FEBS Letters 583 (2009) 2720–2726

FEBS Letters

journal homepage: www.FEBSLetters.org

Hepatitis C virus NS5A protein increases hepatic lipid accumulation via induction of activation and expression of PPARgamma

KyeongJin Kim [a], Kook Hwan Kim [a], Eunsin Ha [a], Jin Young Park [a], Naoya Sakamoto [b], JaeHun Cheong [a,*]

[a] Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan 609-735, Republic of Korea
[b] Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo 113-8519, Japan

ARTICLE INFO

Article history:
Received 11 July 2009
Accepted 17 July 2009
Available online 23 July 2009

Edited by Hans-Dieter Klenk

Keywords:
Hepatitis C virus
NS5A
Lipid accumulation
PGC-1
PPARγ

ABSTRACT

Steatosis is an established risk factor for disease progression in cases of chronic hepatitis C. Recently it was demonstrated that Hepatitis C virus (HCV) core and non-structural (NS) 2 proteins (NS2) induce lipid accumulation in hepatic cells. However, it has yet to be determined whether other HCV proteins are associated with lipid metabolism. The NS5A augmented the transcriptional activity and gene expression of PPARγ. Furthermore, NS5A increased the ability to recruit the transcriptional coactivator PGC-1s to the PPRE with PPARγ, as well as the interaction with PPARγ2 and PGC-1α. Our results indicate that NS5A may exploit multiple strategies that enhance PPARγ-induced lipid accumulation.

Structured summary:
MINT-7229685: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *PGC1 alpha* (uniprotkb:Q9UBK2) by *pull down* (MI:0096)
MINT-7229712: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *NS5A* (uniprotkb:P26662) by *pull down* (MI:0096)
MINT-7229698: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *PGC1 alpha* (uniprotkb:Q9UBK2) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7229731: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *NS5A* (uniprotkb:P26662) by *anti tag coimmunoprecipitation* (MI:0007)

1. Introduction

Hepatitis C virus (HCV) is classified into the genus *Hepacivirus* of the family *Flaviviridae* [1]. As the case with all the members of this family, HCV is an enveloped, single-stranded, and positive-sense RNA virus. Upon translation, HCV polyprotein is proteolytically processed by both cellular and viral proteases into at least 10 individual proteins, including four structural proteins (core, E1, E2 and p7) and six NS proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2]. As the penultimate protein processed from HCV polyprotein precursor, NS5A is a proline-rich hydrophilic phosphoprotein and may exist in dimeric form [3]. Although no intrinsic enzymatic activity has yet been ascribed to NS5A, it likely functions via interactions with other NS proteins and host cell factors [2].

Peroxisome proliferators-activated receptors (PPARs) are ligand-activated nuclear receptors belonging to the steroid/thyroid hormone receptor superfamily; 3 isoforms designated as α, β/δ, and γ exist, all of which are known to be involved in lipid homeostasis. Increased PPARγ expression has been reported in high-fat diet-induced liver steatosis [4].

Chronic HCV infection may result in a wide clinical and prognostic spectrum of severity and progression rates in cases of liver disease, ranging from chronic hepatitis to cirrhosis and hepatocellular carcinoma [5]. Hepatic steatosis is detected in almost 50% of HCV-infected patients, which suggests that it is a crucial contributor to nonalcoholic fatty liver disease (NAFLD) [6], a severe and progressive liver disease resulting in the development of cirrhosis [7]. Recently, it has been reported that HCV core proteins increase hepatic lipid accumulation via the activation of SREBP-1 and PPARγ [8], and that NS2 can upregulate the transcription of SREBP-1c and fatty acid synthase (FAS) [9]. However, the precise molecular mechanisms underlying HCV NS5A-associated steatosis have yet to be clearly characterized. In this study, we explored the possibility that NS5A induces lipid accumulation in hepatocytes and the activity and expression of PPARγ may be deregulated, therefore playing roles in NS5A-induced hepatic triglycerides deposits. Our

* Corresponding author. Fax: +82 51 513 9258.
E-mail addresses: molecule85@pusan.ac.kr, jhcheong2@lycos.co.kr (J. Cheong).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.07.034

results indicate that NS5A-mediated deregulation of PPARγ may be involved in HCV-induced fatty liver disease.

