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SOCS1 abrogates IFN's antiviral effect on hepatitis C virus replication

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ABSTRACT

Suppressor of cytokine signaling 1 (SOCS1) and suppressor of cytokine signaling 3 (SOCS3) have been thought to block type I interferon (IFN) signaling. We have previously reported that SOCS3 suppresses HCV replication in an mTOR-dependent manner. However, the relationship between SOCS1 and HCV replication remains unclear. Here, we found that overexpression of SOCS1 alone did not have an effect on HCV RNA replication. However, suppression of HCV replication by IFN-α was rescued by SOCS1 overexpression. The upregulation of HCV replication by SOCS1 overexpression in the presence of IFN is likely a result of the impairment of IFN signaling by SOCS1 and subsequent induction of ISGs. Knockdown of SOCS1 alone with specific shRNA enhanced the antiviral effect of IFN compared with negative control. Thus, SOCS1 acts as a suppressor of type I IFN function against HCV.

1. Introduction

Hepatitis C virus (HCV) is a major world health problem with an estimated 180 million people infected worldwide (Bostan and Mahmood, 2010). Chronically infected patients have a high risk of progressive liver disease, cirrhosis and hepatocellular carcinoma. Pegylated interferon-α (IFN-α) in combination with ribavirin is standard therapy for patients with chronic hepatitis C (Chung et al., 2004; McHutchison et al., 1998; McHutchison et al., 2009). Newly approved direct acting antivirals significantly enhance HCV treatment responses (Fusco and Chung, 2012), but IFN will likely remain a cornerstone of treatment for many if not most patients. The anti-HCV activity of IFN-α is mediated by the induction of IFN-stimulated genes (ISGs), which establish an antiviral state in cells. Binding of IFN-α to its cell surface receptor subunits IFNAR1 and IFNAR2 leads to the activation of the receptor associated Janus-activated kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2) (Darnell et al., 1994; Stark and Darnell, 2012). The activated kinases phosphorylate the downstream signal transducer and activator of transcription proteins 1 and 2 (STAT1 and STAT2), which combine with IFN-regulatory factor 9 (IRF9) in a complex, ISGF3, that binds to the IFN-stimulated response elements on cellular DNA and stimulates the expression of the multiple ISGs (Fu et al., 1990; Stark and Darnell, 2012).

IFN-α signaling is critical for the generation of an appropriate immune response to viral infections (Theofilopoulos et al., 2005); therefore it is important to understand the regulation of IFN-α signaling. SOCS1 is a potent negative regulator of both type I and type II IFN signaling (Alexander et al., 1999; Fujimoto and Naka, 2010). The SOCS1 protein comprises an N-terminal region that contains the kinase inhibitory region (KIR), a central Src Homology 2 (SH2) domain and a C-terminal SOCS box (Fujimoto and Naka, 2010). The SH2 and KIR domain are involved in binding and inhibition of kinase activity (Giordanetto and Kroemer, 2003), respectively, whereas the SOCS box is involved in targeted degradation. SOCS1 inhibits type I IFN signaling through an interaction with the IFNAR1-associated kinase Tyk2 (Piganis et al., 2011). The SH2 domain of SOCS1 associates with conserved phosphotyrosines 1054 and 1055 of Tyk2 (Piganis et al., 2011). The kinase inhibitory region of SOCS1 is also essential for its interaction with Tyk2 and inhibition of IFN signaling.

We have previously reported that SOCS3 suppresses HCV replication in cell culture in an mTOR-dependent manner (Shao et al., 2010). There are eight SOCS family proteins, which are all negative regulators of the IFN pathway. However, the most relevant question to us was which of these were most likely to be HCV-specific. Based on the literature (Moorman et al., 2009; Sun et al., 2006; Vlotides et al., 2004), we speculated that SOCS1 was the most plausible candidate to mediate this effect. Here, we found that SOCS1 negatively regulated the antiviral effect of IFN in both JFH1 and OR6 models. Moreover, the upregulation of classical ISGs by IFN is reduced when SOCS1 is overexpressed.

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2. Materials and methods

2.1. Cell cultures and infectious viruses

Human hepatocellular carcinoma Huh7.5.1 cells were grown at 37 °C in 5% CO_2 atmosphere in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (0.1 mg/mL streptomycin and 100 U/mL penicillin). Infectious JFH1 plasmid was obtained from Dr. Takaji Wakita and infected as previously described (Wakita et al., 2005).

2.2. Plasmid constructs and transfection

The pCR3.1/SOCS1 constructs were generous gifts from Dr. Christoph J. Auernhammer (Ludwig-Maximilians-University of Munich). Huh7.5.1, OR6 or cured OR6 cells grown on 12-well plates at 60–70% confluency were transfected with 2 μg of pCR3.1, pCR3.1/SOCS1 using Fugene HD (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's protocol. For OR6 or cured OR6 cells, Western blot or RT-PCR were performed 72 h after transfection. For Huh7.5.1 cells infected with JFH1 inoculum Western blot or RT-PCR were performed 48 h after transfection.

