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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Demethylase JMJD6 as a New Regulator of Interferon Signaling: Effects of HCV and Ethanol Metabolism

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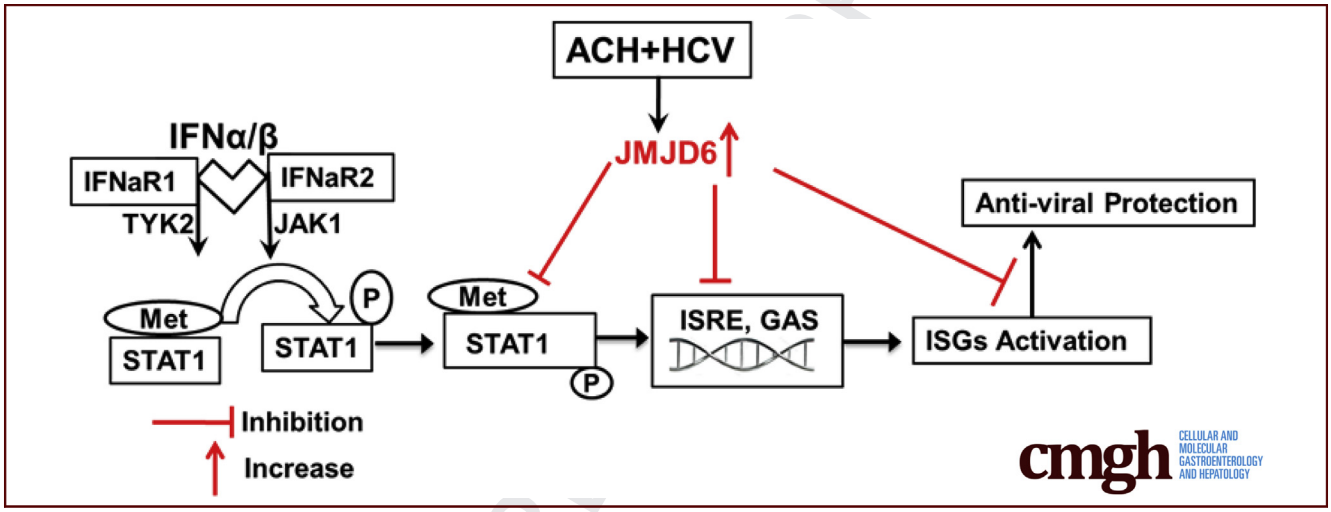

SUMMARY

Demethylase jumonji domain-containing protein 6 expression is enhanced by acetaldehyde and hepatitis C virus. Jumonji domain-containing protein 6 down-regulates signal transducer and activator of transcription 1 methylation in hepatocytes, thereby suppressing interferon α–induced signaling via the Janus kinase–signal transducer and activator of transcription pathway and interferon-sensitive gene activation. This leads to an increase in hepatitis C virus–RNA levels.

BACKGROUND & AIMS: Alcohol-induced progression of hepatitis C virus (HCV) infection is related to dysfunction of innate immunity in hepatocytes. Endogenously produced interferon (IFN)α induces activation of interferon-stimulated genes (ISGs) via triggering of the Janus kinase–signal transducer and activator of transcription 1 (STAT1) pathway. This activation requires protein methyltransferase 1–regulated arginine methylation of STAT1. Here, we aimed to study whether STAT1 methylation also depended on the levels of demethylase jumonji domain-containing 6 protein (JMJD6) and whether ethanol and HCV affect JMJD6 expression in hepatocytes.

METHODS: Huh7.5-CYP (RLW) cells and hepatocytes were exposed to acetaldehyde-generating system (AGS) and 50 mmol/L ethanol, respectively. JMJD6 messenger RNA and protein expression were measured by real-time polymerase chain reaction and Western blot. IFNα-activated cells either overexpressing JMJD6 or with knocked-down JMJD6 expression were tested for STAT1 methylation, ISG activation, and HCV RNA. In vivo studies have been performed on C57Bl/6 mice (expressing HCV structural proteins or not) or chimeric mice with humanized livers fed control or ethanol diets.

RESULTS: AGS exposure to cells up-regulated JMJD6 expression in RLW cells. These results were corroborated by ethanol treatment of primary hepatocytes. The promethylating agent betaine reversed the effects of AGS/ethanol. Similar results were obtained in vivo, when mice were fed control/ethanol with and without betaine supplementation. Overexpression of JMJD6 suppressed STAT1 methylation, IFNα-induced ISG activation, and increased HCV-RNA levels. In contrast, JMJD6 silencing enhanced STAT1 methylation, ISG stimulation by IFNα, and attenuated HCV-RNA expression in Huh7.5 cells.

CONCLUSIONS: We conclude that arginine methylation of STAT1 is suppressed by JMJD6. Both HCV and acetaldehyde increase JMJD6 levels, thereby impairing STAT1 methylation and innate immunity protection in hepatocytes exposed to the virus and/or alcohol. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.10.004)*

Keywords: Alcohol; HCV; JMJD6; STAT1.

