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SOCS1 controls liver regeneration by regulating HGF signaling in hepatocytes

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Background & Aims: Frequent repression of the *Socs1* (suppressor of cytokine signaling 1) gene in hepatocellular carcinoma (HCC) and increased susceptibility of SOCS1-deficient mice to hepatocarcinogens suggest a tumor suppressor role for SOCS1 in the liver, but the underlying mechanisms remain unclear. Here we investigated the role of SOCS1 in regulating hepatocyte proliferation following partial hepatectomy and HGF stimulation.
Methods: Because *Socs1*$^{-/-}$ mice die prematurely due to deregulated IFNγ signaling, we used *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice to study the role of SOCS1 in liver regeneration following partial hepatectomy. We examined the activation of signaling molecules downstream of IL-6 and hepatocyte growth factor (HGF) receptors in the regenerating liver, primary hepatocytes, and in human hepatoma cells. We examined the interaction between SOCS1 and the HGF receptor c-Met by reciprocal immunoprecipitation.
Results: *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice displayed accelerated liver regeneration with increased DNA synthesis compared to *Ifng*$^{-/-}$ and wild type mice. The regenerating liver of *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice did not show increased IL-6 signaling, but displayed earlier phosphorylation of Gab1, a signaling adaptor downstream of c-Met. Following HGF stimulation, hepatocytes from *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice displayed increased phosphorylation of c-Met and Gab1, cell migration and proliferation. Accordingly, SOCS1 overexpression attenuated HGF-induced phosphorylation of c-Met, Gab1, and ERK1/2 in hepatoma cells, and decreased their proliferation and migration. SOCS1 interacted with the Tpr-Met, an oncogenic form of the Met receptor.
Conclusions: SOCS1 attenuates c-Met signaling and thus negative regulation of HGF signaling could be an important mechanism underlying the anti-tumor role of SOCS1 in the liver.

Keywords: Hepatocellular carcinoma; SOCS1; Tumor suppressor; Liver regeneration; HGF, c-Met.
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Abbreviations: SOCS1, suppressor of cytokine signaling 1; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; BrdU, bromodeoxyuridine; PH, partial hepatectomy.

Introduction

Hepatocellular carcinoma (HCC) is an often lethal tumor with limited therapeutic options. Understanding the molecular mechanisms of hepatocarcinogenesis could lead to development of strategies to arrest, retard or even reverse the disease process in HCC patients [1]. Development of HCC follows a series of events involving inflammation, chronic liver injury, and hepatocyte proliferation [2]. The mutagenic environment created by inflammation is believed to facilitate activation of proto-oncogenes and/or inactivation of tumor suppressors, leading to deregulated hepatocyte proliferation and progression toward HCC [1,2]. The *Socs1* gene, encoding suppressor of cytokine signaling 1 (SOCS1), is frequently repressed in human HCC [3], and is suppressed by the core protein of hepatitis C virus, an etiologic agent of HCC [4]. Furthermore, *Socs1*$^{+/-}$ mice display increased HCC formation in response to the hepatocarcinogen diethylnitrosamine [5]. Despite such compelling evidence for a tumor suppressor function of SOCS1 in the liver, the underlying molecular mechanisms remain unknown.

Liver regeneration (LR) following partial hepatectomy (PH) is widely used to study regulatory mechanisms of hepatocyte proliferation that are also relevant for neoplastic growth [6,7]. Liver regeneration involves coordinated action of distinct cytokines and growth factors, which regulate three temporal stages of hepatocyte proliferation, namely, priming, DNA synthesis, and cell division, followed by growth termination. TNFα and IL-6 are critical priming factors, which facilitate G_0 to G_1 transition of hepatocytes, rendering them competent to respond to growth factors. Mice lacking TNF receptor 1 show delayed liver regeneration, which could be reversed by administration of IL-6, whereas IL-6 deficiency induces severe apoptosis because IL-6-induced STAT3 activation is essential for liver regeneration [8–10]. Following priming, growth factors provide mitogenic signals that facilitate

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competent hepatocytes to progress through the cell cycle. Among these mitogenic factors, hepatocyte growth factor (HGF) plays an important role in hepatocyte proliferation and in the pathogenesis of HCC [1,7]. Conditional ablation of HGF or its receptor c-Met in the adult liver impairs liver regeneration [11,12].

In this study, we investigated whether SOCS1 controls hepatocyte proliferation by regulating cytokines and growth factors involved in hepatocyte priming and/or proliferation. To this end, we first evaluated liver regeneration in SOCS1-deficient mice. In parallel, we stimulated SOCS1-deficient and control primary hepatocytes with IL-6 or HGF and compared downstream signaling events. We also examined IL-6 and HGF signaling in murine and human hepatoma cells overexpressing SOCS1. Our findings suggest that the anti-tumor function of SOCS1 in the liver could result, at least partly, from the regulation of c-Met receptor signaling.

Materials and methods

Mice and cell lines

Socs1$^{+/-}$*Ifng*$^{+/-}$ mice, kindly provided by Dr. J. Ihle [13], have been backcrossed with *Ifng*$^{-/-}$ mice in C57BL/6 background (The Jackson Laboratory). All experiments were approved by the institutional Ethics Committee. Murine Hepa1-6 and human Hep3B cells were purchased from ATCC.

Reagents and antibodies

Cytokines and growth factors were from R&D Systems. LPS, bromodeoxyuridine (BrdU), collagen, and Hoechst nuclear stain were from Sigma–Aldrich. Collagenase (Blendzyme), and anti-HA, and anti-BrdU antibodies were from Roche Diagnostics. Phospho-specific antibodies were from Cell Signaling Technology and antibodies against total proteins were from Santa Cruz Biotechnology. Secondary antibodies and enhanced chemiluminescence reagents were from GE Healthcare Life Sciences.

