Prostate Cancer and Prostatic Diseases (2016) 00, 1–12

www.nature.com/pcan

ORIGINAL ARTICLE

SOCS1 inhibits migration and invasion of prostate cancer cells, attenuates tumor growth and modulates the tumor stroma

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BACKGROUND: The suppressor of cytokine signaling 1 (*SOCS1*) gene is repressed in prostate cancer (PCa) by epigenetic silencing and microRNA miR30d. Increased expression of the SOCS1-targeting miR30d correlates with higher biochemical recurrence, suggesting a tumor suppressor role of SOCS1 in PCa, but the underlying mechanisms are unclear. We have shown that SOCS1 inhibits MET receptor kinase signaling, a key oncogenic pathway in cancer progression. Here we evaluated the role of SOCS1 in attenuating MET signaling in PCa cells and tumor growth *in vivo*.

METHODS: MET-overexpressing human DU145 and PC3 PCa cell lines were stably transduced with SOCS1, and their growth, migration and invasion of collagen matrix were evaluated *in vitro*. Cells expressing SOCS1 or the control vector were evaluated for tumor growth in NOD.scid.gamma mice as xenograft or orthotopic tumors.

RESULTS: HGF-induced MET signaling was attenuated in SOCS1-expressing DU145 and PC3 cells. Compared with vector control cells, SOCS1-expressing cells showed reduced proliferation and impaired migration following HGF stimulation. DU145 and PC3 cells showed marked ability to invade the collagen matrix following HGF stimulation and this was attenuated by SOCS1. As xenografts, SOCS1-expressing PCa cells showed significantly reduced tumor growth compared with vector control cells. In the orthotopic tumor model, SOCS1 reduced the growth of primary tumors and metastatic spread. Intriguingly, the SOCS1-expressing DU145 and PC3 tumors showed increased collagen deposition, associated with increased frequency of myofibroblasts.

CONCLUSIONS: Our findings support the tumor suppressor role of SOCS1 in PCa and suggest that attenuation of MET signaling is one of the underlying mechanisms. SOCS1 in PCa cells also appears to prevent the tumor-promoting functions of cancer-associated fibroblasts.

Prostate Cancer and Prostatic Diseases advance online publication, 25 October 2016; doi:10.1038/pcan.2016.50

INTRODUCTION

Prostate cancer (PCa) accounts for 21% of annual cancer incidence and 8% of cancer deaths among men.[1] Despite advances in screening and improved treatment methods,[2] clinical management of PCa remains challenging. Further progress in distinguishing indolent and malignant lesions and developing new therapies for aggressive disease depend on identifying biomarkers with greater prognostic value, elucidating signaling pathways that render PCa refractory to treatment and understanding the mechanisms of metastatic progression.[3]

Besides androgen receptor stimulation, interleukin-6 (IL-6) and other proinflammatory cytokines contribute to PCa pathogenesis.[4,5] In many cell types, IL-6 signaling induces a negative feedback regulator called the suppressor of cytokine signaling 1 (SOCS1).[6] A SOCS1-mimetic peptide has been shown to inhibit IL-6-induced STAT3 phosphorylation and proliferation in PCa cells.[7] SOCS1 induced by IL-6 and androgens inhibits cell growth by downmodulating cyclins and cyclin-dependent kinases.[8] These studies indicated that SOCS1 controls proliferation of PCa cells by attenuating IL-6 and androgen-stimulated oncogenic signaling.

The *SOCS1* gene promoter is repressed by methylation in many cancers including hepatocellular carcinoma, leukemia and pancreatic adenocarcinoma, and hence SOCS1 is considered a tumor suppressor.[9–13] Accordingly, SOCS1-deficient fibroblasts show increased susceptibility to oncogenic transformation and *Socs1*-null mice develop colorectal cancer and hepatocellular carcinoma.[14–16] SOCS1 is also targeted by microRNAs miR-155 and miR-19 in human cancers.[17,18] Although the *SOCS1* promoter is methylated only in 20% of PCa,[19] elevated expression of miR-30d, which also targets SOCS1, often occurs in PCa biopsies and correlates with early biochemical recurrence.[20]