2. Materials and methods

2.1. Plasmids, reagents, and antibodies

pNK/Flag/NS5A, pcDNA4/myc/PGC-1α, and pcDNA3/Flag/PGC-1β were generously donated by Dr. Seishi Murakami, Dr. Toren Finkel, and Dr. Bruce M. Spiegelman [10–12]. pcDNA3/HA/PPARγ, pcDNA3/GFP/PPARγ, pcDNA3/GST/PPARγ, pcDNA3/HA/PGC-1α, pM/PGC-1α, pVP16/PGC-1α, and pcDNA3/HA/NS5A constructs were subcloned via the insertion of the PCR fragments of ORF into pcDNA3/HA, pM (Gal4), pVP16 (Clontech), or pcDNA3/GFP [13,14]. Other constructs were all described previously [8,13].

Rosiglitazone and Ciglitazone were obtained from Cayman. The transfection reagent PolyFect and JetPEI were purchased from QIAGEN and Polyplus-transfection. All other reagents were purchased from Sigma. The antibodies against PPARγ, GST, and GFP were purchased from Santa Cruz Biotechnology Inc. and Actin, Flag, and HA-antibody was obtained from Sigma, Cell Signaling, and Roche, respectively. The anti-NS5A polyclonal antibody was kindly gifted from Dr. Soon B. Hwang (Hallym University, Korea).

2.2. Cell culture and transient ransfection

Chang liver, HepG2, and Huh7 cell lines were maintained in DMEM–10% fetal bovine serum (FBS; Abclone). Transient transfections were conducted using PolyFect or JetPEI with the indicated reporter plasmids and mammalian expression vectors. Total amounts of expression vectors were maintained at constant levels via the addition of empty vectors. Relative luciferase activities were measured with luciferin (BD Biosciences).

2.3. Establishment of Chang liver cells expressing NS5A proteins

Chang liver cells were transfected with 2 μg of pcDNA3/HA or pcDNA3/HA/NS5A using JetPEI reagents in accordance with the manufacturer's instructions. After 48 h, the cells were grown in a medium containing 800 μg/ml G418. Following 2 weeks of selection, sorted single cells were grown under an additional 2 weeks of selection and expanded into stable cells. The candidate clones were analyzed via Western blotting using specific HA-antibody.

2.4. RNA isolation and RT-PCR

Total RNA was prepared using TRIzol (Invitrogen) in accordance with the manufacturer's recommendations. The cDNA was synthesized from 3 μg of total RNA with Moloney murine leukemia virus (MMLV) Reverse Transcriptase (Promega) using a random hexamer (Cosmo, Korea) for 1 h at 37 °C. A one-twenty fifth aliquot of the cDNA was subjected to PCR amplification using gene-specific primers. The PCR primers for PPARγ gene amplification were: 5′-GAAATGACCATGGTTGAC-3′ (sense), 5′-GATGCAGGCTCCACTTTG-3′ (antisense); for NS5A amplification: 5′-TAGCAGTGCTCACTTCCATGCTCA-3′ (sense), 5′-AGGATCTCCGCCGCAATGGATATT-3′ (antisense); for β-actin gene amplification: 5′-GACTACCTCATGAAGATC-3′ (sense), and 5′-GATCCACATCTGCTGGAA-3′ (antisense).

