ELSEVIER

Journal of Hepatology 49 (2008) 787–798

Journal of Hepatology

www.elsevier.com/locate/jhep

Expression of apolipoprotein C-IV is regulated by Ku antigen/peroxisome proliferator-activated receptor γ complex and correlates with liver steatosis ☆,☆☆

Eun Kim, Ke Li, Charmiane Lieu, Shuping Tong, Shigenobu Kawai, Takayoshi Fukutomi, Yonghong Zhou, Jack Wands, Jisu Li*

Liver Research Center, Rhode Island Hospital and Warren Alpert Medical School of Brown University, 55 Claverick Street, Providence, RI 02903, USA

***Background/Aims*: We previously reported that hepatitis C virus (HCV) core protein up regulated transcription of apolipoprotein C-IV (ApoC-IV, 10.7-fold increase), a member of the apolipoprotein family implicated in liver steatosis. Here, we identified host transcription factors regulating the ApoC-IV gene expression.**

***Methods*: Transcriptional regulators were identified by DNA affinity purification and steatosis was detected by oil red O staining and triglyceride assay.**

***Results*: We defined a 163-bp ApoC-IV promoter as a core protein responsive element, and identified Ku antigen complex (Ku70 and Ku80) as well as nuclear receptors PPARγ/RXRα as key regulators of ApoC-IV gene expression. Both Ku70 overexpression and PPARγ agonist significantly increased ApoC-IV promoter activity; conversely, Ku70 silencing or mutation of PPARγ binding site diminished the ApoC-IV promoter activity. Interestingly, transient transfection of ApoC-IV cDNA into a human hepatoma cell line was able to trigger moderate lipid accumulation. In agreement with this *in vitro* study, ApoC-IV transcript level was increased in HCV infected livers which correlated with triglyceride accumulation.**

***Conclusions*: ApoC-IV overexpression may perturb lipid metabolism leading to lipid accumulation. HCV core protein may modulate ApoC-IV expression through Ku antigen and PPARγ/RXRα complex.**

Keywords: **Apolipoprotein; Gene regulation; Liver steatosis; Viral infection**

1. Introduction

Liver steatosis is defined by cytoplasmic accumulation of lipid droplets. It is triggered by a variety of host and environmental factors including obesity, diabetes, and alcohol consumption. In addition, chronic infection with hepatitis C virus (HCV) is also associated with increased prevalence of liver steatosis from 20% to 30% found in

Received 28 January 2008; received in revised form 1 June 2008; accepted 18 June 2008; available online 7 September 2008
Associate Editor: C.P. Day
☆ Data Deposition: GeneBank Accession Nos: EF371377 and EF371378. NIH funded study (DK066950, CA109733, CA95490, CA35711, AA08169, and AA20666).
☆☆ The authors who have taken part in the research of this paper declared that they do not have a relationship with the manufacturers of the materials involved either in the past or present and they did not receive funding from the manufacturers to carry out their research.
* Corresponding author. Tel.: +1 401 444 7387; fax: +1 401 444 2939.
E-mail address: Ji_Su_Li_MD@brown.edu (J. Li).
Abbreviations: ApoC-IV, apolipoprotein C-IV; PPAR, peroxisome proliferators-activated receptor; RXR, retinoid X receptor; VLDL, very low density lipoprotein; SEAP, secreted alkaline phosphatase; EMSA, electrophoretic mobility shift assay; ChIP, chromatin immunoprecipitation; RT-PCR, reverse transcription-polymerase chain reaction; Dox, doxycycline; HNF, hepatocyte nuclear factor; PPRE, PPAR responsive element.

0168-8278/$34.00 © 2008 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jhep.2008.06.029

the general population to 55% [1,2]. It has been reported that sustained response to antiviral therapy led to disappearance of fat from the liver, whereas a relapse of HCV infection correlated with recurrence of steatosis [1,3], thus highlighting the role of virus replication in the induction of steatosis. The HCV expresses three structural proteins (core, E1, and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, 4B, NS5A, and 5B) [4]. Steatosis can be induced experimentally in the mouse model by isolated expression of HCV structural proteins [5], or just the core protein [6].

As a major component of the HCV nucleocapsid, the core protein is required for viral genome packaging and virion formation. Interestingly, it is localized to triglyceride-rich lipid droplets in cytoplasm whether in transfected cell lines or in hepatocytes of HCV-infected chimpanzees [7,8]. The core protein has been reported to physically interact with apolipoprotein AII [9] and apolipoprotein B containing lipoproteins [10], both implicated in the storage of triglyceride inside hepatocytes. Indeed, transgenic mice expressing core protein were impaired in the function of microsomal triglyceride transfer protein and secretion of very low density lipoprotein (VLDL); they also manifested intrahepatic triglyceride accumulation [11]. Similarly, transfection of the core protein coding sequence into the Huh-7 hepatoma cell line led to intracellular triglyceride accumulation [12].