Partial hepatectomy (PH) and hepatocyte DNA synthesis in vivo

Partial hepatectomy was carried out in 8–10 week-old mice following published methods [14]. SOCS1-deficient and control mice were always operated in groups. To evaluate DNA replication in the regenerating liver, hepatectomized mice were injected with BrdU (40 mg/g body weight, i.p.) 4 h prior to euthanasia. Three non-serial sections of paraffin-embedded liver per animal were stained with anti-BrdU-FITC and counterstained with Hoescht nuclear stain. FITC + cells and Hoescht + cells were counted in ten random fields per slide to calculate the proportion of cells that have incorporated BrdU into replicating DNA.

Isolation of primary hepatocytes

Primary hepatocytes were isolated following published methods [11]. Hepatocyte preparations that showed >85% cell viability by trypan blue exclusion, were plated on collagen (Sigma–Aldrich)-coated culture plates in Ham's F-12/DMEM with 10% FCS.

Elisa

The amount of IL-6 in serum collected at the indicated time points after partial hepatectomy was determined by sandwich ELISA using antibody pairs purchased from BD Pharmingen Biosciences.

Expression of exogenous SOCS1 in Hepatoma cells

Hep3B cells do not express SOCS1 due to promoter methylation of both alleles of the *Socs1* gene [3]. Mouse *Socs1* gene, subcloned into pcDNA3.0 with an N-terminal myc-tag, was transiently transfected into Hep3B cells using Qiagen Polyfect® reagent.

Cell proliferation

Primary hepatocytes and Hep3B cells were plated in 96-well culture plates (5×10^3 cells/well) and stimulated with HGF. One μCi of methyl-[^{3}H]-thymidine (New England Nuclear) was added per well during last 8 h of culture. Cells were trypsinized, harvested onto glass fiber filter mats and the incorporated radioactivity was measured using Top Count® microplate reader (Perkin–Elmer).

Wound healing assay

The bottom plates of Petri dishes were marked with parallel lines and 1x10^5 Hep3B cells transfected with SOCS1/pCDNA3.0 or empty vector were plated and cultured in DMEM-10% FCS. For primary hepatocytes, Petri dishes were pre-coated with collagen. When the cells were nearly confluent (>90%), the medium was changed to serum-poor medium (DMEM-0.25% FCS). After 4 h, a scratch wound was made with a sterile pipette tip and serum-poor medium containing HGF was added. In some experiments, hydoxyurea (Calbiochem) was added to induce cell cycle arrest. Wound closure was photographed and the rate of wound healing was quantified by measuring the distance between the edges of the scratch wound at several points.

Cell stimulation, Western blot and co-immunoprecipitation

Primary hepatocytes and Hep3B cells, deprived of serum-derived growth factors by overnight culture in DMEM-0.25% FCS, were stimulated with HGF and lysed in SDS–PAGE sample buffer. Liver tissues were lysed in hypotonic lysis buffer (Tris 10 mM pH 7.6, NaCl 50 mM, sodium diphosphate 30 mM, EDTA 5 mM) containing 1% Triton-X100 and protease and phosphatase inhibitors. Following Western blot, images captured using the VersaDOC® system (Bio-Rad) were densitometrically quantified using NIH ImageJ 1.62 software. To analyze the interaction between SOCS proteins and Met, COS-7 cells were co-transfected with cDNA constructs of SOCS proteins and Tpr-Met, an oncogenic form of the c-Met receptor [15] (provided by Dr. M. Park, McGill University). Protein interaction was analyzed by reciprocal immunoprecipitation.

Real-time RT-PCR

RNA was extracted using Trizol (Invitrogen) from snap-frozen liver samples and cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen). The SOCS1 cDNA, amplified using primers TGGTTGTAGCAGCTTGTGTCTGG (sense) and CCTGGTTTGTGCAAAGATACTGGG (anti-sense), was quantified using SYBR Green Supermix and MyiQ™ real-time PCR detection system (Bio-Rad). Samples from each time point were analyzed in triplicates and normalized for the expression of TATA-box Binding Protein amplified using primers GTTTCTGCG GTCGCGTCATTTT (sense) and TCTGGGTTATCTTCACACACCATGA (anti-sense).

Statistical analysis

Mean + standard deviation values are given. Student's *t*-test was used to determine the *p* values.

Results

SOCS1 deficiency accelerates liver regeneration

To investigate the role of endogenous SOCS1 in regulating hepatocyte proliferation, we resorted to using *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice because *Socs1*$^{-/-}$ mice die within 3 weeks of birth due to deregulated IFNγ signaling [13]. IFNγ-deficient mice were previously shown to display increased rate of liver regeneration [16]. Because IFNγ is a strong inducer of *Socs1* gene expression in hepatocytes (Supplementary Fig. 1), it is possible that the IFNγ-mediated control of liver regeneration might be dependent on SOCS1. Alternatively, SOCS1 may control hepatocyte proliferation in a manner distinct from IFNγ-mediated regulation. To distinguish these possibilities, we used both *Ifng*$^{-/-}$ and C57Bl/6

