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Hypoxia-Inducible Factor 1α Is Up-Regulated by Oncostatin M and Participates in Oncostatin M Signaling

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The interleukin-6–type cytokine oncostatin M (OSM) acts via the Janus kinase/signal transducer and activator of transcription pathway as well as via activation of mitogen-activated protein kinases and is known to critically regulate processes such as liver development and regeneration, hematopoiesis, and angiogenesis, which are also determined by hypoxia with the hypoxia-inducible factor 1α (HIF1α) as a key component. Here we show that treatment of hepatocytes and hepatoma cells with OSM leads to an increased protein level of HIF1α under normoxic and hypoxic conditions. Furthermore, the OSM-dependent HIF1α increase is mediated via Janus kinase/signal transducer and activator of transcription 3 and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase 1/2 pathways. OSM-mediated HIF1α up-regulation did not result from an increase in HIF1α protein stability but from increased transcription from the *HIF1α* gene. In addition, we show that the OSM-induced *HIF1α* gene transcription and the resulting enhanced HIF1α protein levels are important for the OSM-dependent vascular endothelial growth factor and plasminogen activator inhibitor 1 gene induction associated with several diseases. *Conclusion:* HIF1α levels increase significantly after treatment of hepatocytes and hepatoma cells with OSM, and HIF1α contributes to OSM downstream signaling events, pointing to a cross-talk between cytokine and hypoxia signaling in processes such as liver development and regeneration. (HEPATOLOGY 2009;50:253-260.)

Oncostatin M (OSM) is an interleukin (IL)-6–type cytokine produced by monocytes and macrophages, T cells, and several other cell types. OSM receptors are widely expressed and are composed of the common signal transducer gp130 in complex with the LIFR or the OSMR. OSM has pleiotropic effects that in part overlap with those of other IL-6–type cytokines; examples include inflammation, neurogenesis, regulation of cell proliferation, and fibrosis.[1-3] In addition, OSM plays a crucial role in the orchestration of hematopoiesis and liver development.[3]

Upon OSM-induced receptor clustering, Janus kinases—mainly Janus kinase 1—are activated, phosphorylate tyrosines within the receptor that recruit other signaling proteins with matching SH2 domains such as signal transducers and activators of transcription (STATs) or adapter proteins for the mitogen-activated protein kinases to the receptor. The major signaling cascades activated by OSM include STAT3 as well as the extracellular signal-regulated kinase (Erk) 1/2 and p38 pathways.[2-4]

Several processes such as hematopoiesis, angiogenesis, liver development, metabolism, inflammation, and tu-

Abbreviations: Erk, extracellular signal-regulated kinase; HIF, hypoxia-inducible factor; HIF1α, hypoxia-inducible factor 1α; HRE, hypoxia response element; IL, interleukin; mRNA, messenger RNA; OSM, oncostatin M; PAI1, plasminogen activator inhibitor 1; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

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morigenesis are also crucially influenced by the ambient oxygen tension of the tissue. Hypoxia-inducible factors (HIFs) act as master regulators for the expression of genes essential in a hypoxic microenvironment. The best characterized factor is HIF1, which regulates more than 100 genes. HIF1 is formed by dimerization of the oxygen-sensitive hypoxia-inducible factor 1α (HIF1α) subunit with the constitutively expressed beta-subunit, which is also known as ARNT. Under normoxic conditions, the HIF1α subunit is hydroxylated and quickly degraded via the proteasome. Under hypoxic conditions, the activity of the hydroxylases is reduced, and HIF1α protein is stabilized and can bind to hypoxia-response elements (HREs) within the regulatory areas of HIF target genes and efficiently recruit cofactors.[5]

In addition to hypoxia, HIF1α has also been shown to be up-regulated under normoxia in response to growth factors, thrombin, lipopolysaccharide, angiotensin II, insulin, or the cytokines IL-1 and tumor necrosis factor α (TNF-α).[6] Although several details have been unraveled regarding the ability of hypoxia to stabilize HIF1α, the mechanisms by which those factors (especially cytokines) induce HIF1α have not been fully elucidated.

