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Interferon alpha antagonizes STAT3 and SOCS3 signaling triggered by hepatitis C virus

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We aimed to investigate regulation of signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signaling 3 (SOCS3) by interferon alpha (IFN-α) and to analyze the relationship between STAT3 and SOCS3 during hepatitis C virus (HCV) infection. Changes in STAT3 and SOCS3 were analyzed at both mRNA and protein levels in human hepatoma cells infected with HCV (J6/JFH1). At 72 h of HCV infection, STAT3 expression was decreased with sustained phosphorylation, and IFN-α increased such decrease and phosphorylation. HCV increased SOCS3 expression, while IFN-α impaired such increase, indicating different regulation of STAT3 and SOCS3 by IFN-α. IFN-α-induced expression and phosphorylation of upstream kinases of the JAK/STAT pathway, Tyk2 and Jak1, were suppressed by HCV. Moreover, knockdown of STAT3 by RNA interference led to decreases in HCV RNA replication and viral protein expression, without affecting either the expression of Tyk2 and Jak1 or the SOCS3 induction in response to IFN-α. These results show that IFN-α antagonizes STAT3 and SOCS3 signaling triggered by HCV and that STAT3 regulation correlates inversely with SOCS3 induction by IFN-α, which may be important in better understanding the complex interplay between IFN-α and signal molecules during HCV infection.

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1. Introduction

Signal transducers and activators of transcription (STATs) are activated by phosphorylation at tyrosine residues in response to growth factors and cytokines including type I and II interferons (IFNs) and interleukin-6 superfamily [1]. STATs, a family of transcription factors, transmit extracellular signals to the nucleus and initiate transcription [2]. STATs are studied due to their distinct functions in mediating IFN-dependent responses. As with STAT1 and STAT2, STAT3 plays an essential role in IFN signaling [3]. STAT3 has two splice forms, STAT3α (86 kDa) and STAT3β (79 kDa), and both of which are activated by upstream Jak kinases. STAT3 possesses diverse biological functions such as antiviral activity, cytoprotective and anti-inflammatory effects, developmental role, and tumorigenic ability [1,4].

Defining regulation of STAT3 by viruses may provide important insights into how viruses counter host immune responses. Infection with hepatitis C virus (HCV), a positive single-stranded RNA virus, is a major global problem leading to progressive human liver disease. The care for the treatment of chronic hepatitis C, pegylated IFN-α and ribavirin, shows response rates of 40–80% and that such therapy in combination with HCV protease inhibitors will yield higher cure rates and shorter treatment durations [5]. Improved understanding the basis of virus-host interaction and the cellular antiviral processes has led to the identification of potential targets for more effective HCV therapy and prevention. Studies have been performed in HCV-infected patients and in HCV replicons as well as HCV infection systems to investigate the role of STAT3 in viral survival and in therapeutic efficacy of IFN-α against HCV. STAT3 is significantly activated in peripheral blood mononuclear cells from HCV-infected patients and in infected Huh7.5.1 cells [6]. Levels of STAT3 protein are reduced in immune cells and hepatocytes from HCV-infected patients and in Huh7 cells transfected with HCV constructs [7]. In HCV-infected liver tissues, activated STAT3 plays a critical role in hepatocellular response against inflammatory damage [8]. Recently, STAT3 has been shown to positively regulate microtubule dynamics, thereby positively regulating HCV replication [9]. Moreover, STAT3 activation initiates efficient anti-HCV activity of IFN-α [10]. Induction of hepcidin, a protein involved in innate immunity, is mediated by STAT3 signaling following initiation of pegylated IFN-α treatment for HCV infection [11].