Alcohol-induced progression of hepatitis C virus (HCV) infection is related to dysfunction of innate immunity in hepatocytes. Antiviral protection of hepatocytes requires induction of interferon α (IFNα) signaling via the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway to activate interferon–stimulated genes (ISGs), which control viral replication. HCV and alcohol alter the IFN-induced JAK–STAT1 signaling by multiple mechanisms, including the prevention of STAT1 phosphorylation[1–3] and impairment of STAT1 methylation.[4] The latter is attributed to the changes in arginine methylation of STAT1. Indeed, in infected hepatocytes, methylation of STAT1 on Arg-31 is suppressed by HCV.[5,6] Alcohol metabolites, especially acetaldehyde (Ach), further decrease STAT1 methylation, thereby dysregulating ISG activation and enhancing HCV replication.[4]

As shown earlier, STAT1 requires protein methyl transferase 1 (PRMT1)-mediated arginine methylation to attach to DNA and activate protective ISGs.[7] In the liver, activities of many methyltransferases, including PRMT1, are regulated by the changes in the ratio between the methyl donor S-adenosylmethionine (SAM) and its toxic metabolite, S-adenosylhomocysteine. Alcohol and HCV decrease the SAM:S-adenosylhomocysteine ratio[8–10] in liver cells, suggesting suppression of SAM-dependent PRMT1 activity. We in fact observed the reduced STAT1 arginine methylation in HCV-infected hepatocytes exposed to the major ethanol metabolite Ach.[4]

In addition to methyl-transferases, protein methylation levels may be regulated by demethylases. More recently, jumonji domain-containing protein 6 (JMJD6), a nonheme Fe(II) 2-oxoglutarate–dependent oxygenase with arginine demethylase and lysyl hydroxylase activities,[11,12] has been shown to control arginine methylation of another innate immunity factor, TNF-receptor–associated factor 6.[13] However, the role of JMJD6 in altering STAT1 methylation has never been investigated. In this study, we thus examined the effects of HCV and acetaldehyde on JMJD6 levels, which can decrease antiviral protection by impairing STAT1 methylation and subsequent IFNα-induced ISG activation in hepatocytes.

Materials and Methods

Reagents and Media

High-glucose Dulbecco's modified Eagle medium, Williams medium, and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA). Anti–mono-dimethyl arginine antibody (ab412) was obtained from Abcam, Inc (Cambridge, MA). Anti–alcohol dehydrogenase (ADH) was a gift from Dr Michael Felder (University of South Carolina, Columbia, SC). Cytochrome P450 2E1 (CYP2E1) (AB1252) was from EMD Millipore (Temecula, CA). Antibody to the STAT-1 (sc-592) and β-actin (sc-47778) antibodies were from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). JMJD6 antibody (TA306835) and small interfering RNA (siRNA) (SR308094) were from OriGene (Rockville, MD); JMJD6 plasmid (plasmid 31358) was from Addgene. Polymerase chain reaction (PCR) reagents, probes, and primers were from Life Technologies, Inc (Carlsbad, CA). Other reagents, all of analytical-grade quality, were from Sigma (St. Louis, MO).

Cells

Huh7.5 cells were transfected with pIV-G2 (CYP2E1) plasmid as previously described for other cell lines[14,15] using Lipo TAXI (Invitrogen Corp). Recombinant cells, designated *RLW cells*, were selected in culture medium containing G418 at 400 μg/mL. Clones were expanded and screened for CYP2E1 expression and activity. The clones with the highest CYP2E1 activity were designated as RLW cells. Because we were unable to transfect RLW cells with the ADH plasmid, we used acetaldehyde that had been exogenously generated by a special in vitro system (acetaldehyde-generating system [AGS], see description later).

RLW Cell Treatments

RLW cells were infected by Japanese fulminant hepatitis virus-1 (HCV genotype 2a) virus at a multiplicity of infection of 0.1 as previously described[16] or were left uninfected. On day 2 after infection, cells were exposed for up to 48 hours to either 50 mmol/L ethanol or to AGS that contained yeast ADH (0.02 U/mL), 2 mmol/L nicotinAMI-1de adenine dinucleotide, and 50 mmol/L ethanol. In the presence of RLW cells, the levels of generated Ach measured by gas chromatography in the medium fluctuated between approximately 250 (at 1–4 hours of exposure) and 50 μmol/L (at 18–48 hours of exposure). These levels of Ach corresponded to the amounts of Ach produced by ADH-expressing liver cells and were equivalent to the physiological concentrations observed in the liver of ethanol consumers.[15,17] Some treatments were performed in the presence or absence of AMI-1, a PRMT1 inhibitor (100

Abbreviations used in this paper: **Ach, acetaldehyde; ADH, alcohol dehydrogenase; AGS, acetaldehyde-generating system; AMI-1, _____; BHMT, betaine-homocysteine-S-methyltransferase; cDNA, complementary DNA; CYP2E1, cytochrome P450 2E1; 4-MP, 4-methylpirazole; HCV, hepatitis C virus; IFN, interferon; ISG, interferon-stimulated gene; JAK-STAT, Janus kinase–STAT, signal transducer and activator of transcription; JMJD6, jumonji domain-containing 6 protein; mRNA, messenger RNA; OA, okadaic acid; OAS-1, 2'-5'-oligoadenylate synthetase-1; OASL, 2'-5'-oligoadenylate synthetase-like protein; PCR, polymerase chain reaction; PP2A, protein phosphatase 2A; PRMT1, protein methyl transferase 1; RLW, _____; RT, reverse-transcription; SAM, S-adenosylmethionine; siRNA, short interfering RNA; TK-NOG, _____.**

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