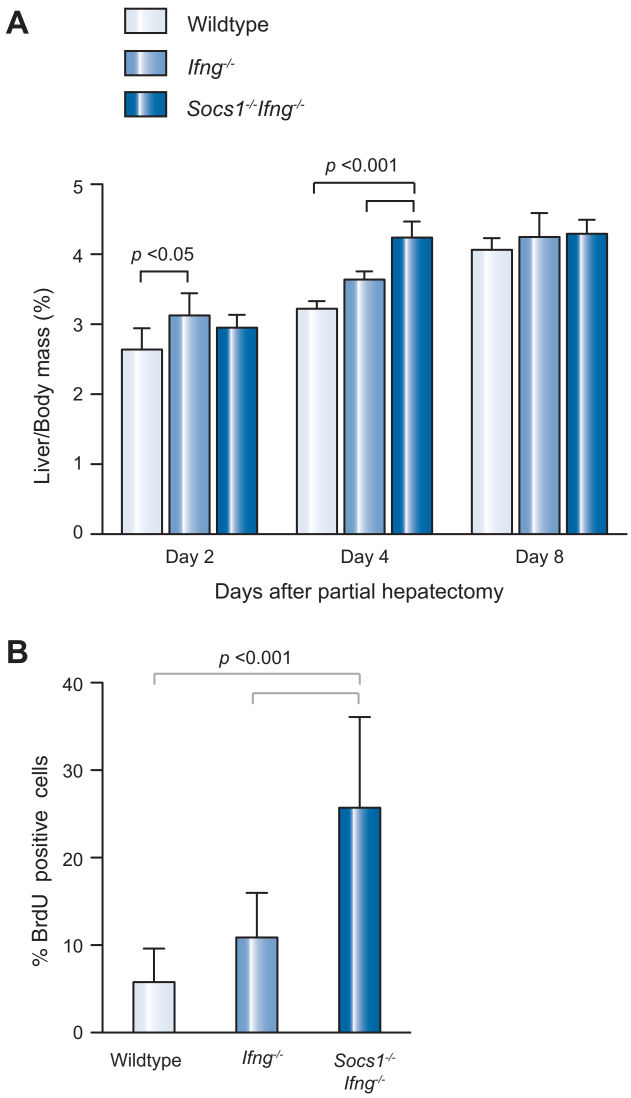

Fig. 1. SOCS1 deficiency increases the rate of liver regeneration and hepatocyte DNA synthesis following partial hepatectomy. (A) The liver/body mass ratio was calculated from 4–8 mice for each of the indicated time points. (B) The BrdU positive cells undergoing DNA synthesis were enumerated at 48 h post-PH from at least three mice per group.

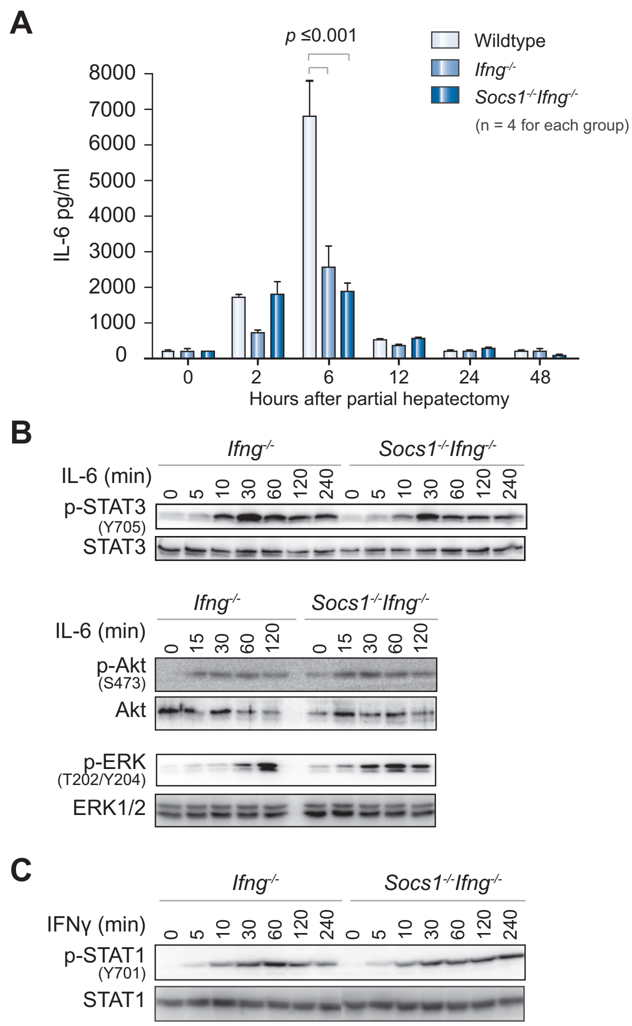

Fig. 2. IL-6 signaling is not enhanced in SOCS1-null primary hepatocytes. (A) Production of IL-6 after PH is decreased in *Socs1*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ mice. (B and C) Primary hepatocytes cultured from SOCS1-deficient and control mice were stimulated with IL-6 (B) or IFNγ (C), and phosphorylation and the total amount of the indicated proteins were evaluated by Western blot. Representative data from three similar experiments are shown.

mice as controls. First, we evaluated the rate of liver regeneration in SOCS1-deficient (*Socs1*$^{-/-}$*Ifng*$^{-/-}$) and control (*Ifng*$^{-/-}$ and C57Bl/6) mice following 65–70% partial hepatectomy. Consistent with the earlier report [16], *Ifng*$^{-/-}$ mice showed significantly increased liver regeneration and DNA synthesis compared to C57Bl/6 mice on day 2 post-PH (Fig. 1A and B). In contrast, *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice displayed significantly faster gain in liver mass compared to both *Ifng*$^{-/-}$ and C57Bl/6 mice on day 4 post-PH (Fig. 1A), suggesting that SOCS1 and IFNγ control liver regeneration via distinct mechanisms. Despite showing an accelerated rate of liver regeneration, the final mass of the regenerated liver in SOCS1-deficient mice was not increased. BrdU incorporation assay revealed significantly elevated proportion of hepatocytes undergoing DNA replication in *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice compared to *Ifng*$^{-/-}$ and C57Bl/6 mice (Fig. 1B). These results suggested that SOCS1 regulates liver regeneration at least partly by controlling hepatocyte proliferation.