2.3. Establishment of a Huh7.5.1 cell line with stable knockdown of SOCS1 expression

Sequences targeting the SOCS1 gene were selected to generate a short hairpin RNA (shRNA) and the following sense oligonucleotide: GCAUCCGCGUGCACUUUCAUU. The short interfering RNA (siRNA) expression vector for SOCS1, pcPURU6-SOCS1i, was designed according to the instructions of the manufacturer (iGENE Therapeutics, Tsukuba, Japan) using the pcPURU6i cassette vector (iGENE Therapeutics), which has been described previously (Shao et al., 2010). The pcPURU6-GFPi cassette vector served as the control. These vectors were introduced into Huh7.5.1 cells to establish stable knockdown of SOCS1. Briefly, Huh7.5.1 cells were transfected with the targeting or the control vectors by using FuGene HD according the manufacturer's protocol, and the clones resistant to puromycin (Sigma, Steinheim, Germany) were selected. Knockdown of SOCS1 was confirmed by qPCR and Western blotting.

2.4. ISRE-luciferase reporter assay

IFN-induced ISRE signaling was monitored as previous described (Zhang et al., 2011). Briefly, OR6 cells were transfected with pCR3.1 or pCR3.1/SOCS1 using Fugene HD following the manufacturer's protocol. HCV replication in OR6 cells was assessed by monitoring *Renilla* luciferase activity (Promega, Madison, WI). Gene expression was monitored by the Promega dual-luciferase reporter assay system (Promega, Madison, WI). To monitor IFN signaling directed by the IFN-stimulated response element (ISRE), the plasmids pISRE-luc (500 ng/well) expressing firefly luciferase and pRL-TK (50 ng/well) expressing *Renilla* luciferase were cotransfected with the appropriate plasmid (2 μg/well) and relative luciferase activity was then assessed by the Promega dual-luciferase reporter assay system (Promega, Madison, WI).siRNA and transfection

2.5. siRNA and transfection

Indicated siRNAs were transfected into cells using Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA). Negative control siRNA was from QIAGEN. All siRNAs used for gene knock-down were from GenePharma and were as follows: SOCS1, #2, UCCGUUCGCACGCCGAUUAUU; SOCS1, #3, GCAUCCGCGUGCACUUUCAUU. The protein expression of each gene knock down was confirmed by Western blotting or QPCR.

2.6. Cell viability assay

Huh7.5.1 cells or OR6 cells were seeded in 96 well plates. Cells were treated according to the different experiment designs described above. Cell viability was monitored using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Madison, WI) Kit according to the manufacturer's protocol.

2.7. Quantitative real time PCR

Total cellular and viral RNA was isolated using RNeasy Mini columns (QIAGEN) with on-column DNase digestion, reverse transcribed by random priming with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Foster City, CA), and then quantitated by real time PCR using the Bio-Rad IQ5 (Bio-Rad Laboratories) and the DyNAmo HS SYBR Green qPCR kit (Finnzyme; Espoo, Finland). The primer sequences are listed in Table 1. The reaction mixture was first denatured at 95 °C for 3 min and then 45 cycles of PCR were performed using the following protocol: 94 °C, 20 s; 60 °C, 30 s; 72 °C, 20 s. Each gene's mRNA level was normalized with actin to obtain mRNA arbitrary units (fold).

2.8. Western blotting

Cells were lysed using a radioimmune precipitation assay (RIPA) buffer containing 0.1% SDS, 0.5% NP-40, 10 mM Tris–HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), and 150 mM NaCl. Whole cell lysates were sonicated, boiled at 95 °C for 5 min, and chilled on ice for 10 min. Proteins were separated by SDS–PAGE with NuPAGE Novex pre-cast 4–12% Bis–Tris gradient gels (Invitrogen, Carlsbad, CA) and transferred to PVDF membranes. The primary antibodies included mouse anti-STAT1, rabbit anti-Phospho-STAT1 (Tyr701) (Cell Signaling Technology, Inc., Beverly, MA), rabbit anti-HCV NS5A, mouse anti-HCV core, C7–50 (Affinity BioReagents Inc., Golden, CO), ISG15, IRF9, DDK, PKR(Abcam, Cambridge, MA), anti-SOCS1 (Cell Signaling Technology, Inc., Beverly, MA), and mouse anti-β-actin (Sigma, Inc., St. Louis, MO). The secondary antibodies were HRP-conjugated ECL donkey anti-rabbit IgG, or HRP-conjugated ECL sheep anti-mouse IgG (Amersham Biosciences, Piscataway, NJ). The ECL Western Blotting Detection Kit (Amersham Biosciences, Piscataway, NJ) was used to detect chemiluminescent signals.

Table 1
Primers used for quantitative RT-PCR.