Interestingly, OSM has been described to orchestrate the hypoxia-influenced processes of hematopoiesis, angiogenesis, liver development, and regeneration.[3,7] This suggests a possible cross-talk of the OSM and hypoxia signaling pathways. The expression of the HIF1 target genes vascular endothelial growth factor (VEGF) and plasminogen activator inhibitor 1 (PAI1)—which are crucial for angiogenesis and tissue remodeling, respectively—can also be up-regulated by the cytokine OSM.[8-12] OSM supports *in vitro* differentiation of fetal hepatic cells into liver-like structures, which is paralleled by enhanced VEGF expression.[11] Moreover, OSM mediates differentiation of oval cells into hepatocytes.[13] OSM plays a crucial, nonredundant role in liver regeneration, as shown for OSM receptor knock-out mice after partial hepatectomy or CCl_4 treatment,[14] and OSM gene therapy attenuates liver damage induced by dimethylnitrosamine.[15] Interestingly, HIF1α is also expressed during liver regeneration,[16] and HIF1α supports the growth of hepatoma cells *in vivo* and *in vitro*.[17,18]

Because both OSM and HIF1α play a pivotal role in liver-related processes (development, regeneration, carcinogenesis), the aim of the present study was to investigate whether the OSM signaling pathway has an impact on the HIF1 system in hepatoma cells and hepatocytes.

Materials and Methods

Cell Culture and Reagents. HepG2 hepatoma cells were maintained in DMEM/NUT-MIX-F12 medium (Lonza) supplemented with 10% fetal bovine serum (PAA), 100 mg/L streptomycin, and 60 mg/L penicillin (Cytogen). The human hepatocyte cell line PH5CH8 has been described.[19] Cells were grown at 37°C in a water-saturated atmosphere at 5% CO_2. Hypoxia treatment was performed at 37°C in a water-saturated atmosphere at 5% CO_2 and 6% oxygen. HepG2 cells were transfected using the Fugene reagent (Roche) according to the manufacturer's recommendations. Cotransfections of small interfering RNA (siRNA) and reporter gene constructs are described in the Supporting Information. Human recombinant OSM was obtained from Peprotech. Actinomycin D and cycloheximide were obtained from Calbiochem. Stattic[20] was from Sigma.

Western Blot Analysis and Antibodies. All steps of cell lysis and immunoprecipitation were performed at 4°C using ice cold buffers. Cells were lysed on a dish with lysis buffer containing 30 mM Tris/HCl (pH 6.7), 5% glycerol, 2.5% mercaptoethanol, and 1% sodium dodecyl sulfate. The lysates were further analyzed via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Antibodies against HIF1α, STAT3, STAT1, and Fin13 were obtained from BD Transduction Laboratories. Antibodies against phospho-STAT3, phospho-STAT1, Erk1/2, phospho-Erk1/2, p38, and phospho-p38 were obtained from Cell Signaling. ECL signals were detected as described.[21] Before reprobing, blots were stripped as described.[4]

Reporter Gene Assays. HepG2 cells were transfected with 1 μg of the β-galactosidase control plasmid (pCH110, Amersham Biosciences) and 1.5 μg of the respective reporter gene construct. Twenty-four hours after transfection, the cells were treated with the different stimuli as described in the figure legends. Cell lysis and luciferase assays were performed using the Promega luciferase assay system (Promega, Madison, WI) (see Supporting Information for further details). All experiments were performed at least in triplicate, and biological triplicates were also performed within one experiment. Luciferase activity values were normalized to transfection efficiency monitored by the cotransfected β-galactosidase expression vector. For some experiments the luciferase activity values after OSM stimulation were additionally normalized to values from control unstimulated cells.

Quantitative Real-Time Polymerase Chain Reaction. The exact protocol is described in the Supporting Information. Total RNA was extracted using the RNeasy Mini Kit (Macherey Nagel) according to the manufacturer's instructions. The concentration of isolated RNA was measured using a NanoDrop spectrophotometer. One microgram of total RNA was reverse-transcribed with a ThermoScript RT-PCR System (Invitrogen). Quantita-

tive real-time polymerase chain reaction (PCR) was performed on an iQ5 Real-Time PCR detection system (Bio-Rad Laboratories). Standard curves using four 10-fold dilutions (1×, 0.1×, 0.01×, 0.001×) were produced to ensure that the amplification efficiencies were similar and in the range of 95% to 105%. The messenger RNA (mRNA) level of each target gene was normalized to the relative amount of the housekeeping gene TBP. The comparative threshold cycles (C_T) method, $2^{-\Delta CT}$, was used to calculate the changes in gene expression for each target gene.

Statistical Analysis. Each experiment was performed at least three times. Representative data are shown and are expressed as the mean ± standard deviation. Depending on datasets, statistical analysis was performed using a *t* test, Mann-Whitney test, or analysis of variance. *P* values of <0.05 were considered significant.