IL-6 signaling is not enhanced in SOCS1-deficient primary hepatocytes

During liver regeneration, IL-6 plays a pivotal role to maintain the viability of hepatocytes and to enhance their responsiveness to growth factors [7,8]. Because SOCS1 deficiency enhances IL-6 production in macrophages [17], we examined whether SOCS1 deficiency facilitated hepatocyte priming via increased IL-6 production. In wild type mice, serum IL-6 concentration increased within 2 h post-PH and attained peak level by about 6 h (Fig. 2A). Surprisingly, the post-PH serum IL-6 level was markedly reduced in *Socs1*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ mice compared to C57Bl/6 controls. These observations suggested that the accelerated rate of liver regeneration in SOCS1-deficient mice did not arise from enhanced IL-6 production, and that IL-6 production following PH is modulated by IFNγ-dependent mechanisms.

Next, we examined whether SOCS1 deficiency enhanced IL-6 signaling, thereby compensating for decreased IL-6 availability. In Hepa cells, IL-6 induced *Socs1* gene transcription and forced expression of SOCS1 inhibited STAT3 phosphorylation (Supplementary Fig. 1). However, the magnitude and kinetics of IL-6-induced STAT3 phosphorylation were comparable between SOCS1-deficient and control hepatocytes (Fig. 2B), whereas IFNγ-induced STAT1 phosphorylation remained elevated in SOCS1-deficient cells for a prolonged period (Fig. 2C). The kinetics of IL-6-induced phosphorylation of AKT and ERK1/2 were also similar in SOCS1-deficient and control hepatocytes (Fig. 2B, lower panel). Collectively, these results indicated that endogenous SOCS1 is dispensable for the control of IL-6 signaling in hepatocytes.

SOCS1-null liver shows increased phosphorylation of Gab1and ERK1/2 during regeneration

Next, we examined the key phosphorylation events induced by cytokine receptor signaling in the regenerating liver. The magnitude and kinetics of STAT3 phosphorylation was comparable in the regenerating livers of *Socs1*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ mice (Fig. 3A and B). However, phosphorylation of ERK1/2 occurred early and was higher in magnitude in the regenerating livers of *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice compared to *Ifng*$^{-/-}$ controls (Fig. 3B). Phosphorylation of p38 MAPK also showed a similar kinetic difference. Because ERK1/2 is activated not only by IL-6 but also by growth factors in hepatocytes, we examined phosphorylation of Gab1, an adaptor molecule downstream of HGF and EGF receptors [7,18]. Gab1 is phosphorylated on multiple tyrosine residues, which could serve as docking sites for signaling proteins containing an SH2 domain [19]. As shown in Fig. 3B, phosphorylation of Gab1 occurred early in the regenerating liver of SOCS1-deficient mice, however it was dephosphorylated by 48 h in both control and SOCS1-deficient mice. The increased phosphorylation of Gab1 and ERK1/2 corroborated with the kinetics of SOCS1 gene expression, reaching its peak at 6 h post-PH and returning to the base level by 12 h (Fig. 3C), largely in agreement with SOCS1 gene expression in regenerating rat liver [20]. These results suggested that the increased rate of liver regeneration in SOCS1-deficient mice could result from increased growth factor signaling.

Primary hepatocytes lacking SOCS1 show increased HGF-induced Met signaling

Among the growth factors that promote hepatocyte proliferation following PH, HGF plays an important role in liver regeneration [7,11,12]. Evaluation of HGF expression in the regenerating livers did not show appreciable difference between SOCS1-deficient and control mice (data not shown). Because HGF-induced STAT3 activation was inhibited in the liver following *Socs1* gene transfer [21], we investigated whether endogenous SOCS1 regulates HGF signaling in hepatocytes. HGF-induced phosphorylation of c-Met, Gab1 and AKT, but not that of ERK1/2, was significantly increased in SOCS1-deficient hepatocytes compared to control cells (Fig. 4A). Lack of c-Met was shown to impair hepatocyte migration in a wound-healing assay [11]. As shown in Fig. 4B, SOCS1-null cells displayed faster wound healing in the presence of HGF. Furthermore, SOCS1-deficient hepatocytes proliferated