2.5. Establishment of Huh7 cells expressing HCV replicons and interferon-cured cells

An HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally referred to as pHCVIbneo-delS) [15]. The pRep-Feo expressed a fusion gene comprised of *firefly* luciferase (Fluc) and neomycin phosphotransferase, as described else-

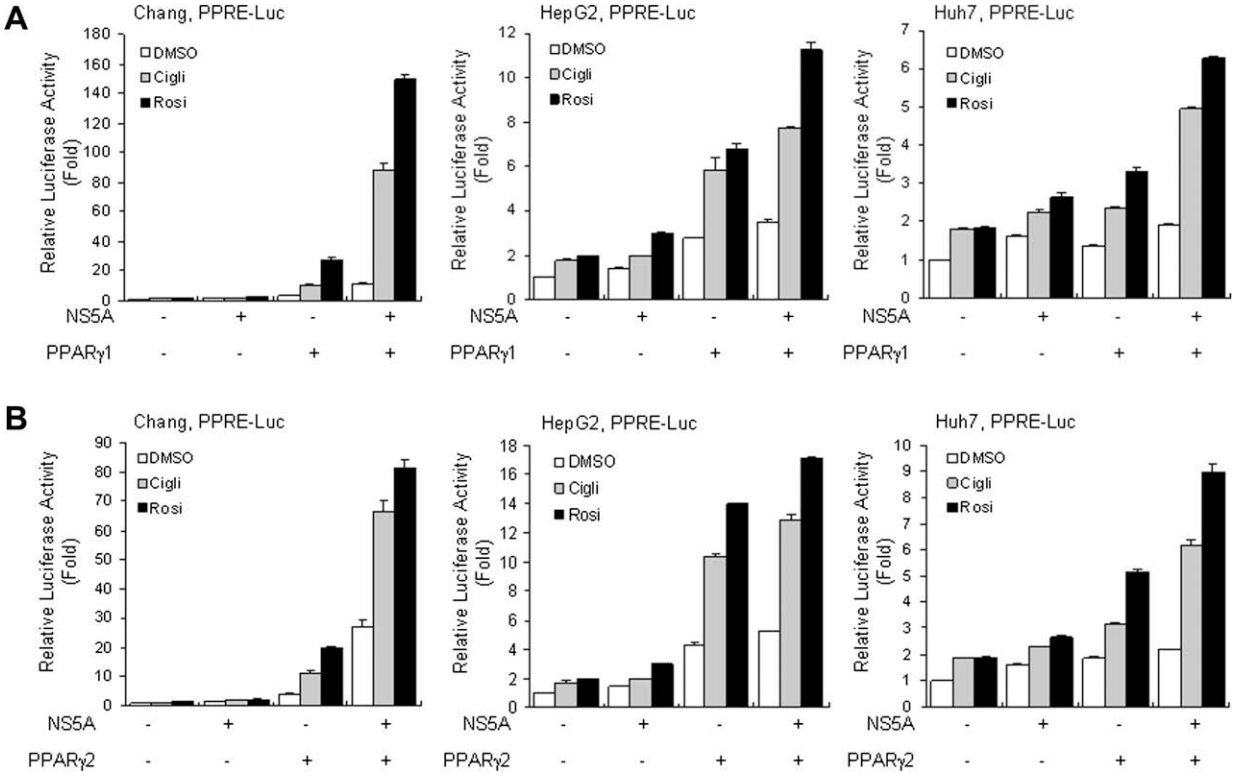

Fig. 1. NS5A induces the transcriptional activity of PPARγ in hepatocytes. (A) PPRE-tk-Luc was cotransfected with the expression vectors for PPARγ1 and NS5A into Chang liver, HepG2, and Huh7 cells. Transfected cells were incubated in the presence or absence of 10 μM Ciglitazone and Rosiglitazone. (B) Cells were cotransfected with a PPRE-tk-luciferase reporter, PPARγ2, and NS5A. Transfected cells were incubated for 24 h in the presence or absence of 10 μM Ciglitazone and Rosiglitazone. Luciferase activity was measured and values are expressed as means ± S.D. for at least two or more independent experiments.