Target gene	Primer[a]	Nucleotide sequence
β-Actin	F	5′-GCACTCTTCCAGCCTTCCT-3′
β-Actin	R	5′-AGGTCTTTGCGGATGTCCAC-3′
IRF9	F	5′-CCCGAAAACTCCGGAACTG-3′
IRF9	R	5′-CAGCACACTCCGGGAAACT-3′
ISG15	F	5′-GGTGGACAAATGCGACGAA-3′
ISG15	R	5′-ATGCTGGTGGAGGCCCTTA-3′
JFH1	F	5′-TCTGCGGAACCGGTGAGTA-3′
JFH1	R	5′-TCAGGCAGTACCACAAGGC-3′
PKR	F	5′-TCTACGCTTTGGGGCTAAT-3′
PKR	R	5′-AGATGATGCCATCCCGTAG-3′
SOCS1	F	5′-GGTCCCCCTGGTTGTTGTA-3′
SOCS1	R	5′-TAGGAGGTGCGAGTTCAGGT-3′
STAT1	F	5′-GTGGAAAGACAGCCCTGCA-3′
STAT1	R	5′-ACTGGACCCCTGTCTTCAA-3′

[a] F, forward; R, reverse.

2.9. Statistical analysis

Data analysis was carried out using the Student's t test with pooled variance. Data were expressed as an average of at least quadruplicate, unless stated otherwise. The significance of differences was calculated by two-tailed unpaired t test. In all analyses, $*$ indicates $p < 0.05$, $**$ indicates $p < 0.005$ and $***$ indicates $p < 0.0005$.

3. Results

3.1. SOCS1 knockdown accentuates the antiviral effects of IFN

Robust HCV viral replication in hepatocytes requires expression of host proviral factors or attenuation of host antiviral factors. Previous work from our lab showed that SOCS3 negatively regulated HCV replication in two different HCV replication models: the tissue culture infectious JFH1 strain in Huh7.5.1 cells, and the full length HCV replicon OR6 cells. In order to evaluate the effect of SOCS1 on HCV replication, we designed two siRNA oligos against SOCS1. Both siRNA oligos knocked down SOCS1 mRNA and protein as shown in Fig. 1A and Fig. 1C. The antiviral effect of IFN-α was enhanced by knocking down SOCS1 in both JFH1 infected cells (Fig. 1B) and OR6 cells (Fig. 1C). Next we constructed an shRNA using the same sequence to one of the siRNA oligos (#3) and stably transfected it into Huh 7.5.1 cells. We then infected the stable SOCS1 knockdown cells with genotype 2a HCV full-length infectious virus JFH1. Knockdown of SOCS1 was confirmed by RT-qPCR (Fig. 1D). In the presence of shRNA against SOCS1, JFH1 RNA levels were unchanged (Fig. 1E). Because SOCS1 is a known negative regulator of type I interferon signaling, we evaluated whether SOCS1 could impact the antiviral effects of IFN-α. As shown in Fig. 1F, IFN-α did not upregulate the expression of SOCS1, however the antiviral effect of IFN-α was enhanced by knocking down SOCS1 (Fig. 1E). These data indicate that SOCS1 is not an ISG and that knockdown of SOCS1 enhances the anti-HCV effects of IFN.

3.2. SOCS1 overexpression reverses the antiviral effects of IFN

Having observed that SOCS1 knockdown enhanced the antiviral activity of IFN-α, we subsequently investigated the role of SOCS1