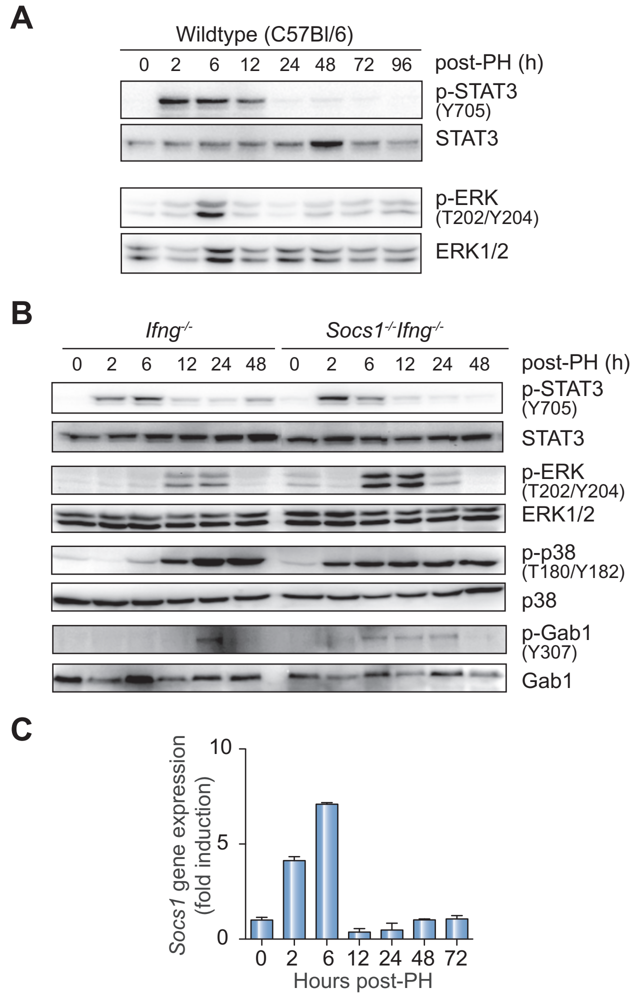

Fig. 3. Regenerating liver of SOCS1-deficient mice show evidence of increased growth factor signaling. Whole tissue lysates of liver samples, snap frozen at different time point after PH of (A) wild type, *Ifng*$^{-/-}$ and (B) *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice, were examined by Western blot using the antibodies. Representative results from three independent experiments for each group of mice are shown. (C) Real-time RT-PCR analysis of *SOCS1* gene expression in the liver following PH. Data from two separate experiments, done in triplicates, are shown.

strongly following HGF stimulation (Fig. 4C). These results indicate that endogenous SOCS1 regulates a subset of HGF-induced signaling events that promote hepatocyte proliferation and migration.

Constitutive expression of SOCS1 attenuates HGF signaling in human hepatoma cells

Cell-permeable constructs of SOCS1 peptide mimic and full-length SOCS1 inhibits cytokine signaling in inflammatory cells and cancer cells [22,23]. To explore the possibility of using such an approach for HCC therapy, we overexpressed SOCS1 in Hep3B cells and evaluated their responses to HGF. In Hep3B cells overexpressing SOCS1, phosphorylation of c-Met, Gab1, and ERK was markedly diminished, although AKT phosphorylation was not affected (Fig. 5A). Hep3B cells showed constitutive phosphorylation of STAT3, which was reduced by SOCS1. Functionally,