The suppressor of cytokine signaling (SOCS) family consists of SOCS1–SOCS7 and CIS members and is a negative regulator of cytokine signaling [12]. Among these proteins, SOCS1 and SOCS3 are

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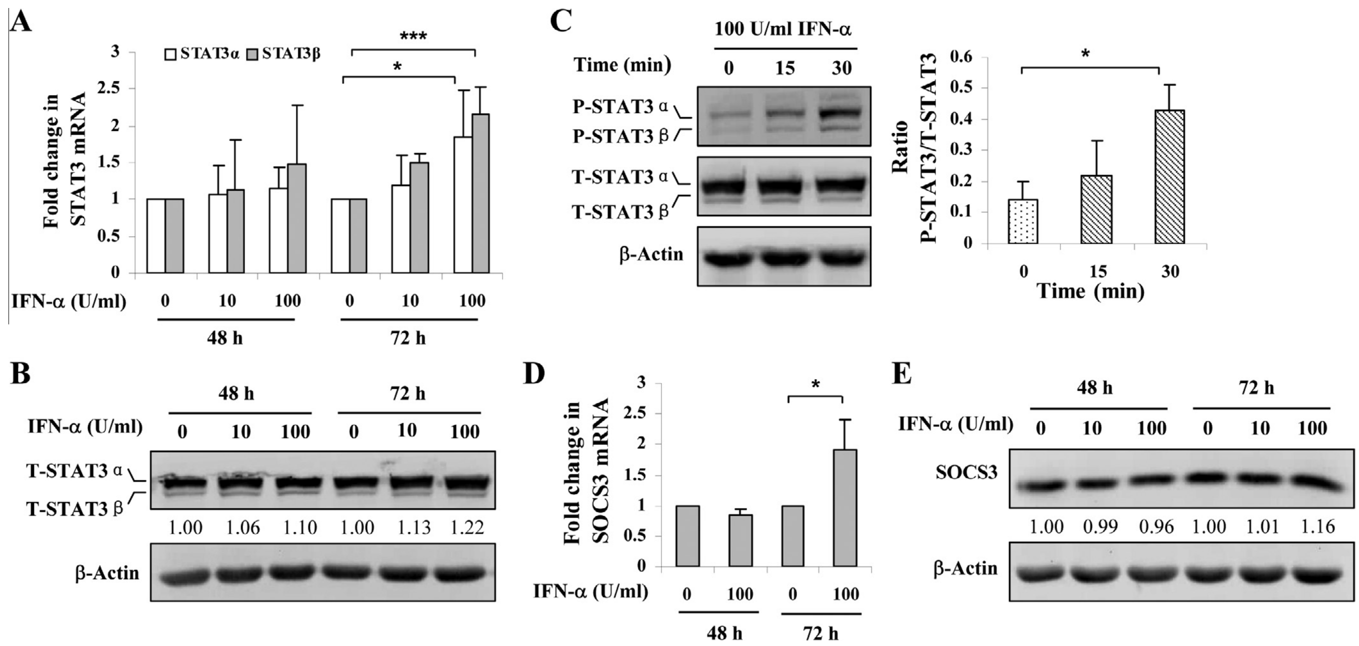

Fig. 1. Effect of IFN-α on STAT3 and SOCS3. (A) Huh7.5.1 cells were cultured with IFN-α for 48 h and 72 h. Cellular RNA was assessed by real-time PCR to determine STAT3 mRNA levels. Data are expressed as fold induction of the mRNA levels of STAT3α and STAT3β relative to the corresponding levels of untreated control (0, set to 1) and represent the means ± SD of 4 experiments. $^{*}P < 0.05$; $^{***}P < 0.001$. (B) Cell lysates were analyzed by Western blotting using anti-total STAT3 (T-STAT3) and β-Actin antibodies. The signals for T-STAT3 (STAT3α and STAT3β) from the shown experiment were quantified. Fold induction of the amounts of T-STAT3 relative to the corresponding amounts of untreated control (0, set to 1) is shown below each blot. (C) Huh7.5.1 cells were stimulated with IFN-α for the indicated time periods. Cell lysates were analyzed by Western blotting using antibodies against total STAT3 and phosphorylated STAT3 (P-STAT3). The signals for T-STAT3 and P-STAT3 (STAT3α and STAT3β) from 3 experiments were quantified. Data are expressed as the means of the ratio of P-STAT3/T-STAT3 ± SD (right panel). (D) SOCS3 mRNA levels were determined in Huh7.5.1 cells cultured with IFN-α. Data are expressed as fold induction of the mRNA levels of SOCS3 relative to the corresponding levels of untreated control (0, set to 1) and represent the means ± SD of 3 experiments. (E) Western blotting was performed to detect SOCS3. The signals for SOCS3 from the shown experiment were quantified. Fold induction of the amounts of SOCS3 relative to the corresponding amounts of untreated control is shown below each blot. Representative of 3 experiments is shown.

