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Downregulation of autophagy-related gene ATG5 and GABARAP expression by IFN-λ1 contributes to its anti-HCV activity in human hepatoma cells

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ABSTRACT

Type-III interferon (IFN-λ), the most recently discovered family of IFNs, shares common features with type I IFNs, but also has many distinctive activities. It is not clear that whether IFN-λ has additional antiviral mechanisms. In this study, we investigated the effects of IFN-λ on autophagy, a cellular process closely related to hepatitis C virus (HCV) infection in human hepatoma Huh7 cells. Our results showed that IFN-λ1 treatment inhibit autophagic activity in Huh7 cells, as evidenced by the decreased expression of microtubule-associated protein 1 light chain 3B (LC3B)-II and conversion of LC3B-I to LC3B-II, decreased formation of GFP-LC3 puncta and accumulation of autophagosomes. IFN-λ1 could also inhibit HCV-induced or tunicamycin (a known inducer of autophagy with similar mechanism to HCV infection) -induced LC3B-II expression and autophagosome formation. Through PCR array, real time RT PCR, and western blot, two autophagy-related genes, ATG5 and GABARAP, were identified and verified to be down-regulated by IFN-λ1 treatment, either in HCV-uninfected Huh7 cells or in HCV JFH-1-infected cells. Overexpression of ATG5 and/or GABARAP could partly recover the IFN-λ1-inhibited HCV replication. Mechanism research demonstrated that IFN-λ1 could induce the expression of miR-181a and miR-

Abbreviations: IFN-λ, interferon-λ; HCV, hepatitis C virus; LC3B, microtubule-associated protein 1 light chain 3B; HCC, hepatocellular carcinoma; DAAs, directly acting antivirals; SVR, sustained virological response; PEG-IFN/RBV, polyethylene glycol interferon-α/ ribavirin; IFN-λR1, IFN-λ receptor 1; GWASs, genome-wide association studies; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HSV-1, herpes simplex virus 1; PRRs, pattern-recognition receptors; JAK, Janus kinase; TYK, tyrosine kinase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; NEAA, non-essential amino acids; MOI, multiplicity of infection; TEM, Transmission electron microscopy; ISGs, interferon-stimulated genes; ER, endoplasmic reticulum; PE, phosphatidylethanolamine; PAS, pre-autophagosomal structure; PAMP, pathogen-associated molecular pattern; RIG-I, retinoic acid−inducible gene I; Tu, tunicamycin.

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214 (targeting ATG5 and GABARAP respectively), by which down-regulates ATG5 and GABARAP expression. Taken together, our results indicate that suppression of the autophagy response by IFN-λ1 contributes to IFN-λ1 anti-HCV activity. The results also provide a theoretical basis for improving the effectiveness of IFN treatment of HCV infection through inhibition of the HCV-induced autophagy response.

1. Introduction

Hepatitis C virus (HCV) infection is a major health problem affecting nearly 170 million people worldwide. As a leading cause of chronic viral hepatitis, HCV establishes chronic infection in more than 70% of infected individuals, which is associated with a chronic inflammatory disease that ultimately leads to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Maasoumy and Wedemeyer, 2012). Recently, licensing of directly acting antivirals (DAAs) has considerably improved therapeutic options (Tse, 2013), and sustained virological response (SVR) rate in HCV chronically infected patients has been increased tremendously (Koh and Liang, 2014). However, due to high cost, the standard therapy to treat HCV infection is still polyethylene glycol interferon-α/ribavirin (PEG-IFN-α/RBV) in most developing countries (Sarrazin et al., 2012). Furthermore, this therapy has suboptimal SVR rates in treatment resistant patients as well as deleterious side effects (Feld, 2014). Moreover, natural or treatment-induced virus elimination does not prevent reinfection by HCV. In consideration of the limitations of current PEG-IFN-α/RBV therapy, developing new alternative drugs is an important goal in HCV research.