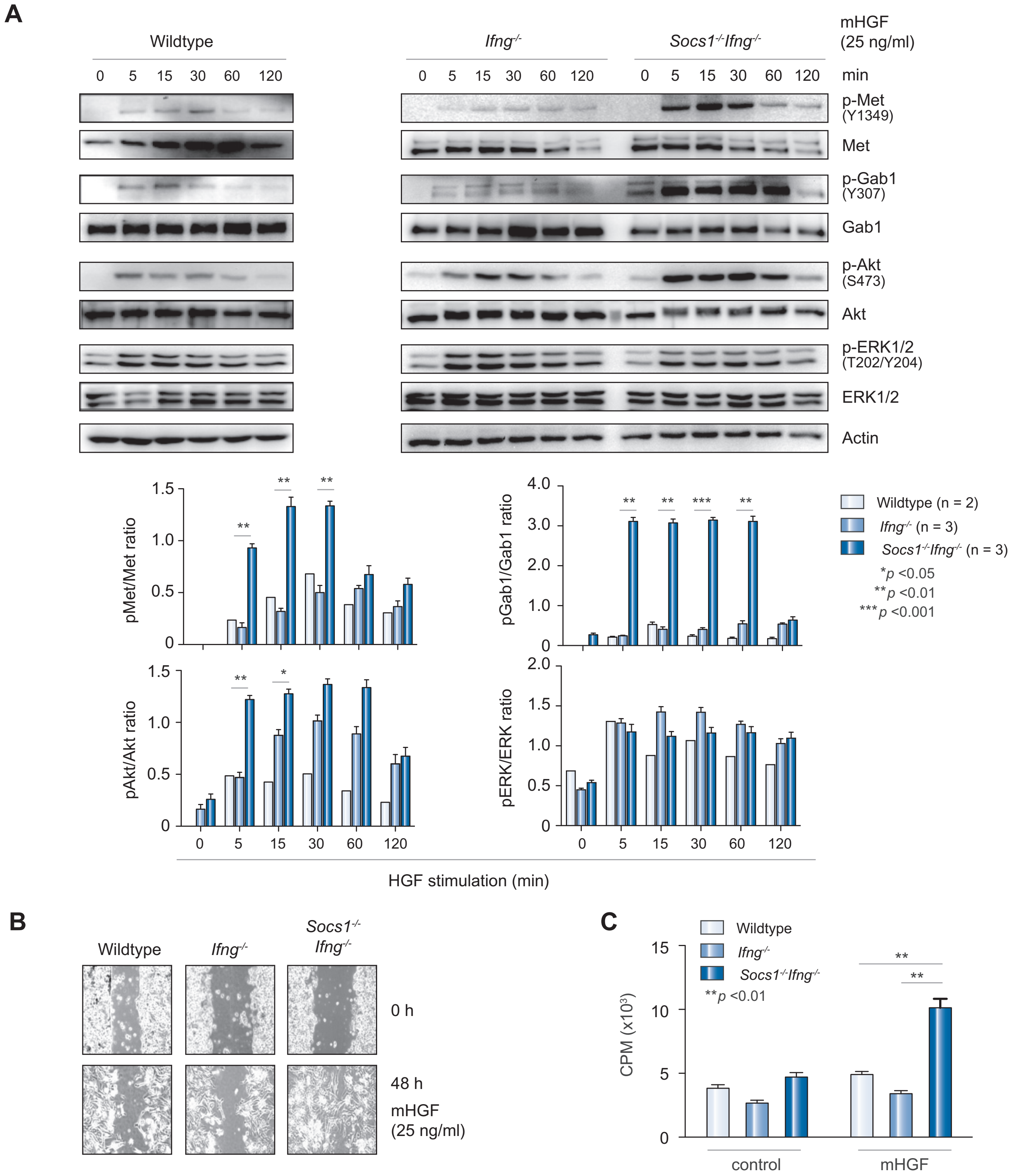

Fig. 4. Primary hepatocytes lacking SOCS1 show increased HGF signaling and HGF-induced cell migration. (A) Primary hepatocytes of the indicated genotypes, stimulated with mHGF for the indicated period, were lysed and analyzed by Western blot. Representative results from three experiments are shown. Lower panels show densitometric data from three experiments. (B) Confluent cultures of primary hepatocytes were tested for wound healing ability in the presence or absence of mHGF. Representative results from two identical experiments are shown. (C) HGF-induced proliferation of primary hepatocytes cultures in triplicates was evaluated by ^{3}H-thymidine incorporation assay. Data from three experiments are shown.

SOCS1 overexpression diminished the proliferation of Hep3B cells cultured in the presence of HGF (Fig. 5B) and impaired the HGF-induced cell migration during wound healing (Fig. 5C). SOCS1-overexpressing cells also showed delayed migration when cell

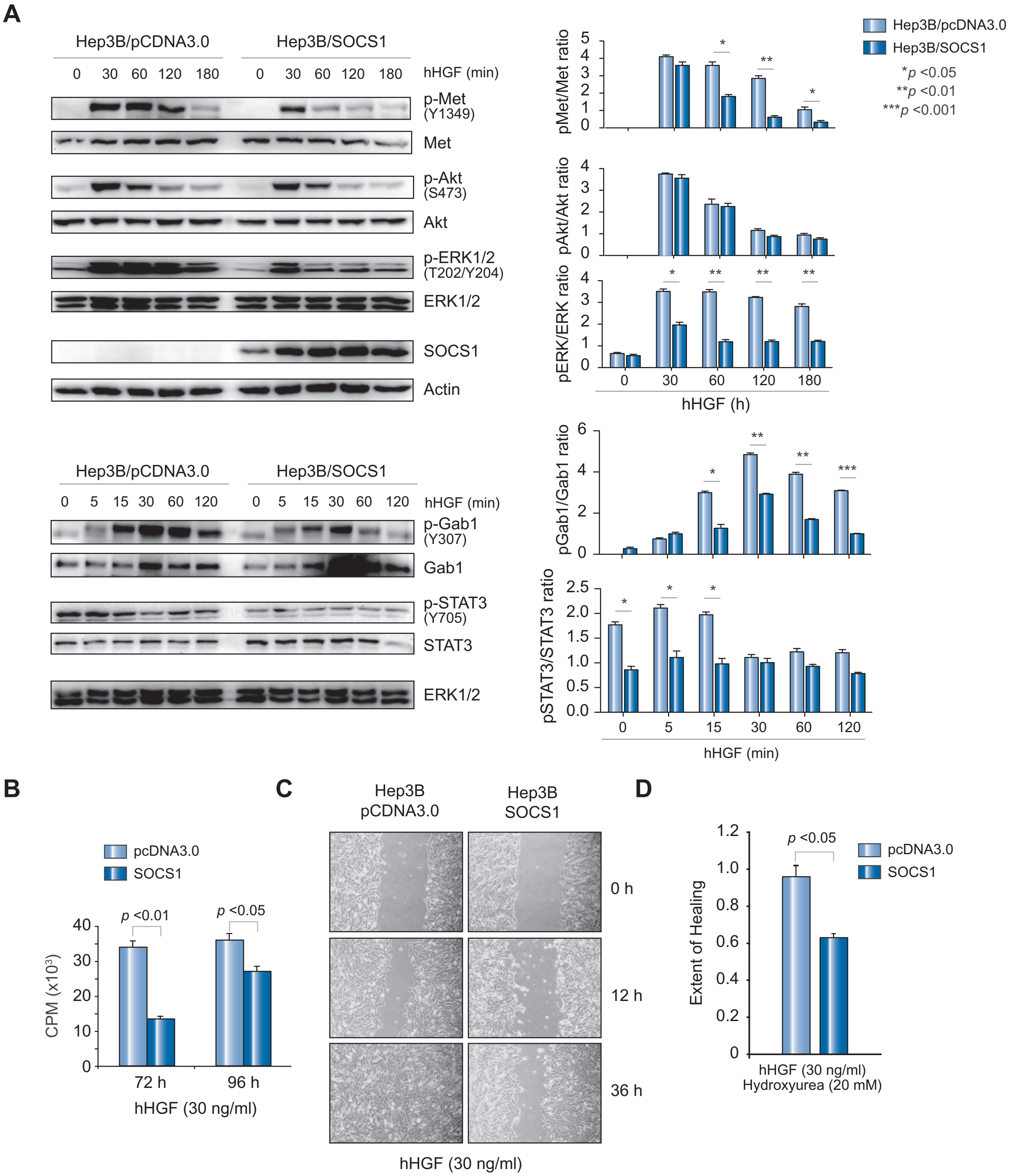

Fig. 5. Forced expression of SOCS1 inhibits HGF signaling in human hepatoma cells and attenuates their proliferation and migration. (A) Hep3B cells transfected with SOCS1 or the control vector were stimulated with hHGF. At the indicated time points, phosphorylation and total amount of the indicated proteins were evaluated. Representative Western blot and normalized protein phosphorylation data from three independent experiments are shown. (B) Cell proliferation following HGF stimulation was evaluated by ^{3}H-thymidine incorporation. Data shown are derived from quadruplicates of two experiments. (C and D) Healing of the scratch wound made on confluent cultures of Hep3B cells expressing SOCS1 and control cells was measured in the presence of HGF, in the absence (C) or presence (D) of hydroxyurea. Representative results from two identical experiments (C) and the extent of wound closure at 36 h (D) are shown.