Results

OSM Increases Expression of Functional HIF1α Under Normoxic Conditions. Exposure of HepG2 hepatoma cells to OSM for different times led to a profound and transient increase in HIF1α protein levels that lasted up to 24 hours. The up-regulation reached a maximum after about 6 hours of stimulation. As expected, OSM induced STAT3, Erk1/2, and p38 phosphorylation (Fig. 1A). HIF1α was also induced upon stimulation of the human hepatocyte cell line PH5CH8 with OSM (Fig. 1B). Quantitation of western blots showed that upon OSM stimulation, the HIF1α protein is up-regulated by factors of 2.66 ± 0.3 in PH5CH8 cells and 2.7 ± 0.9 in HepG2 cells.

To investigate whether OSM-induced HIF1α is functional, we tested the effect of OSM on HepG2 cells transfected with a HIF1-responsive luciferase reporter gene construct. OSM treatment increased luciferase activity three-fold (Fig. 1C). Thus, OSM-induced HIF1α is transcriptionally active even under normoxic conditions.

OSM Augments Hypoxia-Dependent HIF1α Induction and Hypoxia-Mediated Target Gene Expression. Next, we compared the induction of HIF1α by OSM under normoxia with that induced by hypoxia. In addition, we examined whether OSM may affect the hypoxia-dependent induction of HIF1α. We found that HIF1α levels induced by OSM under normoxia were slightly lower than those induced by hypoxia after 4 and 6 hours of induction, respectively. Interestingly, when cells were treated with OSM under hypoxia, the increase in HIF1α protein levels was higher than under each treatment alone (Fig. 2A). Quantitation of western blots showed that the HIF1α protein is up-regulated by factors of 3.6 ± 0.19 upon hypoxia, 2.7 ± 0.9 upon OSM, and 6.4 ± 1.8 upon combined treatment with hypoxia and OSM.

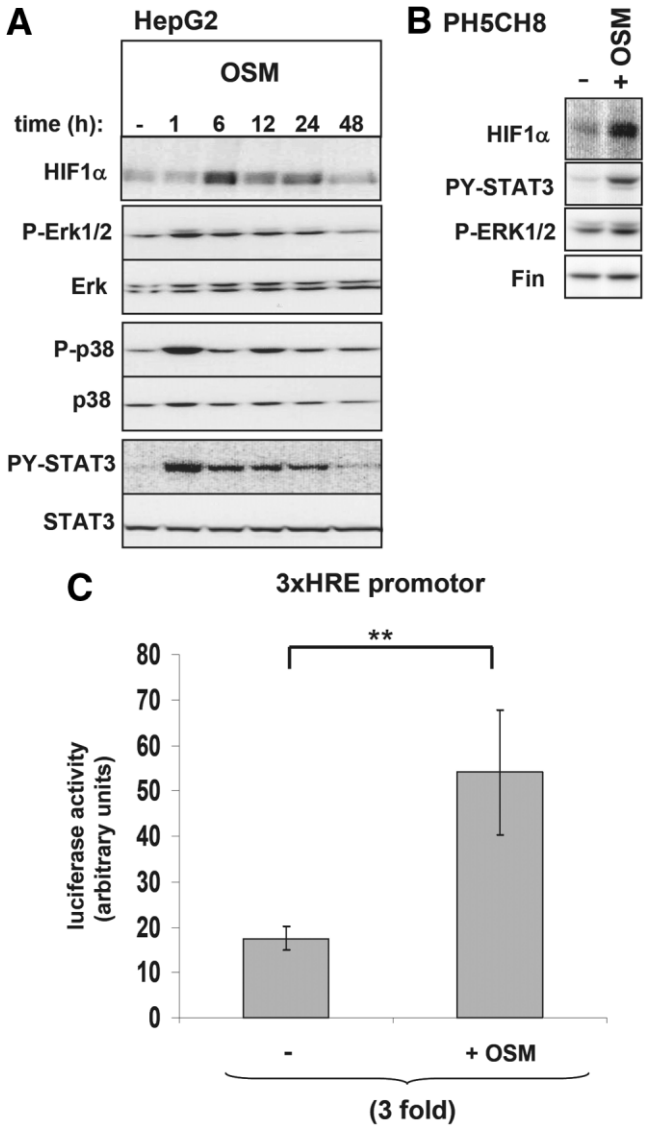

Fig. 1. OSM induces HIF1α protein levels over an extended period, and the induced HIF1α is transcriptionally active. (A) HepG2 cells were treated for the indicated periods with OSM (10 ng/mL). Lysates of the cells were separated via SDS-PAGE, and western blots of the membranes were detected with HIF1α, phospho-STAT3, phospho-Erk1/2, phospho-p38, STAT3, Erk1/2, and p38 antibodies. (B) PH5CH8 cells were stimulated for 4 hours with OSM (10 ng/mL) or left untreated. Western blots of the membranes were detected as described in (A). (C) HepG2 cells were transfected with the luciferase reporter gene plasmids pGL3-EPO-HRE-Luc and the β-galactosidase expression vector pCH110. Twenty-four hours after transfection, the medium was exchanged and the cells were treated for an additional 16 hours with OSM (10 ng/mL) before lysates were prepared, and the reporter gene activity was measured as described in Experimental Procedures. $^{**}P < 0.01$.