closely involved in regulation of IFN-dependent JAK/STAT signaling [13]. SOCS3 is responsible for suppression of IFN signaling and IFN production during herpes simplex virus infection [14]. Similarly, HCV core protein impairs IFN-α-induced signal transduction via induction of SOCS3 expression [15]. SOCS3 also represents a novel biomarker for prediction of antiviral treatment response in chronic hepatitis C [16]. However, how SOCS3 induction correlates with STAT3 regulation by IFN-α remains to be determined during HCV infection.

Previously, we found that IFN-α participated in the regulation of STAT3 by ribavirin [17]. Given the roles of STAT3 and SOCS3 in HCV infection and in the anti-HCV activity of IFN-α, we investigated how IFN-α targeted STAT3 and SOCS3 signaling. We used Huh7.5.1, a permissive human hepatoma cell line for cell culture-derived HCV (HCVcc) replication, to monitor dynamic changes in STAT3 status and SOCS3 induction. Our results have shown antagonistic effects of IFN-α on STAT3 and SOCS3 signaling triggered by HCV.

2. Materials and methods

2.1. Reagents

Recombinant human IFN-α2a was obtained from PBL Interferon Source (Piscataway, NJ, USA). STAT3 siRNA (cat. no. 6580), control siRNA (unconjugated, cat. no. 6568), and rabbit polyclonal antibodies for Tyk2, Jak1, STAT3, phospho-Tyk2 (Tyr1054/1055), phospho-Jak1 (Tyr1022/1023), phospho-STAT3 (Tyr705), SOCS3, and β-Actin were purchased from Cell Signaling Technology (Beverly, MA, USA). HCV nonstructural protein 3 (NS3) mouse mAb (Clone H23) and core protein mouse mAb (Clone C7-50) were obtained from Abcam (Cambridge, UK). Horseradish peroxidase-conjugated goat anti-rabbit IgG or anti-mouse IgG was purchased from Cell Signaling Technology or Bio-Rad (Hercules, CA, USA).

2.2. Cell culture and HCVcc infection

Human hepatic cell line Huh7.5.1 was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). For HCVcc (J6/JFH1) infection, Huh7.5.1 cells were incubated for 2 h at 37 °C with HCVcc stock (multiplicity of infection of 0.3) in 10% FBS–DMEM. After the virus inoculum was removed, fresh medium with or without 100 U/ml IFN-α was added to the cells.

2.3. RNA interference

To down-regulate STAT3 expression, STAT3 siRNA was transfected into Huh7.5.1 cells using Lipofectamine™2000 following the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Cells at 30–50% confluence were transfected with STAT3 siRNA or control siRNA at a final concentration of 100 nM. At 72 h post-transfection, cells were stimulated with 100 U/ml IFN-α for 30 min and lysed for assessment of STAT3 expression and activation.

2.4. IFN-α treatment

Huh7.5.1 cells were stimulated with 100 U/ml FN-α dissolved in 0.1% bovine serum albumin for short time periods (0, 15, and 30 min) at 37 °C. In addition, Huh7.5.1 cells were cultured in 10% FBS–DMEM with increasing concentrations of IFN-α (0, 10, and 100 U/ml) for 48 h and 72 h, respectively. Seventy-two hours after transfection with siRNA, cells were passaged, infected with the

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