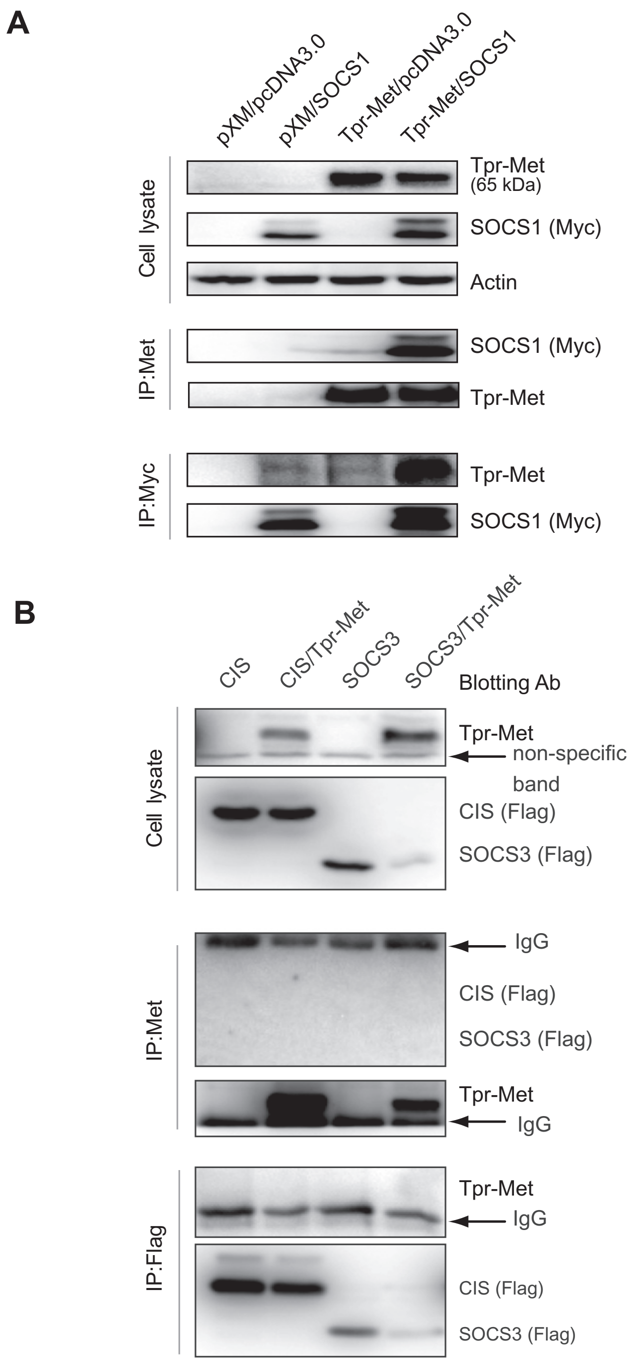

Fig. 6. SOCS1 interacts with the Met receptor. Trp-Met, a constitutively active oncogenic form of c-Met, was co-transfected with (A) N-terminal myc-tagged SOCS1 or (B) Flag-tagged CIS or SOCS3 in COS7 cells. Whole cell lysate and immunoprecipiated Tpr-Met and SOCS family proteins were immunoblotted using anti-Met Ab to detect Tpr-Met or using Ab against myc and Flag epitope tags to detect the SOCS proteins. Representative data from four (for A) and two (for B) independent experiments are shown.

proliferation was inhibited by the cell cycle inhibitor hydoxyurea (Fig. 5D). These results indicate that restoration of SOCS1 expression in hepatoma cells does not completely abrogate c-Met activation, but selectively attenuates a subset of HGF-induced signaling pathways, resulting in decreased proliferation and migration.

SOCS1 interacts with Tpr-Met, an oncogenic form of the Met receptor

The SH2 domain of SOCS1 interacts with several growth factor receptors, including c-Kit and EGFR, and inhibits their signaling capacity [24–27]. To investigate whether SOCS1 similarly interacts with c-Met, we used Tpr-Met, a constitutively active oncogenic form of the Met receptor. Tpr-Met arose from fusion of the intracellular region of c-Met with the leucine zipper domain of a translocated promoter region (Tpr) [15]. In COS-7 cells transfected with SOCS1 and Tpr-Met, immunoprecipitates of Tpr-Met contained SOCS1 and vice versa (Fig. 6). Because SOCS3 is also implicated in the pathogenesis of HCC and in liver regeneration, and SOCS3 was shown to inhibit HGF-induced STAT3 phosphorylation [21,28–30], we examined whether SOCS3 also interacted with Tpr-Met. Neither SOCS3 nor another member of the SOCS family proteins, CIS, interacted with Tpr-Met (Fig. 6B). These results suggest that attenuation of HGF signaling in hepatocytes by SOCS1 might rely on its ability to interact with the Met receptor.