We then investigated HIF1α mRNA expression under the same conditions and found that OSM induces HIF1α mRNA levels stronger (3.4-fold) than hypoxia (2.3-fold). Combined treatment with hypoxia and OSM led to an

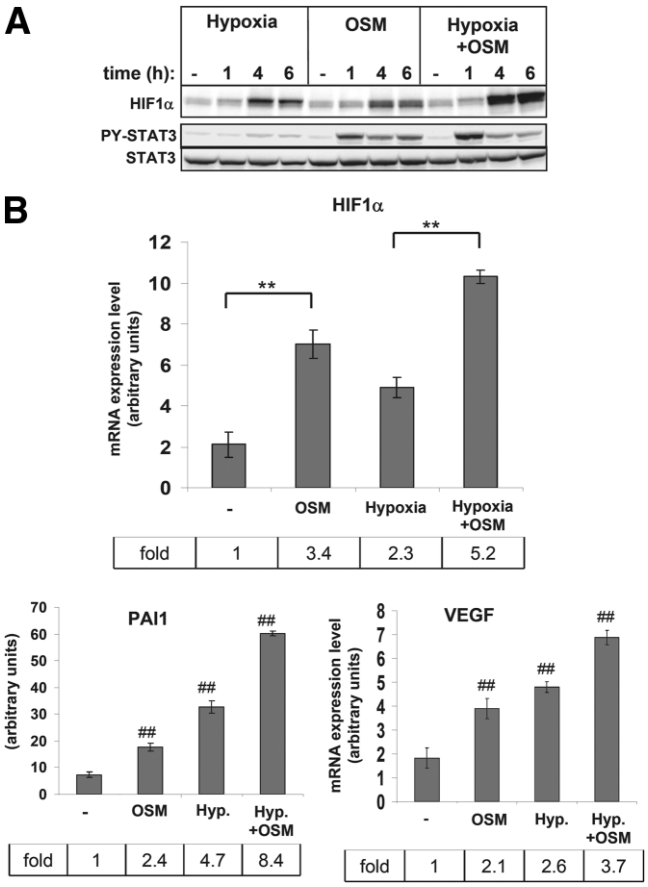

Fig. 2. OSM enhances hypoxia-induced HIF1α, PAI1, and VEGF expression. (A) HepG2 cells were treated for the indicated periods with hypoxic conditions and/or OSM (10 ng/mL). Lysates of the cells were separated via SDS-PAGE, and western blots of the membranes were detected with antibodies directed against HIF1α, phospho-STAT3, and STAT3. (B) HepG2 cells were stimulated for 4 hours with hypoxic conditions and/or OSM (10 ng/mL). RNA was prepared, and HIF1α mRNA levels were analyzed via quantitative PCR. $^{**}P < 0.01$. (C) HepG2 cells were treated as described in (B) and VEGF and PAI1 mRNA levels were analyzed via quantitative PCR. $^{\#\#}P < 0.01$ versus untreated controls.

even higher induction of HIF1α mRNA (5.2-fold) (Fig. 2B). Thus OSM significantly increases HIF1α mRNA under normoxia and hypoxia, which matches with the protein up-regulation seen in the western blots (Fig. 2A). Two important target genes of the hypoxic response, PAI1 and VEGF, were also significantly up-regulated by OSM, and a combined treatment with hypoxia and OSM led to an even stronger induction of PAI1 and VEGF mRNA (Fig. 2C).

OSM-Mediated Up-Regulation of HIF1α Protein Levels Is Due to De Novo Transcription but not Regulation of Protein Stability. To find out whether OSM influences HIF1α protein stability, we aimed to measure HIF1α protein half-life. Therefore, we stimulated HepG2 cells with OSM or $CoCl_2$ (a hypoxia mimetic) for 6 hours to induce a robust HIF1α expression before the translation inhibitor cycloheximide was added for different periods. We found that the OSM-induced HIF1α protein disappeared completely after a 10-minute treatment with cycloheximide (Fig. 3A, right panel). In contrast, $CoCl_2$-induced HIF1α was still well detectable after 1 hour; it disappeared after 3 hours of cycloheximide treatment (Fig. 3A, left panel). There was no difference in HIF1α protein stability between $CoCl_2$ and the combined treatment with OSM and $CoCl_2$. These data show that the OSM-mediated HIF1α up-regulation is not due to an enhanced stability of the protein.