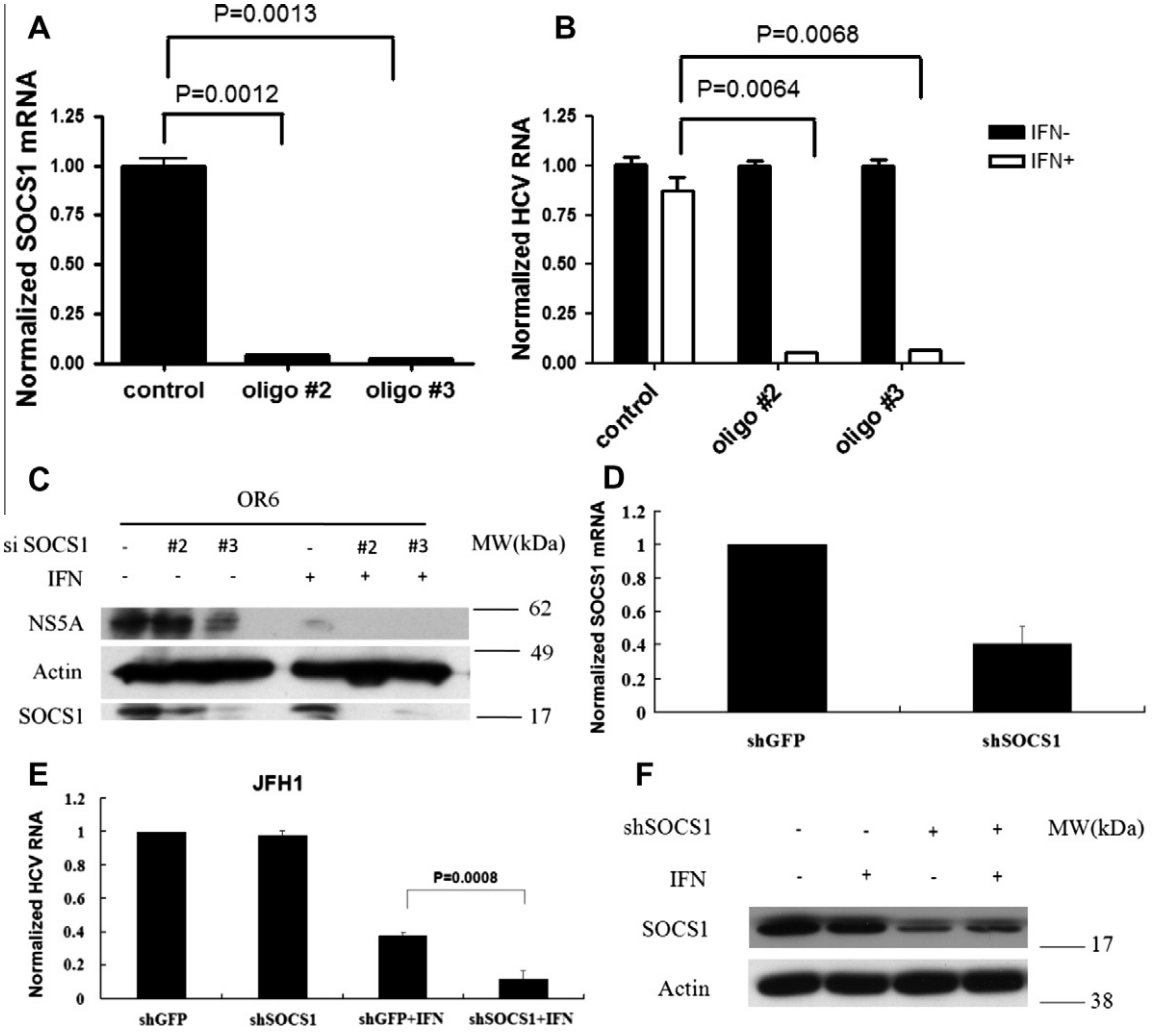

Fig. 1. Knockdown of SOCS1 accentuates IFN's antiviral effects. Huh7.5.1 cells were treated with siRNA against SOCS1 (#2 or #3) or control siRNA for three days and then infected with JFH1 in the presence of 1IU/ml IFN or mock for another 2 days. Total RNA was harvested and reverse transcribed and mRNA expression of SOCS1 (A) and JFH1 (B) was determined by quantitative real time PCR normalized to β-actin. (C) OR6 cells were treated with siRNA against SOCS1 (#2 or #3) or control siRNA for three days and then incubated with 100IU/ml IFN or mock for another 36 h and cell lysates were analyzed by immunoblotting with the indicated antibodies. (D) Two days after JFH1 infection, shGFP and shSOCS1 stable transfected Huh7.5.1 cells were collected and total RNA was harvested and reverse transcribed. mRNA expression of SOCS1 was determined by quantitative real time PCR normalized to β-actin. (E) Two days after JFH1 infection, shGFP and shSOCS1 stable transfected Huh7.5.1 cells were treated with 100 IU/ml IFN for 24 h and total RNA was harvested and reverse transcribed. mRNA expression of SOCS1 and JFH1 was determined by quantitative real time PCR normalized to β-actin. (F) Two days after JFH1 infection, shGFP and shSOCS1 stably transfected Huh7.5.1 cells were treated with IFN for 24 h and cell lysates were analyzed by immunoblotting with the indicated antibodies.

overexpression on the anti-HCV effects of IFN. This was accomplished by comparing HCV replication in the OR6 and JFH1 models in the presence of either a SOCS1 expression vector or empty vector. As shown in Fig. 2A, IFN treatment significantly repressed HCV replication in OR6 cells. However, incubation of IFN caused less repression of HCV replication in cells overexpressing SOCS1 (Fig. 2A). Similarly, HCV inhibition by IFN-α in JFH1 infected Huh7.5.1 cells was also attenuated in SOCS1-transfected cells compared to cells transfected with empty vector (Fig. 2B). Taken together, SOCS1 overexpression reverses the antiviral effects of IFN, indicating that SOCS1 is a functional negative regulator of the IFN-α signaling pathway.

3.3. SOCS1 overexpression blocks IFN-induced ISRE-luciferase activity

The anti-HCV effect of IFN-α is mediated by the induction of ISGs. To monitor the activity of interferon-induced signaling pathway in cells, we applied the interferon stimulated response element (ISRE) reporter system, which encodes the firefly luciferase reporter gene under the control of a minimal CMV promoter and tandem repeats of the ISRE. We assessed whether SOCS1 overexpression could regulate IFN-induced ISRE-luciferase activity. As shown in Fig. 3A and B, IFN-α induced ISRE-luciferase activities in both OR6 and JFH1 infected Huh7.5.1 cells whereas overexpression of SOCS1 blocked ISRE-luciferase activity induced by IFN. Those data imply that SOCS1 promotes HCV replication by blocking the activation of the interferon stimulated response element of ISGs.