Recently, we performed microarray analysis of Huh-7 cells transiently transfected with HCV core gene, and observed transcriptional up regulation of a significant number of genes responsible for fat/lipid metabolism [13]. Of particular interest was a marked up regulation of ApoC-IV, a member of apolipoprotein family that is frequently overexpressed in steatotic livers [14] and HCV-associated HCC samples [15]. In the present study, we identified host transcription factors regulating the ApoC-IV gene expression, evaluated effect of HCV core protein on ApoC-IV promoter activity, and measured ApoC-IV transcripts in HCV infected liver samples. We demonstrate that ApoC-IV was regulated by Ku antigen/PPARγ and its overexpression could increase lipid accumulation in Huh-7 cells.

2. Materials and methods

2.1. ApoC-IV promoter and expression constructs

A 1.7-kb DNA fragment upstream of the ApoC-IV coding sequence was amplified from genomic DNA of Huh-7 cells using Expand High Fidelity PCR System (Roche Diagnostics, Indianapolis, IN), and cloned to pGL3-Basic luciferase reporter plasmid (Promega, Madison, WI). From this construct, a panel of 5′ deletion mutants and a site-directed mutant of PPARγ/RXRα binding site was generated by PCR. The 384-bp ApoC-IV coding sequence and 1830-bp Ku70 coding region were amplified by RT-PCR from total RNA extracted from Huh-7 cells and cloned into pcDNA3.1/Zeo$^{-/+}$ vector (Invitrogen, Carlsbad, CA). All constructs were verified by DNA sequencing.

2.2. Cell culture and transfection

Culture of Huh-7 and Huh-7.5 cells and transfection of HCV replicons have been described [13]. Huh-7 Tet-on cells, which harbor HCV core coding sequence of 1b genotype under the transcriptional control of tetracycline regulatory elements (Li et al., unpublished), were cultured in tetracycline-free medium. Core protein expression was induced by 1 μg/ml of doxycycline (Sigma, St. Louis, Missouri). A reporter plasmid for the secreted alkaline phosphatase (SEAP) was co-transfected to evaluate the transfection efficiency. Polyamine (Mirus, Madison, WI) was used as a transfection reagent for DNA constructs [13], and lipofectamine 2000 for siRNA transfection (Invitrogen, Carlsbad, CA). Differences among the groups were examined by Student's *t* test.

2.3. Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP)

EMSA was performed according to User Manual (ProteinOne, Bethesda, MD and Promega, Madison, WI). Chromatin was immunoprecipitated with antibodies against Ku70, Ku80 or irrelevant IgG (Active Motif, Carlsbad, CA). Presence of ApoC-IV promoter sequence in the purified DNA was verified by PCR.

2.4. Western blot and IP-Western blot analyses

Experiments were performed using standard procedures or as recommended by manufacturers. Antibodies: Ku70 and Ku80 (NeoMarkers, Fremont, CA), RNA polymerase II (Active Motif, Carlsbad, CA), actin (Sigma, St. Louis, Missouri), PPARα, β, γ and histone-1 (Santa Cruz Biotechnology, Santa Cruz, CA), respectively.

2.5. Detection of ApoC-IV mRNA and triglyceride in liver samples

Liver tissues were obtained from the Tissue Bank at University of Minnesota. Total RNA was extracted (Invitrogen, Carlsbad, CA) followed by reverse transcription (Roche Diagnostics, Indianapolis, IN) and real time PCR detection of ApoC-IV mRNA (Qiagen, Valencia, CA) using primers listed in Table 1. The ApoC-IV mRNA in transfected Huh-7 cells was detected by RT-PCR (see Table 1 for primers). Triglyceride was detected by Oil-Red O staining and quantified by enzymatic assay which measures the concentration of glycerol release after hydrolysis of the triglyceride (Zen-Bio, Inc., Research Triangle Park, NC).

3. Results

3.1. Identification of a core protein responsive element in the ApoC-IV promoter

We cloned a 1.7-kb genomic sequence upstream of ApoC-IV gene to examine the molecular mechanism by which HCV core protein up regulates ApoC-IV gene transcription. The promoter was inserted to a luciferase reporter plasmid so as to measure the promoter activity by luciferase assay. Progressive shortening of the 5′-end led to fluctuation of luciferase expression (Fig. 1A) consistent with the presence of multiple positive elements (nt −1672 to −1355, −1223 to −1027, −644 to −472, and −333 to −240, −122 to −52) and negative regulatory sequences (nt −1355 to −1223, −847 to −644, −472 to −333, and −242 to −122). The impact of