In contrast, experiments with the transcription inhibitor actinomycin D showed that less HIF1α protein was detectable when cells were treated with OSM in the presence of actinomycin D relative to cells treated with OSM alone (Fig. 3B). This was even more remarkable because actinomycin D treatment prevents the STAT3-induced up-regulation of the feedback inhibitor SOCS3, which suppresses STAT3 activation upon OSM. Despite the higher STAT3 activation observed in actinomycin D–treated cells, no HIF1α expression could be observed. Actinomycin D did not affect HIF1α expression in cells exposed to hypoxia, which was expected because hypoxia essentially increases protein stability. Here, the HIF1α

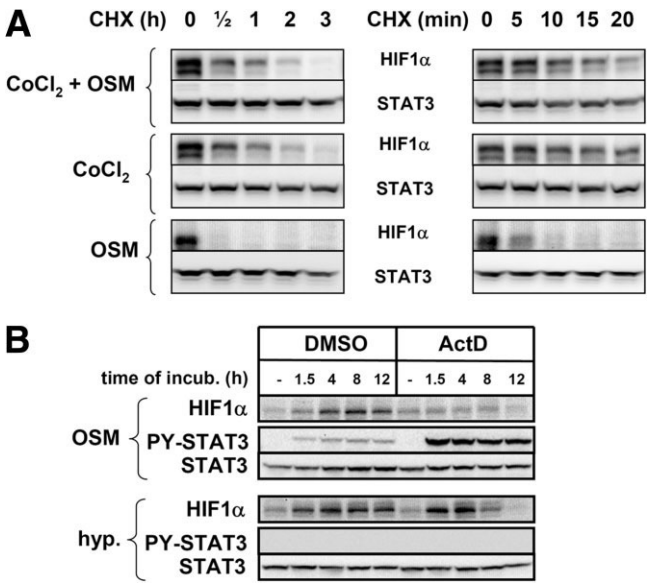

Fig. 3. OSM increases transcription of HIF1α but does not affect HIF1α protein stability. (A) HepG2 cells were stimulated for 6 hours with $CoCl_2$ (50 μM) and/or OSM (10 ng/mL). Cycloheximide (10 μg/mL) was then added for the indicated periods before lysates were prepared. Western blots were detected with HIF1α and STAT3 antibodies. For HIF1α detection, the western blots were exposed so that the band intensity of the untreated lane for all treatments was comparable. (B) HepG2 cells were treated for the indicated periods with OSM (10 ng/mL) or hypoxia (hyp.) in the presence of dimethyl sulfoxide alone or actinomycin D (5 μg/mL). Western blots of lysates separated via SDS-PAGE were detected with HIF1α, phospho-STAT3, and STAT3 antibodies.