3.4. SOCS1 overexpression decreases IFN-induced IRF9, ISG15, PKR and STAT1 mRNA and protein levels

A number of proteins, including IRF9, ISG15, PKR and STAT1, induced by the JAK/STAT pathway play a role in the antiviral responses of IFN-α. We directly tested whether SOCS1 overexpression could inhibit the expression of these ISGs. Initially

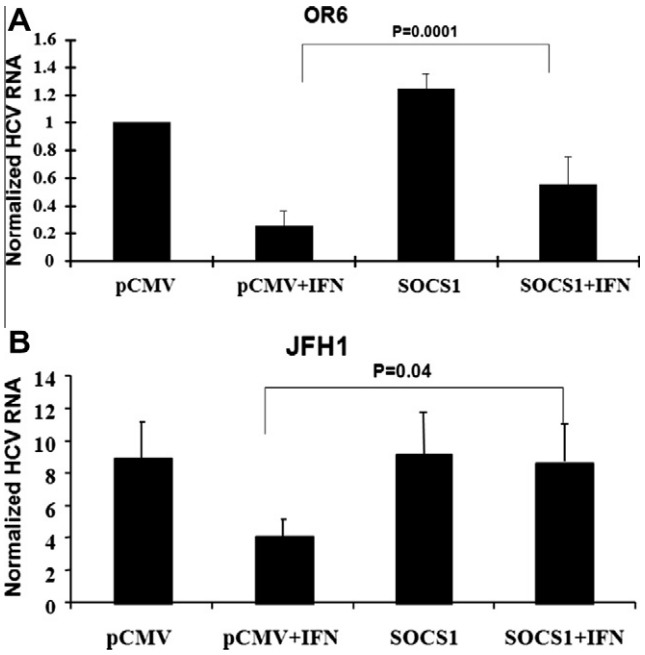

Fig. 2. SOCS1 overexpression reverses IFN's antiviral effects. OR6 cells (A) or JFH1-infected Huh7.5.1 cells (B) were transfected with pCR3.1 or pCR3.1SOCS1 for 24 h and then treated with 100 IU/ml IFN for 24 h. Total RNA was harvested and reverse transcribed. The mRNA expression of JFH1 was determined by quantitative real time PCR normalized to β-actin.

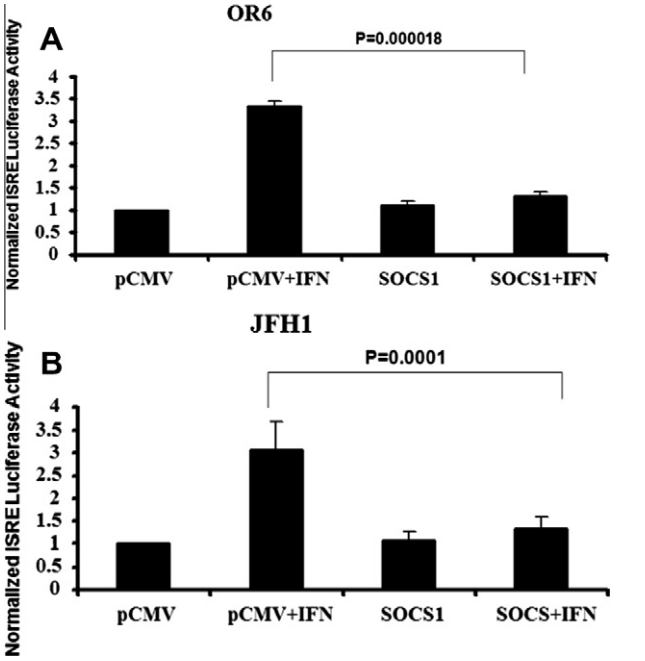

Fig. 3. SOCS1 overexpression blocks IFN-induced ISRE-luciferase activity. OR6 cells (A) or JFH1-infected Huh7.5.1 cells (B) were transfected with pCR3.1 or pCR3.1SOCS1 for 24 h and then transfected with pISRE-luc encoding firefly luciferase under the control of the ISRE and pRL-TK expressing *Renilla* luciferase for 24 h and 100 IU/ml IFN was added to the cells for 6 h. The firefly and *Renilla* luciferase activity was measured.

OR6 cells were transfected with SOCS1 construct or empty vector and then treated with IFN-α before analyzing IRF9, ISG15, PKR and STAT1 mRNA expression by RT-qPCR. SOCS1 overexpression reduced IFN-induced mRNA levels of IRF9 (Fig. 4A), ISG15 (Fig. 4C), PKR (Fig. 4B), and STAT1 (Fig. 4D) from 15.2 to 7.7-fold, from 44.7 to 26.7-fold, from 8.3 to 5.8-fold, from 4.9-fold to 3.9-fold, respectively.

To further analyze the induction of those ISGs at the protein level, OR6 cells were transfected with SOCS1 construct or empty vector and then treated with IFN-α before lysates were harvested and analyzed by Western blotting. As predicted, DDK-tagged- SOCS1 (SOCS1-DDK) overexpression reduced protein levels of those ISGs (Fig. 4E).