Recently, we have shown that SOCS1 is dispensable to control IL-6 signaling in hepatocytes, but is essential to attenuate HGF signaling via its receptor, the MET receptor tyrosine kinase (RTK).[16,21] Indeed, MET and other RTKs activated by growth factors are implicated in PCa progression.[22–24] MET expression occurs in 40% of localized PCa that correlates with Gleason score, and MET positivity increases with lymph node metastasis, reaching nearly 100% in bone metastases.[25–28] Serum HGF and urinary MET levels, which increase during metastatic PCa, predict aggressive disease and recurrence even in the absence of elevated PSA levels.[29–31] Therefore, we investigated whether SOCS1 regulates MET signaling in human PCa by stably expressing SOCS1 in DU145 and PC3 cell lines.

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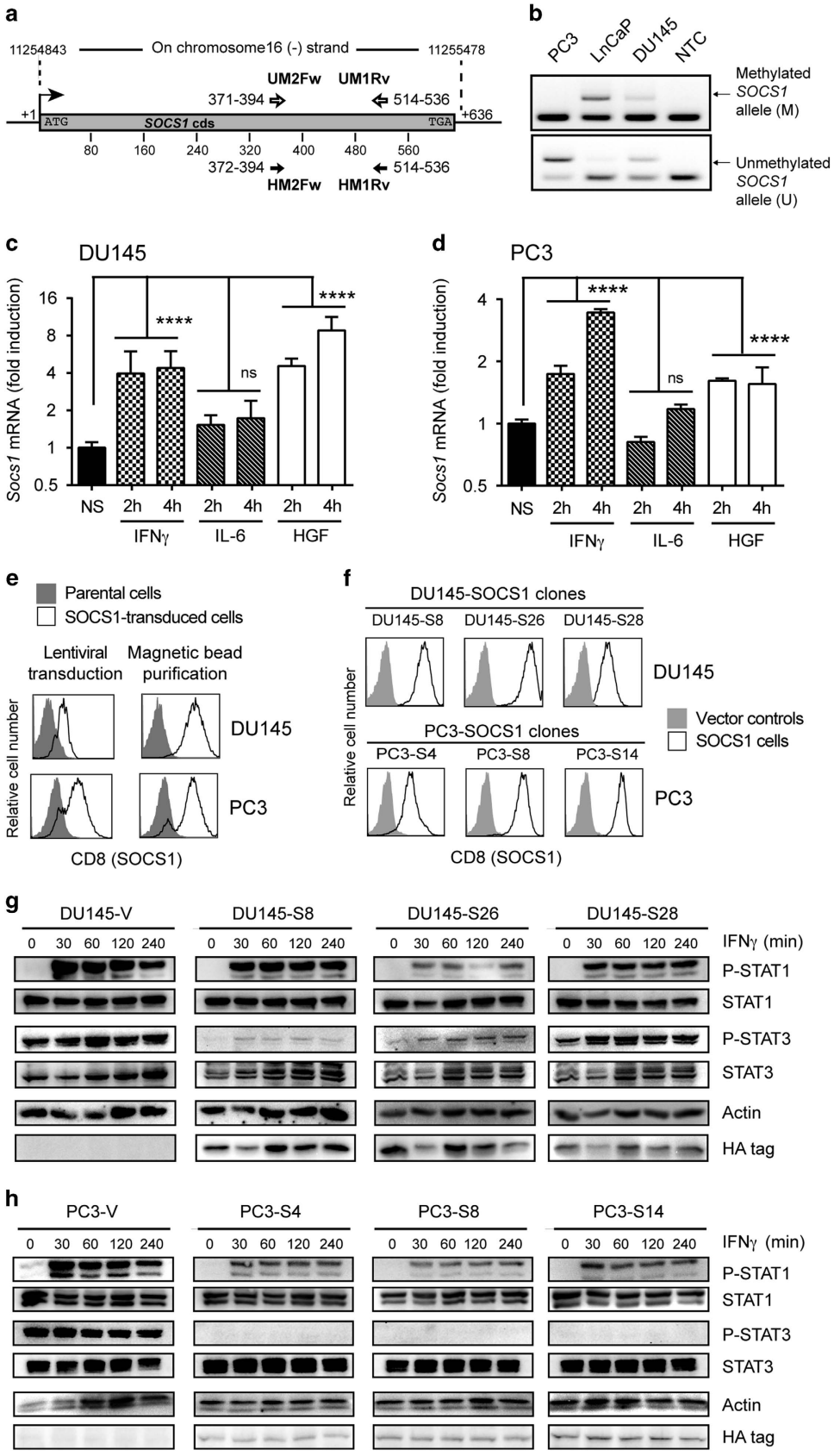
Received 13 July 2016; revised 20 August 2016; accepted 11 September 2016