Type III IFNs (IFN-λs), the most recently discovered IFN family, shares some common features with type I IFNs, but also has many distinctive activities. Unlike the type I IFN receptor that is commonly expressed in most types of cells, IFN-λ receptor (IFN-λR1) that is unique to IFN-λ displays a restricted cellular distribution. In non-hematopoietic cells, it is expressed in epithelial cells including hepatocytes (Durbin et al., 2013; Mahlakoiv et al., 2015). IFN-λs might share the therapeutic benefits but avoid the clinical side effects of type I IFNs (Donnelly et al., 2011; Hermant and Michiels, 2014). Furthermore, genome-wide association studies (GWASs) have revealed that multiple IFN-λ polymorphisms are associated with HCV clearance and might improve outcomes in other viral infections, including hepatitis B virus (HBV), human cytomegalovirus (HCMV), and herpes simplex virus 1 (HSV-1) (Griffiths et al., 2015; Lampertico et al., 2013; Manuel et al., 2015). Due to its anti-HCV activity and fewer side effects (Friborg et al., 2013), IFN-λ is being tested in several clinical trials with promising preliminary data (Hruska et al., 2015; Muir et al., 2010). Currently, it is considered that IFN-λs share antiviral mechanisms with type I IFNs, including induction of IFNs through pattern-recognition receptors (PRRs), activation of Janus kinase (JAK) 1/tyrosine kinase (TYK) 2, upregulation of IFN-stimulated genes (ISGs), and so on. However, other mechanism(s) involved in anti-HCV of IFN-λs remain unclear and need to be further studied, because IFN-λs are after all quite different from type I IFNs.

Recently, a number of papers have shown close relationship between HCV and cellular autophagy (Dreux et al., 2009; Wang et al., 2014, 2015b). Autophagosomes may act as a scaffold for intracellular membrane-associated replication of certain cytoplasmic RNA viruses including HCV. HCV could modulate the autophagy pathway, for example, inducing autophagy vesicles in hepatocytes, for its survival and replication (Dreux et al., 2009; Shinohara et al., 2013; Taguwa et al., 2011; Tanida et al., 2009). On the other hand, several autophagy proteins have the capacity to positively regulate HCV productive infection (Guevin et al., 2010; Shrivastava et al., 2012; Su et al., 2011; Wang et al., 2015b). Furthermore, inhibition of autophagy results in enhancement of the innate immune response and reduces the production of HCV viral particles (Shrivastava et al., 2011; Tanida et al., 2009). Thus, autophagy has been considered a potential target for the development of novel anti-HCV drugs (Fabri et al., 2011; Panigrahi et al., 2015; Subauste, 2009). In this study, we investigated whether IFN-λ inhibits HCV replication through its impact on cellular autophagy as well as mechanism(s) involved in.

2. Materials and methods

2.1. Reagents

IFN-λ1, tunicamycin, bafilomycin A1, mouse anti-LC3 antibody, and secondary antibodies for Western blot (horseradish peroxidase -conjugated goat-anti-rabbit IgG, goat-anti-mouse IgG) were purchased from Sigma-Aldrich China (Shanghai, China). Rabbit anti-β-actin, mouse anti-ATG5, and mouse anti-GABARAP antibodies were purchased from Abcam Trading China (Shanghai, China). Mouse anti-HCV core antibody was purchased from Thermo Fisher Scientific China (Shanghai, China).

2.2. Cells, virus, plasmids, miRNA inhibitors

The human hepatoma Huh7 cell line and HCV JFH-1 virus were kindly provided by Dr. Wenzhe Ho (Temple University, Philadelphia, USA). ATG5 plasmid (pCMV-myc-Atg5) was purchased from www.addgene.org (Cambridge, MA, USA). GABARAP plasmid (pcDNA3.1-GABARAP) was purchased from the Land Biotechnology Company (Guangzhou, China). Transfection of plasmid DNA to Huh7 cells was carried out with Lipofectamine3000 from Thermo Fisher Scientific China (Shanghai, China). miR-181a inhibitor, miR-214 inhibitor and miRNA inhibitor control were purchased from Ruibo Biotechnology (Guangzhou, China) and the transfection was carried out according to manufacturer's instructions.

2.3. Cell culture and virus infection

Huh7 cells were routinely maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 nM non-essential amino acids (NEAA), 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were cultured at 37 °C in a humidified incubator with an atmosphere of 5% CO_2. Infectious HCV JFH-1 was generated by transfection of *in vitro* transcribed genomic JFH-1 RNA to Huh7 cells as previously described (Wakita et al., 2005). Infection of Huh7 cells with HCV JFH-1 was carried out at a multiplicity of infection (MOI) of 0.1.

2.4. RNA preparation and quantitative real-time RT-PCR (qPCR)

Total cellular RNA was extracted from cells with Qiagen RNeasy Mini Kit (Qiagen). For mRNA analysis, cDNA was synthesized with