Since we found that overexpression of SOCS1 decreased the induction of IRF9, ISG15, PKR and STAT1 in OR6 cells, we wondered whether similar effects would be observed in JFH1 infected Huh7.5.1 cells. Therefore, we transfected the Huh7.5.1 cells with SOCS1 construct or empty vector and infected those cells with JFH1 and then treated with IFN-α. The mRNA levels of IRF9, ISG15, PKR and STAT1 were analyzed by RT-qPCR and protein levels were analyzed by Western blotting. Overexpression of SOCS1 in JFH1 infected Huh7.5.1 cells decreased the IFN-α-induced of IRF9 (Fig. 5A), ISG15 (Fig. 5B), PKR (Fig. 5C) and STAT1 (Fig. 5D) by from 9.1 to 6.2-fold, from 43.6 to 31.4-fold, from 9.9 to 5.6-fold, from 8.2-fold to 5.1-fold, respectively. As shown in Fig. 5E, SOCS1-DDK overexpression reduced protein levels of those ISGs.

Taken together, these findings indicate that SOCS1 overexpression represses the expression of several important anti-HCV factors including IRF9 and STAT1 which correlates with increased HCV replication.

4. Discussion

SOCS1 was originally identified as a negative regulator of STAT3 and JAK2 (Fujimoto and Naka, 2010; Hong et al., 2002). Later, it was

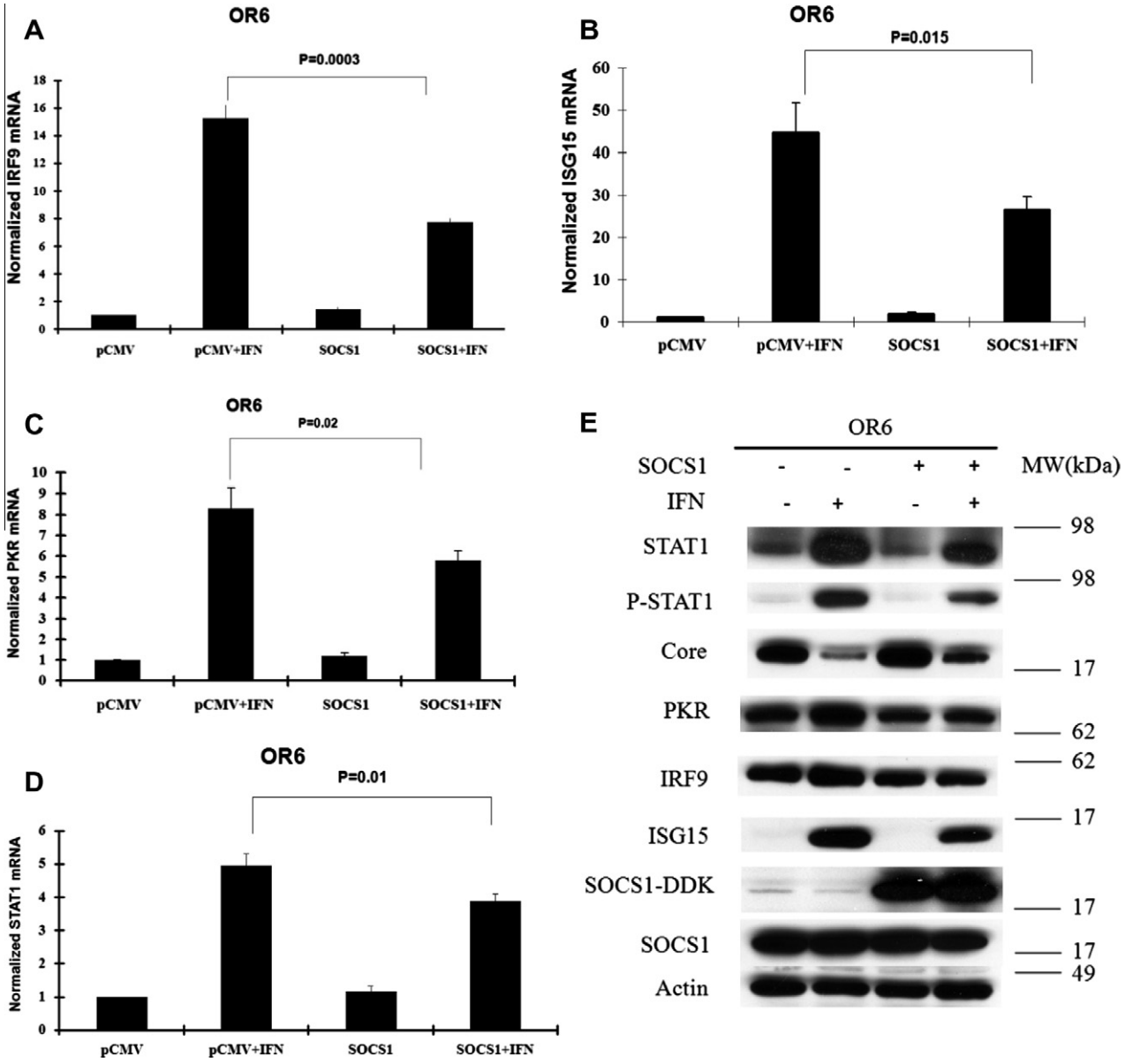

Fig. 4. SOCS1 overexpression decreases IFN-induced IRF9, ISG15, PKR and STAT1 mRNA and protein levels in OR6 cells. OR6 cells were transfected with pCR3.1 or pCR3.1SOCS1 for 24 h and then treated with 100 IU/ml IFN for 24 h and the cells were collected. Total RNA was harvested and reverse transcribed. mRNA expression of IRF9 (A), ISG15 (B), PKR (C) or STAT1 (D) were determined by quantitative real time PCR normalized to β-actin. (E) Cell lysates were analyzed by immunoblotting with the indicated antibodies. SOCS1-DDK indicates the DDK tagged form of SOCS1.

