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Down-regulation of IFIT3 protects liver from ischemia-reperfusion injury

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ABSTRACT

Hepatic ischemia-reperfusion injury (IRI) could result in severe liver damage and dysfunction during liver surgery and transplantation. As one of the Interferon (IFN)-stimulated genes, IFIT3 exerted antitumor activity but its roles in hepatic IRI are still unknown. In this study, roles of IFIT3 in hepatic IRI were investigated using a mouse hepatic IRI model and a cellular hypoxia-reoxygenation model. Firstly, our results showed that IFIT3 was up-regulated in reperfused liver tissues of patients undergoing liver transplantation and was positively correlated with serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Secondly, knockdown of IFIT3 could significantly ameliorate hepatic IRI and suppress ischemia and reperfusion-induced release of inflammatory cytokines in vivo and in vitro. Furthermore, knockdown of IFIT3 inhibited phosphorylation of STAT1 and STAT2, and decreased expressions of IFN-stimulated genes induced by ischemia and reperfusion in vivo and in vitro. These data highlight the importance and potential clinical use of IFIT3 in hepatic IRI.

1. Introduction

Ischemia-reperfusion injury (IRI) is a common and serious complication during liver, heart, kidney and brain surgery [1]. IRI of liver mainly occurs during hepatic resection and liver transplantation. Despite significant progress in recent years, hepatic IRI remains a main cause of liver dysfunction and contributes to poor prognosis of patients [2,3]. In view of the facts above, hepatic IRI is still an important clinical problem which should be further explored and solved. Hepatic ischemia and reperfusion could elevate the levels of pro-inflammatory cytokines and the production of reactive oxygen species, both of which would lead to liver damage [4,5]. Many studies showed that gene targeting strategies for inhibiting inflammatory or apoptotic signaling pathways might exert protective effects against IR-induced liver damage [6–8].

In the past few years, many studies investigated the mechanisms underlying hepatic IRI. It was revealed that the inhibition of interferon (IFN) signaling and IFN-stimulated genes (ISGs) could significantly ameliorate hepatic IRI, which suggested important roles of IFN signaling and ISGs in the progression of hepatic IRI [9–11]. As one of the ISGs, IFIT3 was widely expressed in human organs including liver [12]. IFIT3 was also revealed to act as a tumor suppressor gene in leukemia, lung cancer and hepatocellular carcinoma [13–15]. Mechanistic investigations showed that IFIT3 could exert its antiproliferative activity by increasing the levels of cell cycle inhibitors p21 and p27 [13]. It was also reported that IFIT3 could strengthen IFN signaling pathway by interacting with STAT1 and STAT2 during IFN-α treatment [15]. Notably, little is known about the functions and mechanisms of IFIT3 in inflammation and hepatic IRI.

Consequently, in this study, we aimed to investigate the expressions, roles and mechanisms of IFIT3 in hepatic IRI using clinical samples of patients with liver transplantation, mouse hepatic IRI model and cellular hypoxia-reoxygenation (HR) model. Our findings not only provided new insights into the roles of IFIT3 in hepatic IRI, but also suggested the potential clinical use of IFIT3 for ameliorating hepatic injury caused by ischemia and reperfusion.

2. Materials and methods

2.1. Patients and samples

Tissue and serum samples were collected from patients with liver transplantation at Organ Transplantation Center, the Affiliated Hospital of Qingdao University, from January 2015 to December 2017. Liver tissues were obtained from liver grafts before liver transplantation and after hepatic artery reperfusion. The clinicopathological data of patients were shown in Table 1. All human materials were obtained with

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Table 1
Clinicopathological characteristics of patients with liver transplantation categorized according to the IFIT3 levels of liver tissues.

Characteristic	IFIT3 levels of liver tissues		*p*-Value
	Low (n = 10) (After/Before reperfusion) ≤2	High (n = 30) (After/Before reperfusion) > 2	
Gender (Male/female)	10/0	27/3	0.5597
Age (years)	54.5 ± 2.774	49.83 ± 2.831	0.3670
Albumin (g/L)	42.7 ± 2.156	41.74 ± 1.175	0.6876
Total bilirubin (μmol/L)	57.9 ± 6.84	67.68 ± 11.84	0.6396
ALT (U/L)	423 ± 84.1	1005 ± 118.8	**0.0086**
AST (U/L)	704.1 ± 188.4	2097 ± 233	**0.0018**
Glutamyl transpeptidase (U/L)	47.7 ± 7.165	80.46 ± 10.42	0.0783
Alkaline phosphatase (U/L)	60.10 ± 7.213	88.25 ± 13.87	0.2446
Lactate dehydrogenase (U/L)	256 ± 41.46	699 ± 171.1	0.1353
Leucine aminopeptidase (U/L)	41.6 ± 4.385	52.57 ± 2.997	0.0608
Adenosine deaminase (U/L)	16.7 ± 2.055	17.37 ± 1.137	0.7671
Prothrombin time (s)	17.51 ± 0.6672	18.07 ± 0.5719	0.6167

ALT: alanine aminotransferase; AST: aspartate aminotransferase.
Bold numbers indicate significant differences ($p < 0.05$).

informed consents and approved by the Ethics Committee of Affiliated Hospital of Qingdao University.

