/pnas.1211805110 PMID:23288901
16. Schindler CW. Series introduction. JAK-STAT signaling in human disease. J Clin Invest. 2002; 109:1133–137. PMID:11994400
17. Jacoby JJ, Kalinowski A, Liu MG, Zhang SS, Gao Q, Chai GX, et al. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. Proc Natl Acad Sci USA. 2003; 100: 12929–12934. PMID:14566054
33. Lou LX, Uemura T, Mani H, Yang C, Li W, Kadry Z, et al. Endogenous signal transducer and activator of transcription 3 (Stat3) regulates liver ischemia/reperfusion injury. Am J Transplant. 2014; 14: 1552–1561. doi:10.1111/ajt.12711 PMID: 24903305

34. Ropeleski MJ, Tang J, Walsh-Reitz MM, Musch MW, Chang EB. Interleukin-11-induced heat shock protein 25 confers intestinal epithelial-specific cytoprotection from oxidant stress. Gastroenterology. 2003; 124: 1358–1368. PMID: 12730876

35. Nielsen M, Kaestel CG, Eriksen KW, Woetmann A, Stokkedal T, Kaltoft K, et al. Inhibition of constitutive activated Stat3 correlates with altered Bcl-2/Bax expression and induction of apoptosis in mycosis fungoides tumor cells. Leukemia. 1999; 13: 735–738. PMID: 10374878

36. Dinasarapu AR, Gupta S, Ram Maurya M, Fahy E, Min J, Sud M, et al. A combined omics study on activated macrophages-enhanced role of STATs in apoptosis, immunity and lipid metabolism. Bioinformatics. 2013; 29: 2735–2743. doi:10.1093/bioinformatics/btt469 PMID: 23981351

37. Spehlmann ME, Manthey CF, Dann SM, Hanson E, Sandhu SS, Liu LY, et al. Trp53 deficiency protects against acute intestinal inflammation. J Immunol. 2013; 191: 837–847. doi:10.4049/jimmunol.1201716 PMID: 23772033

38. Lee TL, Yeh J, Friedman J, Yan B, Yang X, Yeh NT, et al. A signal network involving coactivated NF-kappaB and STAT3 and altered p53 modulates BAX/BCL-XL expression and promotes cell survival of head and neck squamous cell carcinomas. Int J Cancer. 2008; 122:1987–1998. doi:10.1002/ijc.23324 PMID: 18172861
39. Shyu WC, Lin SZ, Chiang MF, Chen DC, Su CY, Wang HJ, et al. Secretoneurin promotes neuroprotection and neuronal plasticity via the Jak2/Stat3 pathway in murine models of stroke. J Clin Invest. 2008; 118:133–148. PMID: 18079966

40. Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology. 2000; 32: 169–173. PMID: 10915720

41. Liu J, Ren F, Cheng Q, Bai L, Shen X, Gao F, et al. Endoplasmic reticulum stress modulates liver inflammatory immune response in the pathogenesis of liver ischemia and reperfusion injury. Transplantation. 2012; 94: 211–217. doi: 10.1097/TP.0b013e318259d38e PMID: 22790388

42. Ren F, Duan Z, Cheng Q, Shen X, Gao F, Bai L, et al. Inhibition of glycogen synthase kinase 3 beta ameliorates liver ischemia reperfusion injury by way of an interleukin-10-mediated immune regulatory mechanism. Hepatology. 2011; 54: 687–696. doi: 10.1002/hep.24419 PMID: 21567437

43. Sano T, Izuishi K, Hossain MA, Inoue T, Kakinoki K, Hagiike M, et al. Hepatic preconditioning using lipopolysaccharide: association with specific negative regulators of the Toll-like receptor 4 signaling pathway. Transplantation. 2011, 91: 1082–1089. doi: 10.1097/TP.0b013e31821457cb PMID: 21403587