found to be an inhibitor of additional signaling pathways (Fujimoto and Naka, 2010), such as IL6 (Croker et al., 2003), IL2 (Sporri et al., 2001), IFN-α (Vlotides et al., 2004) and IFN-γ (Alexander et al., 1999). The engagement of SOCS1 with its binding partners has been linked to a wide variety of physiological and pathologic functions. Much effort has been devoted to understanding its biochemical interactions with the JAK-STAT pathway (Piganis et al., 2011). However, the role of SOCS1 in IFN mediated suppression of HCV has remained largely unknown. In the present study, we used two distinct HCV infection models and examined the role of SOCS1 during HCV replication.

The genes encoding SOCS act in a negative-feedback loop to inhibit IFN signal transduction. Eight SOCS proteins including SOCS1–7 and CIS have been identified (Akhtar and Benveniste, 2011). Previously, we showed that SOCS3 repressed HCV replication in an mTOR-dependent manner (Shao et al., 2010); however in this study, we found that SOCS1 itself could not inhibit HCV replication. Our results are consistent with previous studies from other groups. It has been found that transfection of HCV core protein in mouse liver silenced SOCS1 transcription leading to permanent activation of the JAK-STAT signaling pathway. In our study, we found that silencing SOCS1 impaired ISRE luciferase activity induced by IFN. In HepG2 cells, SOCS1 has been shown to inhibit type I and III IFN-induced activation of the JAK–STAT pathway and expression of antiviral proteins 2′,5′-OAS and MxA (Vlotides et al., 2004). Here, we examined several additional ISGs including IRF9, ISG15, PKR and STAT1 and found that these IFN-induced ISGs were also inhibited by SOCS1 overexpression in both Huh7.5.1 and OR6 cells. Since type-III-IFN uses similar JAK-STAT pathway as type-I IFN (Zhang et al., 2011), the inhibitory role of SOCS1 on IFN-α-based anti-HCV effect may also apply to IFN-λs.

The precise mechanism by which IFN exerts its anti-HCV effect has not yet been fully elucidated. Two distinct strategies have been applied to identify those important host proteins. The first has

