Upregulation of caveolin-1 and SR-B1 in mice with non-alcoholic fatty liver disease

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BACKGROUND: **Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent causes of liver diseases, with markedly increased prevalence. However, its mechanisms are not clear. The present study was undertaken to illustrate the role of caveolin-1 (cav1) and the scavenger receptor class B type 1 (SR-B1) in NAFLD.**

METHODS: **Adult male C57BL/6 mice were fed with a normal diet or high fat and cholesterol (HFC) diet for 14 weeks. The mice were sacrificed to collect plasma and harvest the liver; their plasma lipid concentration was measured. Hepatic cav1 and SR-B1 mRNA and protein expression were determined by real-time quantitative polymerase chain reaction (qPCR) and Western blotting, respectively. In order to study cav1 and SR-B1 distribution and change in hepatocytes, immunohistochemical analysis was performed.**

RESULTS: **HFC diet increased plasma lipids, induced NAFLD and increased the liver/body weight ratio. Compared to the control mice (n=6), the mRNA and protein levels of cav1 and SR-B1 in liver tissue of the NAFLD mice (n=12) increased significantly (cav1 mRNA: 1.536±0.226 vs 0.980±0.272, P<0.05; protein: 0.643±0.240 vs 0.100±0.130, P<0.01; SR-B1 mRNA: 1.377±0.125 vs 0.956±0.151, P<0.01; protein: 2.156±0.507 vs 0.211±0.211, P<0.01). Furthermore, both cav1 and SR-B1 immunoreactivity increased and their distribution was also changed, mainly in the plasma membrane of hepatocytes, cytoplasm and membrane of lipid droplets and around.**

CONCLUSION: **NAFLD is associated with increased concentration of plasma lipids and upregulation of hepatic cav1 and SR-B1 gene and protein expressions, which indicate that cav1 and SR-B1 might play crucial roles in the pathogenesis of NAFLD.**

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KEY WORDS: **non-alcoholic fatty liver disease; caveolin-1; scavenger receptor class B type 1; pathogenesis; high fat and cholesterol diet**

Introduction

Non-alcoholic fatty liver disease (NAFLD) as a clinical syndrome without excess alcohol intake is characterized pathologically by such symptoms as liver steatosis. The prevalence of this disease increasingly exceedings that of hepatitis B, hepatitis C and alcoholic liver disease. It has been the most common liver disease. However, the mechanisms involving in the pathogenesis of NAFLD have not been thoroughly investigated.

Caveolin-1 (cav1) is the main structural protein of caveolae, which has now emerged as a plasma membrane protector, organizer and sensor that can respond to plasma membrane stresses and remodel the extracellular environment.[1] Recent studies[2-4] also identified the role of cav1 in cholesterol transport, lipogenesis and lipolysis. However, how cav1 implicates in the pathogenesis of NAFLD has not been clarified. Reports[5, 6] have shown that upregulation of cav1 enhances the oxidized low density lipoprotein (LDL) absorption of HepG2 cell markedly, and that downregulation of cav1 inhibits HepG2 uptake of long chain fatty [^{3}H]-oleic acid. Although the involvement of cav1 in lipogenesis has been investigated, the role of cav1 in mice with NAFLD has rarely been found.

Recent evidences have shown that scavenger receptor class B type 1 (SR-B1) ameliorates hepatic lipid metabolism disorder.[7] Moreover, SR-B1 is also

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implicated in plasma lipid metabolism. Apart from the surface receptor of high density lipoprotein (HDL) in the liver, SR-B1 binds to the receptors of non-HDL, i.e. very low density lipoprotein (VLDL) and LDL. Hoekstra et al[8] found that SR-B1 regulated plasma VLDL and LDL levels in mice. But the role of SR-B1 in the formation of lipid droplets in NAFLD is still controversial.[9, 10] Matveev and coworkers[11] showed that cav1 is a negative regulator of SR-B1-dependent selective cholesteryl ester uptake.

Our study aimed to determine the mRNA and protein expressions of cav1 and SR-B1 in the liver of NAFLD mouse induced by HFC diet. We also detected the distribution associations of cav1 and SR-B1 by immunohistochemistry.