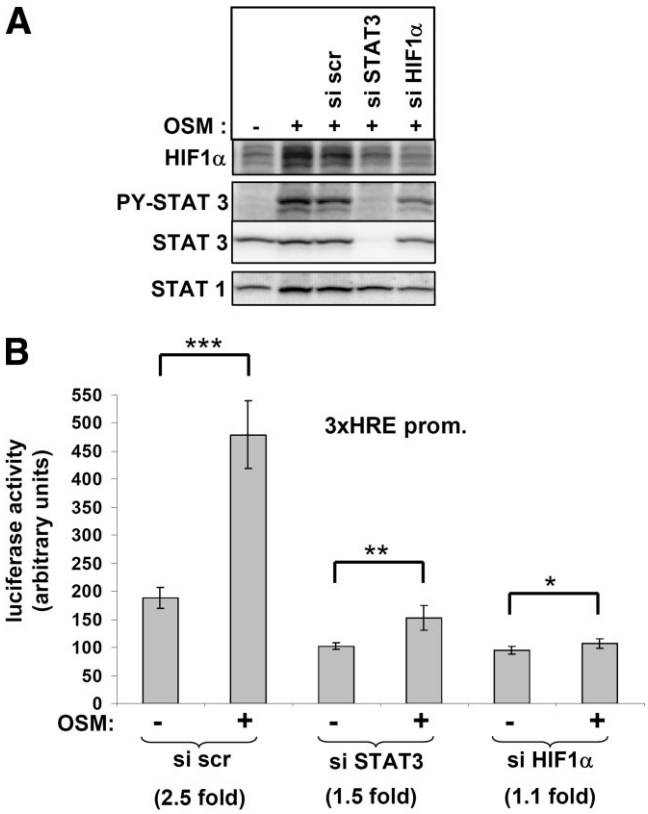

Fig. 4. Knockdown of STAT3 by siRNA decreases HIF1α protein levels and HIF1α-responsive reporter gene activity upon OSM treatment. (A) HepG2 cells were transfected with siRNAs as indicated. Forty-eight hours after transfection, cells were treated for 4 hours with OSM (10 ng/mL). Lysates of the cells were separated via SDS-PAGE, and western blots of the membranes were detected with HIF1α, phospho-STAT3, STAT3 and STAT1. (B) HepG2 cells were transfected with siRNAs as indicated, the luciferase reporter gene plasmids pGL3-EPO-HRE-Luc and the β-galactosidase expression vector pCH110. The cells were treated for additional 16 hours with OSM (10 ng/mL) before lysates were prepared. $***P < 0.001$. $**P < 0.01$. $*P < 0.05$.

levels decreased only after 8 hours, in accordance with the normal half-life of HIF1α under hypoxia (8-10 hours).[22]

In addition, our data in the Supporting Information show that OSM, in contrast to hypoxia, does not affect hydroxylation and ubiquitination (Supporting Fig. 2). Together, these data provide evidence that OSM enhances HIF1α via transcriptional regulation.

STAT3 Protein Is Crucial for OSM-Induced Up-Regulation of HIF1α. After having shown that OSM-mediated up-regulation involves transcriptional regulation, we addressed the role of STAT3, playing a pivotal role in signaling of IL-6–type cytokines. Suppression of STAT3 by siRNA led to a loss of HIF1α expression upon OSM treatment, similar to the HIF1α siRNA used as a positive control (Fig. 4A). The unspecific control siRNA did not show these pronounced effects. Upon transfection of STAT3 or HIF1α siRNAs together with the 3xHRE reporter gene construct, OSM only weakly induced the reporter gene activity, whereas OSM induced reporter gene activity 2.5-fold when the unspecific control siRNA was transfected (Fig. 4B). In addition, the STAT3 inhibitor Stattic or dominant negative STAT3 also inhibited OSM-dependent effects (increase of HIF1α protein and mRNA levels, HRE promotor activity, or target gene induction) (Supporting Figs. 3-5). Furthermore, we provide evidence for the relevance of Erk signaling in OSM-mediated induction of HIF1α protein and activity (Supporting Figs. 3-5).

OSM-Induced HIF1α Is Crucially Involved in the Transcriptional Regulation of the Genes for VEGF and PAI1. To investigate the relevance of HIF1α expression in OSM signal transduction, we examined the effects of HIF1α suppression on the target genes PAI1 and VEGF. We found that the OSM-dependent induction of both the VEGF and PAI1 mRNA were decreased upon HIF1α suppression (Supporting Fig. 6). In addition, the OSM-mediated induction of the VEGF and PAI1 promotor was down-regulated by HIF1α siRNA and by STAT3 siRNA (Fig. 5A), while a control siRNA had no effect. Consistent with this finding, we found that the OSM-dependent induction of the VEGF promoter was reduced by about 50% when a VEGF promoter construct mutated at the HRE was used for transfection (Fig. 5B). Because the robust induction of the VEGF promoter by hypoxia requires the integrity of the HRE and the AP1 site, we also used a construct where the AP1 site was mutated; the OSM-dependent induction of this promoter was reduced by about 25% (Fig. 5B). Importantly, mutation of the previously described STAT3 binding site within the VEGF promoter had no effect on the OSM-dependent induction of reporter gene activity (Fig. 5B). Thus, OSM-induced VEGF transcription seems to be mediated via HIF1 and AP1, rather than by STAT3.

In addition, HIF1 seems to play a crucial role in OSM-mediated activation of the PAI1 promotor, for which a reduction of about 40% was observed when the HRE was mutated (Fig. 5C).

Together, these data indicate that *de novo*–transcribed HIF1α importantly contributes to the OSM-induced VEGF and PAI1 transcription.