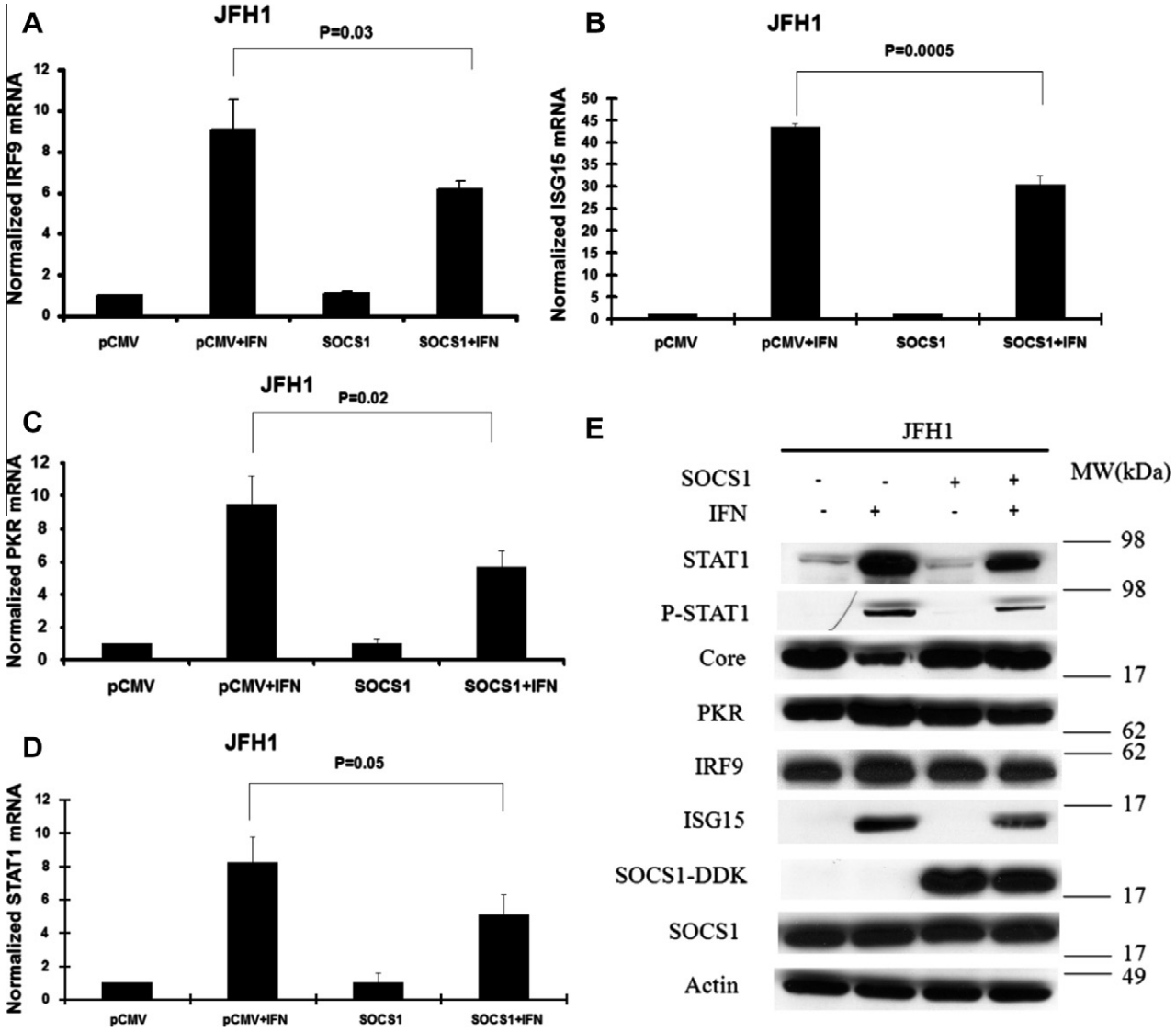

Fig. 5. SOCS1 overexpression decreases IFN-induced IRF9, ISG15, PKR and STAT1 mRNA and protein levels in JFH1 infected Huh7.5.1 cells. JFH1-infected Huh7.5.1 cells were transfected with pCR3.1 or pCR3.1SOCS1 for 24 h and then treated with 100 IU/ml IFN for 24 h and the cells were collected. Total RNA was harvested and reverse transcribed. mRNA expression of IRF9 (A), ISG15 (B), PKR (C) or STAT1 (D) were determined by quantitative real time PCR normalized to β-actin. (E) Cell lysates were analyzed by immunoblotting with the indicated antibodies. SOCS1-DDK indicates the DDK tagged form of SOCS1.

been to conduct an unbiased whole-genome siRNA library screen. Recently our group identified an unexpected role of mRNA processing machinery in promoting the antiviral state of IFN. Nine genes, including SART1, which is a component of the U4/U6.U5 tri-snRNP, were identified (Zhao et al., 2012). In the same study, depletion of ISG15 and PKR with siRNA did not alter IFN's anti-HCV effect. In a separate study from our lab (Zhang et al., 2011), we found that depletion of IRF9 or STAT1 with siRNA attenuated IFN's anti-HCV effect. The second strategy was an overexpression screen approach. Schoggins et al. (Schoggins et al., 2011) tested more than 380 human ISGs and found a diverse range of ISGs to be effectors of the type I interferon antiviral response.

Our current finding implies that SOCS1 is a double-edged sword in the potential contribution of IFN-based anti-HCV therapy. On one hand, SOCS1 may relieve the toxicity of IFN-based therapy. Although stimulating transcription of antiviral ISGs provides a beneficial effect, controlling the magnitude and duration of IFN signaling is crucial to minimize side effects of IFN. SOCS1 negatively regulates the IFN signaling pathway, which might modulate the systemic toxicity of IFN. Recent study found that SOCS1 transcription quantified in peripheral blood mononuclear cells (PBMC) increased significantly in treated HCV-infected patients, in contrast to untreated patients (Sedeno-Monge et al., 2010).

On the other hand, SOCS1 expression during HCV therapy may reduce treatment response. Despite great advances in treatment strategies for HCV, a significant proportion of patients fail to achieve viral clearance following treatment with pegylated interferon (IFN) plus ribavirin. While new directly acting antiviral therapies are being introduced (Fusco and Chung, 2012; Schaefer and Chung, 2012), IFN will likely remain a part of standard therapy for most patients worldwide. Here we have examined the interplay between SOCS1 and IFN antiviral effects in the context of HCV infection, and find evidence that could contribute to our understanding of the basis for low SVR rates in some patients. Further studies of hepatic SOCS1 expression levels and activity in responders and non-responders may be warranted.

In summary, we evaluated the impact of SOCS1 on the antiviral effect of IFN in the genotype 1 HCV replicon OR6 and the fully infectious genotype 2 HCV JFH1. We demonstrated that silencing of SOCS1 by shRNA resulted in an increased antiviral effect of

IFN. Furthermore, overexpression of SOCS1 prior to IFN treatment inhibited JAK-STAT signaling, including ISRE reporter activity and induction of several classical ISGs. Our data therefore suggest that SOCS1 negatively regulates IFN signaling during HCV treatment and thus suppression of SOCS1 may help overcome IFN resistance in HCV-infected patients.

Acknowledgements

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