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Suppression of NF-κB activation by PDLIM2 restrains hepatic lipogenesis and inflammation in high fat diet induced mice

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis, insulin resistance, dyslipidemia and a systemic pro-inflammatory response, a leading cause of cirrhosis and hepatocellular carcinoma. Here, we showed that PDZ-LIM domain-containing protein 2 (PDLIM2) was an effective suppressor of steatohepatitis. After 16 weeks on a high fat diet (HFD), obesity, insulin resistance, hepatic dyslipidemia and inflammation were markedly aggravated in PDLIM2-knockout (KO) mice. PDLIM2 deletion resulted in lipid accumulation in liver tissue samples of HFD-induced mice, as evidenced by the significant increase of hepatic TG and TC through reducing the expression of lipogenesis- and transcriptional regulators of lipid metabolism-related genes and enhancing fatty acid oxidation-associated molecules. In addition, PDLIM2-ablation promoted the expression of pro-inflammatory cytokines by activating nuclear factor kappa-B (NF-κB) signaling pathway, as supported by the remarkable increase of phosphorylated IKKβ, IκBα and NF-κB expressions in liver of HFD-fed mice. Of note, the in vitro study demonstrated that PDLIM2 ablation-enhanced inflammatory response and disorder of lipid metabolism were abrogated by suppressing NF-κB activity. Collectively, the findings could lead to the development of potential therapeutic strategy to prevent NAFLD and associated metabolic disorders by targeting PDLIM2.

1. Introduction

Obesity and its associated metabolic consequences, such as insulin resistance (IR) and non-alcoholic fatty liver disease (NAFLD) are global health threats to human with increasing prevalence [1,2]. Increasing evidences have indicated that hepatic steatosis is a chronic inflammatory response and often observed in individuals with obesity and IR [3]. In liver, the disturbed insulin pathway leads to insulin insensitivity and glucose intolerance, which is accompanied with inflammatory response, enhancing hepatic lipid accumulation and steatosis, in turn, contributing to liver inflammation and IR [4,5]. Therefore, IR, hepatic steatosis, lipid accumulation and inflammation are interconnected pathological events observed in individuals with obesity [6]. Despite a large number of researches conducted in the field, the underlying molecular mechanisms that occur during the process are not fully understood.

PDLIM2, also known as Mystique or SLIM, is located on chromosome 8p21.2 and encodes a PDZ-LIM domain protein [7]. As reported before, PDLIM2 could modulate inflammation, oxidative stress and cell migration [8,9]. In addition, the expression of PDLIM2 has been implicated in tumorigenesis and tumor suppression [10]. The transcription factor of NF-κB has been proposed to play an essential role in impairing normal microenvironmental cues necessary for sustaining tissue organization [11]. PDLIM2 was reported to modulate the stability of several transcription factors including NF-κB [12,13]. Accordingly, PDLIM2 is involved in the inactivation of NF-κB [12]. Considering the close implication of NF-κB in insulin function, and inflammatory response that intimately related to fatty acid metabolism in hepatocytes [14–16], we hypothesize that PDLIM2 might be included in the pathogenesis of hepatic steatosis, and NAFLD progression.

In the present study, the wild type (WT) and PDLIM2 knockout (KO) mice were generated and subjected to HFD to calculate the effects of PDLIM2 on hepatic steatosis, lipid metabolism and inflammation. We identified a negative relationship between PDLIM2 and NF-κB. PDLIM2 deletion promoted NAFLD progression by accelerating hepatic steatosis, lipid accumulation and inflammatory response through activating NF-κB.

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2. Materials and methods

2.1. Patients and samples

Human steatotic liver samples were obtained from patients with simple steatosis (SS) or NASH undergone either liver biopsy or liver transplantation. Non-steatotic liver samples were collected from healthy regions of the livers from donors undergone liver resection due to a liver hemangioma or hepatic cyst [17]. Simple steatosis (SS) and NASH were diagnosed independently using standard histological criteria by two pathologists in a blinded fashion [18]. All procedures involved human samples were approved by the Renmin Hospital of Wuhan University (Wuhan, China).

2.2. Animals and culture

All animal experiments were performed in accordance with the approval obtained from the Renmin Hospital of Wuhan University Animal Ethics Committee. Mice were housed in a specific pathogen-free (SPF) barrier facility on a regular light-dark cycle (12 h light-12 h dark) at 22 ± 1 °C with free access to water and normal chow diet (ND, D12450B, Research Diets, New Brunswick, USA) or HFD (D12492, Research Diets). All studies were conducted in male mice on a C57Bl6J background. Male ob/ob mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China) and fed a ND. The wild type (WT) mice and PDLIM2 knockout (KO) mice were purchased from the Model Animal Research Center of Nanjing University and Cyagen Biosciences (Guangzhou, China), respectively, fed with ND or HFD for 16 weeks. All mice were divided into 4 groups (n = 8/group): 1) ND-WT; 2) ND-KO; 3) HFD-WT and 4) HFD-KO. The change of body weight and food intake was recorded. After 16 weeks, all mice were sacrificed for blood collection. Subcutaneous, epididymal and total white adipose tissue weights were measured. Liver tissue samples were isolated for further study.