Methods

Animals and diet

Eight-week C57BL/6 male mice were obtained from Shanghai Laboratory Animal Center (Shanghai, China). The mice were kept under room temperature (22 ℃) and constant light-dark cycles with free access to water and normal diet (<0.02% cholesterol). Twelve mice were fed on a high fat and cholesterol (HFC) diet consisting of 15% fat and 1.25% cholesterol after receiving two-week normal diet, while another six mice were still fed with normal diet (control). Both normal diet and HFC chaw were purchased from the Zhejiang Academy of Medical Science (Hangzhou, China). All mice were subjected to experimental protocols adhered to ethical standards and under the care of animal and licensing guidelines. Samples were collected between 9 and 10 AM at the end of the dark phase of the diurnal cycle.

Materials

Standard molecular biological techniques were applied. TRIzol reagent was purchased from Invitrogen™ (Carlsbad, CA, USA). ReverTra Ace qPCR RT Kit and Thunderbird SYBR qPCR Mix were obtained from TOYOBO (Osaka, Japan). Primers were designed by AlleleID 7.0 and synthesized by Sangon Biotech Co., Ltd., (Shanghai, China). Total protein extraction kit was also purchased from Sangon Biotech Co., Ltd. Rabbit monoclonal antibody to glyceraldehyde phosphate dehydrogenase (GAPDH) and rabbit antibody to cav1 were purchased from Cell Signaling Technology (Beverly, MA, USA). Rabbit antibody to SR-B1 and goat anti-rabbit IgG-HRP (horseradish peroxidase) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). PageRuler prestained protein ladder was purchased from Fermentas Life Science (Shanghai, China). Diaminobenzidine was provided by Dr. HT Yao from the First Affiliated Hospital, Zhejiang University School of Medicine, China. Unless previous indicated, materials were obtained from Amresco (Ohio, USA) or BBI (Nova Scotis, Canada). Micro BCA™ protein assay kit was purchased from Thermo Pierce Scientific (Shanghai, China).

Plasma and liver sampling

All mice fasted overnight were anesthetized with 4% chloral hydrate (8 mL/kg body weight, intraperitoneally) after 14-week feeding with HFC diet or normal diet (control). Blood was collected from the inferior vena cava, centrifuged at 3000 rpm for 8 minutes at room temperature and finally the supernatant was collected. The plasma samples were analyzed immediately. The harvested liver was weighed, cut into pieces, washed extensively in cold phosphate-buffer saline (PBS) and aliquoted. Part of the aliquots was snap-frozen in liquid nitrogen and stored at -80 ℃ until use, and part was fixed in formaldehyde solution.

Plasma lipid concentration measurement

Once the plasma of all mice was collected, it was aliquoted into special test tube with a fixed volume, and mounted to an automated analysis equipment (Hitachi 7600, Japan) which has been set parameters according to lipid test kit protocols, such as triglyceride test reagent kit, cholesterol test kit, and so on.

Real-time quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from control and HFC liver according to TRIzol reagent protocol. The concentration of total RNA was measured using a Nanodrop Spectrophotometer 2000 (Thermo Scientific, USA). About 0.5 μg RNA was subjected to reverse transcription using ReverTra Ace qPCR Kit. Thunderbird SYBR qPCR Mix was used in a 7500 instrument (ABI Real-time system, USA) according to the manufacturer's instructions. The primers used are listed in Table 1. The expression of each gene of interest was normalized with

Table 1. Primers of qPCR performed in the control and NAFLD mice

Primers	Sense	Antisense
Cav1	5'-AGA CTC CGA GGG ACA TCT C-3'	5'-GCG TCA TAC ACT TGC TTC TC-3'
SR-B1	5'-GCC TGT TTG TTG GGA TGA A-3'	5'-ATC TTG CTG AGT CCG TTC C-3'
β-actin	5'-GAA GAT CAA GAT CAT TGC TCC T-3'	5'-TGG AAG GTG GAC AGT GAG-3'