Discussion

Among the SOCS family proteins, SOCS1 and SOCS3 are implicated in hepatocellular carcinoma [3,5,30]. Several lines of evidence suggest that SOCS1 and SOCS3 proteins may regulate proliferation of hepatocytes. Transcription of *Socs1* and *Socs3* genes in the liver is induced by PH and following systemic administration of IL-6 [20,31,32]. IL-6-induced SOCS3 expression occurs early and lasts longer, whereas SOCS1 expression begins later and occurs transiently, suggesting very tight regulation of SOCS1 expression. Nevertheless, *Socs1* and *Socs3* genes appear to be induced during hepatocyte priming and be down-modulated before DNA replication. SOCS3 deficiency increases liver regeneration through increased activation of STAT3 and ERK signaling induced by IL-6 and EGF [28,29,33]. Consistent with the important role for SOCS3 in regulating hepatocyte proliferation, repression of the *Socs3* gene by CpG methylation was found in 30–50% of HCC cell lines and human HCC biopsies [30]. In comparison, repression of the *Socs1* gene occurs at a much higher frequency (~65%) in primary HCC tissues [3,30], yet mechanisms underlying the putative anti-tumor role of SOCS1 remain unknown.

In this report, we show an increased rate of liver regeneration in SOCS1-deficient mice during the early phase that encompasses DNA synthesis and cell division. However, the mass of the completely regenerated liver was comparable between SOCS1-deficient and control groups of mice, suggesting SOCS1-independent regulatory mechanisms operating at later stages of liver growth. Cessation of hepatocyte proliferation following restoration of the functional liver mass is mediated, at least partly, by TGFβ [7,34]. This inhibitory effect of TGFβ could be relieved by IFNγ [35]. Because SOCS1-deficient mice used in our study also lacked IFNγ, the TGFβ-mediated growth control could have occurred more efficiently. Nevertheless, the accelerated rate of liver regeneration in SOCS1-deficient mice, within a narrow time window during the rapid growth phase, suggests that SOCS1 primarily controls cytokines and growth factors that promote hepatocyte proliferation.

IL-6-induced STAT3 activation is not only essential for liver regeneration but is also implicated in hepatocarcinogenesis [8,36]. Because SOCS1 is a negative regulator of LPS-induced IL-6 production in macrophages [17], we expected an increase in serum IL-6 level following PH in SOCS1-deficient mice. Contrarily, both *Socs1*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ mice produced significantly less IL-6 than wild type controls. The IFNγ-deficient mice also showed increased rate of liver regeneration, in agreement with an earlier report [16]. The reason for decreased IL-6 production in the absence of IFNγ is currently unclear. One possible explanation could be that IL-6 production following partial hepatectomy might rely on IFNγ derived from NK cells, which are implicated in liver regeneration [16]. Despite the lower level of IL-6, STAT3 activation occurs efficiently in the regenerating liver of both *Socs1*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ mice (Fig. 3A and B), suggesting that the IL-6-dependent hepatocyte priming is not compromised in these mice. In this context, it is noteworthy that *MyD88* knockout mice show severely impaired IL-6 production, STAT3 activation and decreased liver regeneration at 2–3 days after PH, yet display a normal recovery phase by day 4 [37], indicating that minimal IL-6 is sufficient to achieve complete liver regeneration.

A recent study showed that adenoviral vector-mediated delivery of SOCS1 to hepatocytes *in vivo* inhibited STAT3 phosphorylation induced by HGF [21]. However, this study did not address whether endogenous SOCS1 was necessary to attenuate HGF signaling in hepatocytes. This possibility is supported by our findings showing increased phosphorylation of Gab1 and ERK1/2 in the regenerating liver of SOCS1-deficient mice (Fig. 3B). Furthermore, we observed strong phosphorylation of c-Met, Gab1 and AKT in SOCS1-deficient primary hepatocytes stimulated with HGF (Fig. 4). Thus, our findings demonstrate that endogenous SOCS1 is a critical regulator of at least a subset of HGF-induced signaling pathways.

SOCS1 is implicated in the regulation of several growth factor receptors in different cell types. Overexpressed SOCS1 inhibited stem cell factor (SCF)-induced proliferation of hematopoietic cells via binding to c-Kit as well as its downstream signaling molecules Grb2 and Vav [24]. Similarly, SOCS1 was shown to interact with insulin receptor and attenuate phosphorylation of ERK and AKT [25]. SOCS1 also attenuates proliferation of hematopoietic cells induced by limiting concentrations of M-CSF by interacting with its receptor [26]. In chondrocytes, SOCS1 interacts with the FGF receptor FGFR3 and inhibits STAT1 activation [27]. In the light of these reports, the interaction of SOCS1 with Trp-Met suggests that SOCS1 could diminish the kinase activity of c-Met and/or interfere with the recruitment of downstream signaling molecules. Clearly, further studies are needed to elucidate the molecular determinants of the interaction between SOCS1 and c-Met.

In this study, we have shown that HGF stimulation induces strong proliferation and increased cell migration in SOCS1-deficient hepatocytes. Accordingly, overexpression of SOCS1 attenuates Met signaling and significantly diminishes HGF-induced proliferation and migration of human hepatoma cells. It has been reported that exogenous addition of cell-permeable SOCS1 mimetic peptide or full-length SOCS1 molecule blocked IFNγ signaling in macrophages and inhibited proliferation of prostate cancer cells [22,23]. We envisage that cell-permeable analogs of SOCS1 or its peptide derivatives that attenuate HGF signaling in hepatoma cells could be tested for their ability to hinder the growth of experimental HCC and eventually be evaluated for treatment in human patients.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.03.027.

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