2.3. Cells isolation and treatments

Primary hepatocytes from 6- to 8-week-old WT or PDLIM2-KO mice were isolated by liver perfusion [19]. The obtained hepatocytes were suspended in DMEM (GIBCO, USA) supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin-streptomycin in a 5% CO_2/water-saturated incubator at 37 °C. Palmitate (PAL, Sigma-Aldrich, USA) was added to the medium for 24 h. SC75741 (Selleck Biotech, USA) was pre-treated to cells or not for 5 h.

2.4. Tolerance tests

Glucose tolerance test (GTT) was monitored in mice that were fasted for 8 h. For i. p. GTT, mice received an i. p. injection of glucose (1.5 g/kg body weight, Sigma-Aldrich, USA). Blood was taken by tail vein puncture and glucose levels were measured at the indicated time points (0, 30, 30, 90 and 120 min) using a glucometer (One Touch Ultra Easy, Life Scan, USA).

2.5. Biochemical analysis

The concentrations of cytokines (TNF-a, IL1b, Il6, Il10, Il6 and Ccl2) in serum were examined using ELISA (R&D; USA). Total glucagon in liver was measured following the methods of the Glycogen Assay Kit (BioVision, USA). The serum levels of ALT and AST were measured using commercial kits (Nanjing Jiancheng Biological Engineering Institute, Nanjing, China) to evaluate the liver function of mice according to the manufacturer's instructions. Triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDLC) and free fatty acid (FFA) levels in serum or liver tissue samples were determined using commercial kits (BeyoTime, Nantong, China) following the manufacturer's instructions. Serum insulin levels were measured by ELISA (Millipore, USA), and the HOMA-IR was evaluated with the equation (blood glucose (mmol/L) × serum insulin (mI/L))/22.5.

2.6. Histological analyses

The frozen liver sections were subjected to oil red O staining to visualize lipid droplets in liver, whereas haematoxylin and eosin (H&E) staining was performed on paraffin sections (5 μm-thickness) after deparaffinization and rehydration. Digital images were obtained under a light microscope. For immunohistochemical (IHC) analysis, liver samples were fixed in 10% buffered formalin. After paraffin embedding, tissue sectioning, and slide mounting, tissues were incubated with CD36 (1:200, #ab133625, Abcam, Cambridge, UK) and Plin5 (1:200, #ab194128, Abcam) [20].

2.7. Immunofluorescence (IF)

Phosphorylated NF-κB expression and localization in the liver and cells were investigated using immunofluorescent analysis as previously described [21]. The mouse p–NF–κB antibody (ab97726; Abcam) was used as primary antibody, and goat anti-rabbit IgG (Abcam) was used as the corresponding secondary antibody. IF images were obtained using fluorescence microscope.

2.8. Quantitative RT-PCR and western blot analysis

For RT-PCR analysis, total mRNA was isolated from liver or cells using TRIzol Reagent (Invitrogen, USA) and reverse-transcribed into cDNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche, USA) following the manufacturer's instructions. SYBR Green (Roche) was used to quantify PCR amplification. The mRNA expression levels were normalized to GAPDH expression [21]. The primers used in the study were listed in Supplementary Table 1.

For western blot analysis, total protein was isolated from tissue or cells using RIPA lysis buffer (BeyoTime). The protein concentrations were measured using BCA Protein Assay Kit (Thermo Fisher Scientific, USA). 50 μg protein samples was separated in a 10% SDS-PAGE gel and then transferred to a PVDF membrane (Millipore, USA). After being blocked with 5% skim milk, the membrane was incubated with primary antibodies at 4 °C overnight, followed by the corresponding secondary antibodies. Immunoreactive proteins were visualized using ECL Western blotting detection reagents (Thermo Fisher Scientific). The antibody information and working dilutions were listed in Supplementary Table 2. Protein expression levels were quantified with ImageJ Software and normalized to the loading control GAPDH.

2.9. Statistical analysis

Data are expressed as mean ± SEM. GraphPad Prism 6.0 software was used to analyze the results. For comparisons between two groups, unpaired *t*-test was performed; for comparisons among multiple groups, One-way analysis of variance (ANOVA) test was performed. $P < 0.05$ was considered statistically significant.

3. Results

3.1. PDLIM2 deficiency promotes HFD-induced mice obesity and insulin resistance

In order to gain a comprehensive understanding of potential