2.2. Cell culture

The mouse macrophage cell line RAW264.7 was obtained from Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). Cells were cultured in a humidified environment with 5% CO_2 at 37 °C.

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

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNAs of liver tissues and cells according to the manufacturer's instruction. After reverse transcription using Primescript RT Master Mix with gDNA Eraser (Takara, Dalian, China), cDNA was amplified using SYBR-Green Premix (Takara). β-actin was used as an internal control. Primers used in this study were listed in Supplementary Table S1.

2.4. Hypoxia/reoxygenation (HR) model

RAW264.7 cells were seeded in a plate and cultured for 24 h at 37 °C. Then 100% mineral oil was applied to cover the cells for 4 h. Next the mineral oil was removed and the cells were replenished with fresh media under normal culture conditions. Media and cells were collected for further analysis at indicated time points after reoxygenation.

2.5. Cell transfection

SiRNAs targeting IFIT3 and negative control (NC) were obtained from GenePharma Company (Shanghai, China). Oligonucleotides were transfected into cells by Hiperfect transfection reagent (Qiagen, Valencia, CA, USA) according to the instructions provided by the manufacturer. The siRNA sequences used in this study are listed as follows: IFIT3 siRNAs: 5′-GCACAAUGAUCAAUCAGAATT-3′ (sense) and 5′-UUCUGAUUGAUCAUUGUGCTT-3′ (antisense); NC siRNAs: 5′-UUC UCCGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACA CGUUCGGA GAATT-3′ (antisense).

2.6. Enzyme-linked immunosorbent assay (Elisa)

To evaluate the inflammatory cytokines released from tissues and cells, serum and cell culture supernatants were collected and the levels of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) were measured by the Elisa kit (Invitrogen) according to the instructions provided by the manufacturer.

2.7. Western blot

Total proteins in liver tissues and cells were extracted using RIPA buffer (Solarbio, Beijing, China), separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto 0.45 μm PVDF membranes (Millipore, Billerica, MA, USA). The following primary antibodies were used in the immunoblotting assays: anti-IFIT3 antibody (sc-393396, Santa Cruz Biotechnology, Santa cruz, CA, USA), anti-STAT-1 antibody (#14994, Cell Signaling Technology, Danvers, MA, USA), anti-phospho-STAT-1 antibody (ab29045, Abcam, Cambridge, MA, USA), anti-STAT-2 antibody (ab231184, Abcam), anti-phospho-STAT-2 antibody (ab53132, Abcam), anti-albumin (ab106582, Abcam), anti-CD68 (ab125047, Abcam) and anti-β-actin antibody (20536-1-AP, Proteintech, Chicago, IL, USA).

2.8. Animals

Male C57BL/6J mice (7–9 weeks old) were obtained from Vital River Laboratory Animal Technology Company (Beijing, China). The animals were provided with food and water, and were housed in a temperature environment with 12 h light/dark cycles. All animal experiments were conducted in the Animal Institute of Affiliated Hospital of Qingdao University according to the protocols approved by the Medical Experimental Animal Care Commission of Qingdao University.

2.9. Mouse model of hepatic ischemia-reperfusion injury (IRI)

A non-lethal model of segmental hepatic ischemia was established. C57BL/6J mice were injected with the corresponding lentiviruses through caudal vein. About two weeks later, the mice were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal). Then a clip was used to interrupt the blood supply from hepatic artery and portal vein to the left and middle liver lobes of mice. The clip was removed after 60 min. Sham animals underwent laparotomy without hepatic ischemia. At indicated time points after reperfusion, the mice were euthanized for liver tissue collection.

2.10. Isolation of Kupffer cells and hepatocytes

Kupffer cells and hepatocytes were isolated from livers of mice. Mice were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal). Then the livers were perfused and digested with collagenase solution (Sigma, St Louis, MO, USA) and passed through a cell strainer. The hepatocytes were centrifuged with a low speed, washed and collected. After separation by 25% and 50% Percoll gradient centrifugation for 30 min with a high speed at 4 °C, non-parenchymal liver cells were collected from the interface and cultured in a cell culture plate. After about 3 h, the nonadherent cells were removed and the adherent Kupffer cells were remained and collected.

2.11. Assessment of liver damage

Hepatic injury of patients or mice was assessed by measuring the levels of serum alanine aminotransferase (ALT) and aspartate