Discussion

The first major finding of the present study is that the cytokine OSM, which activates the STAT3, Erk1/2, and p38 signaling pathways, can induce a robust up-regulation of HIF1α protein levels in hepatocytes and hepatoma cells under normoxic conditions and leads to the formation of transcriptionally active HIF1 complexes. OSM-induced HIF1α protein up-regulation was stronger compared with other cytokines (IL-6, IL-1β, TNF-α, interferon-γ), some of which have been implicated in

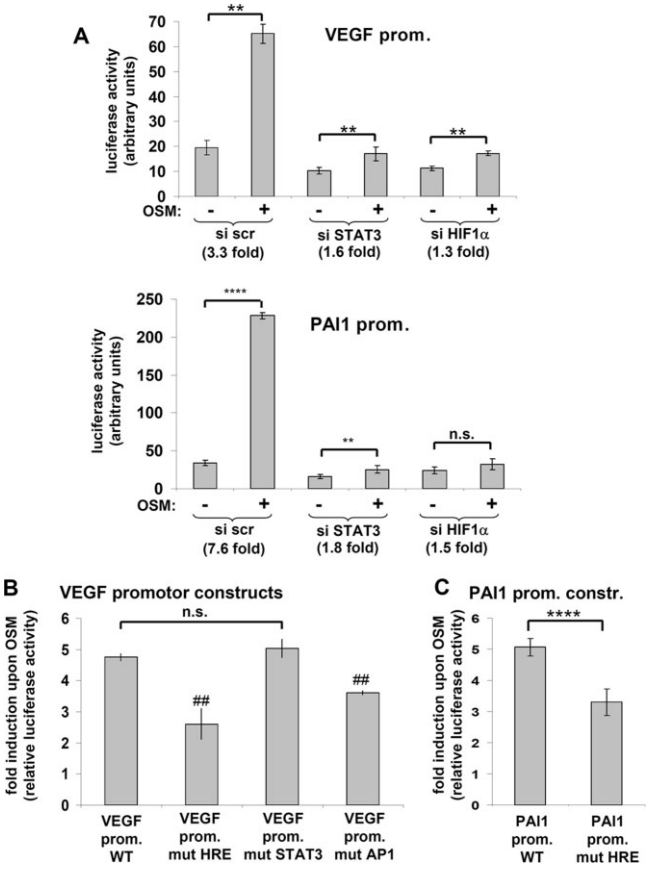

Fig. 5. Regulation of the VEGF and PAI1 gene promotors by OSM. (A) HepG2 cells were transfected with siRNAs as indicated, the luciferase reporter gene plasmids pGL3-VEGF-Luc or pGL3-hPAI1-796 and the β-galactosidase expression vector pCH110, and processed as described in Fig. 4B. $^{****}P < 0.0001$. $^{**}P < 0.01$. n. s., not significant. (B) HepG2 cells were transfected with the luciferase reporter gene plasmids pGL3-VEGF-Luc or the following mutants thereof: pGL3-VEGFmutHRE-Luc (containing a mutated HIF1α binding site), pGL3-VEGFmutSTAT3-Luc (containing a mutated STAT3 binding site), or pGL3-VEGFmutAP1-Luc (containing a mutated AP1 binding site). The β-galactosidase expression vector pCH110 was cotransfected. Twenty-four hours after transfection, the medium was exchanged, and the cells were treated for an additional 16 hours with OSM (10 ng/mL) before lysates were prepared. Values for luciferase activity (relative to β-galactosidase activity) are shown as fold induction compared with untreated samples. $^{\#\#}P < 0.01$ versus wild-type construct. (C) HepG2 cells were transfected with pGL3-hPAI1-796 or with pGL3-hPAI1-796-M2 containing a mutation in the HRE and processed as described in (B). $^{****}P < 0.0001$.

HIF1α up-regulation before (IL-6, IL-1β, TNF-α) (Supporting Fig. 1). Moreover, the OSM-increased HIF1α protein was shown to be involved in the enhanced expression of the HIF1 target genes PAI1 and VEGF.

Our study also shows that OSM and hypoxia differ in their mechanism of HIF1α up-regulation. Regulation of HIF1α activity is complex and under normoxic conditions, HIF1α has an extremely short half-life because it is continuously degraded due to the initial hydroxylation at two proline residues and transcriptional activity is reduced due to hydroxylation of asparagine 803. Hypoxia reduces the activity of the oxygen-utilizing hydroxylases, thereby stabilizing the protein and increasing its transactivity.[5] However, our data provide evidence that OSM does not contribute to an increased stability or increased transactivity as shown in the experiments with the Gal-HIF1α-TADN or TADC gene constructs (Supporting Fig. 2C). Furthermore, we could show that OSM-induced HIF1α was clearly ubiquitinated, which was less the case for hypoxia-stabilized HIF1α (Supporting Fig. 2A,B). We propose that transcriptional mechanisms are responsible for the OSM-mediated HIF1α protein up-regulation because it was inhibitable by the transcriptional inhibitor actinomycin D, whereas this was not the case for hypoxic treatment. The importance of HIF1α regulation at the mRNA level is further supported by findings showing that hepatocyte growth factor, angiotensin-II, lipopolysaccharide, IL-1, thrombin, or hypoxia also enhance HIF1α mRNA levels in different cell types (see Bonello et al.[23] and references therein).

Moreover, our tests with inhibitors for Janus kinases, STAT3, and mitogen-activated protein kinase kinase indicate that these pathways play an important role in OSM-dependent induction of HIF1α mRNA and protein expression (Supporting Fig. 3), HIF1α-dependent reporter gene activity (3x HRE promotor) (Supporting Fig. 4A), as well as in the regulation of the target genes VEGF and PAI1 (Supporting Fig. 5). In contrast, inhibitor tests suggested that p38 mitogen-activated protein kinases or the PI3K/Akt pathway are not involved in OSM-induced HIF1α expression (data not shown).

Experiments with siRNA further revealed that STAT3 plays a crucial role in OSM-regulation of HIF1α protein levels and HIF1α-dependent transcriptional activity. Moreover, dominant negative STAT3 (STAT3-DN) also led to a down-regulation of HIF1α-dependent transcriptional activity (Supporting Fig. 4B). It has been shown that HIF1α transcription is regulated by SP-1[24] and NF-κB transcription factors.[23] In addition to that, the present study indicates that the transcriptional regulator STAT3 appears to be a key player for HIF1α transcription in response to OSM. STAT3 was also found to be involved in HIF1α mRNA expression in tumor cells and tumor-associated myeloid cells.[25]

Our study provides evidence that HIF1α is important for OSM signal transduction. Experiments with HIF1α siRNA showed that the expression of the OSM target genes VEGF and PAI1 involves regulation by HIF1α. OSM and other IL-6–type cytokines have been shown to induce expression of the HIF1 target gene VEGF.[10,12,26-29] The VEGF gene is also considered a STAT3 target gene, because a dominant negative STAT3 reduced[10,27,30,31] and constitutively active forms of

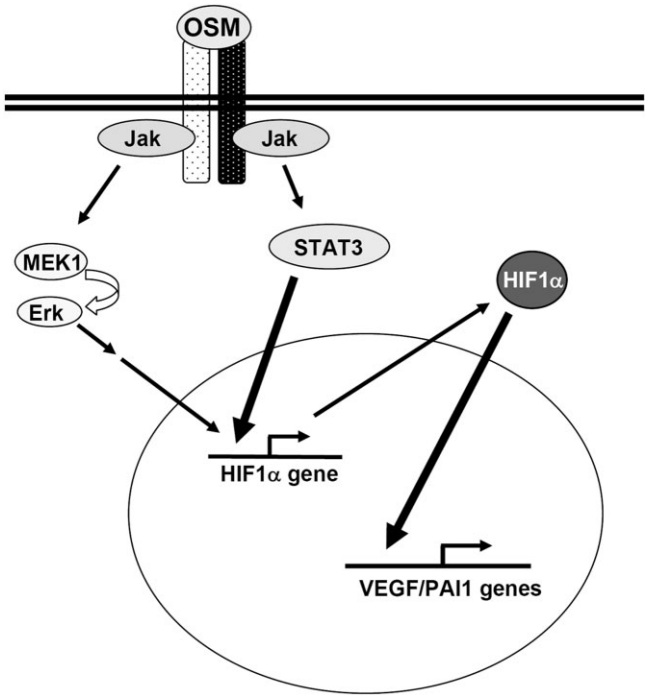

Fig. 6. Schematic representation of VEGF gene regulation by OSM.

STAT3 induced the VEGF promoter,[27,30,31] and a STAT3 binding element was detected at site −848.[30,31] We found that mutation of the described STAT3 binding site in the VEGF promoter at −848 did not affect OSM-dependent induction of reporter gene activity. These data are consistent with those of another study in which deletion of this site also did not affect IL-6–induced reporter gene activity.[27] Instead, our results with the VEGF reporter gene constructs clearly demonstrate a relevance of the HIF1 and AP1 binding elements for OSM-mediated regulation, because the constructs mutated at the HRE and the AP1 sites displayed significantly reduced induction in response to OSM. Thus, we conclude from our data that STAT3 regulation of the VEGF gene may rather be mediated indirectly via Erk1/2 and STAT3-dependent induction of HIF1α transcription. HIF1α then regulates VEGF and PAI1 transcription (Fig. 6), which leads to increased secretion of VEGF and PAI1 proteins (Supporting Fig. 7).

Although the present study was performed with hepatoma cells and nontransformed hepatocytes, it will be interesting to further clarify the role of HIF1α in OSM-mediated signal transduction and regulation of secreted factors involved in tissue remodeling (such as VEGF and PAI1) in processes such as liver development, regeneration, and inflammation.

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