MATERIALS AND METHODS

Establishment of stable SOCS1-expressing PCa cell lines

Human PCa cell lines PC3, DU145 and LNCaP, purchased from American Type Culture Collection (Manassas, VA, USA), were kindly provided by Dr Robert Day (Université de Sherbrooke). Cells were transduced with a lentiviral vector expressing HA-tagged SOCS1 also carrying mouse CD8 marker, or the control vector expressing green fluorescent protein.[21] The SOCS1-transfected cells were enriched by magnetic selection using anti-mouse CD8 antibody and then cloned by limiting dilution. Cells transduced with the control vector were sorted by flow cytometry based on green fluorescent protein expression.

Cytokines and antibodies

Human INFγ, IL-6 and HGF were from R&D Systems (Minneapolis, MN, USA) or Peprotech (Rocky Hill, NJ, USA). Antibodies used for western blotting and immunohistochemistry are listed in Supplementary Table 1.

Tumor growth in NOD.scid.gamma mice

NOD.*scid.gamma* mice were from the Jackson Laboratory (Bar Harbor, ME, USA). Male mice (8–12 weeks old) were used to evaluate xenograft and orthotopic tumor growth under protocols approved by the Université de Sherbrooke ethics committee in accordance with Canadian Council on Animal Care guidelines (Protocol number 117-15B) as detailed in Supplementary Methods.

Histopathology

Sections of formalin-fixed paraffin-embedded tumors and prostate glands were stained with hematoxylin and eosin. Sirius red, Masson's trichrome and immunohistochemical staining is detailed in Supplementary Methods. All histopathology images were acquired using Nanozoomer 2.0-RS scanner (Hamamatsu Photonics, Hamamatsu, Japan) and analyzed using the Nanozoomer digital pathology (NDP) software (Hamamatsu Photonics). Positively staining areas were quantified using the ImageJ software (NIH).

Supplementary methods

Methylation-specific PCR of the *SOCS1* gene, PCR with reverse transcription, western blot, thymidine incorporation, wound-healing assay and analysis of the PCa data sets are detailed in Supplementary Methods.

Statistical analysis

Statistical analyses were done using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

RESULTS

Generation of stable SOCS1-expressing PCa cells

The androgen-resistant PC3 and DU145 cells express high levels of MET compared with the androgen-sensitive LNCaP cells.[32] Therefore, we selected DU145 and PC3 cells to investigate the role of SOCS1 in regulating MET signaling in PCa. First, we examined the methylation status of the *SOCS1* gene in PCa cell lines by methylation-specific PCR (Figure 1a). The *SOCS1* gene was not methylated in PC3 cells, whereas DU145 cells harbored one methylated *SOCS1* allele and LNCaP cells had both alleles methylated (Figure 1b). Following exposure to IFNγ, a potent inducer of *SOCS1*, both DU145 and PC3 cells showed comparable *SOCS1* expression (Figures 1c and d), even though DU145 cells harbored only one unmethylated allele compared with the two in PC3 cells. IL-6 failed to induce *SOCS1* in both PC3 and DU145 cells, despite being an autocrine growth factor and its known ability to induce *SOCS1*.[6,33] HGF elicited strong *SOCS1* gene expression in DU145 cells and caused only modest upregulation in PC3 cells (Figures 1c and d). These observations indicate that *SOCS1* is an HGF-responsive gene in PCa cells and suggest differential induction and/or post-transcriptional control of *SOCS1* mRNA in these cells.

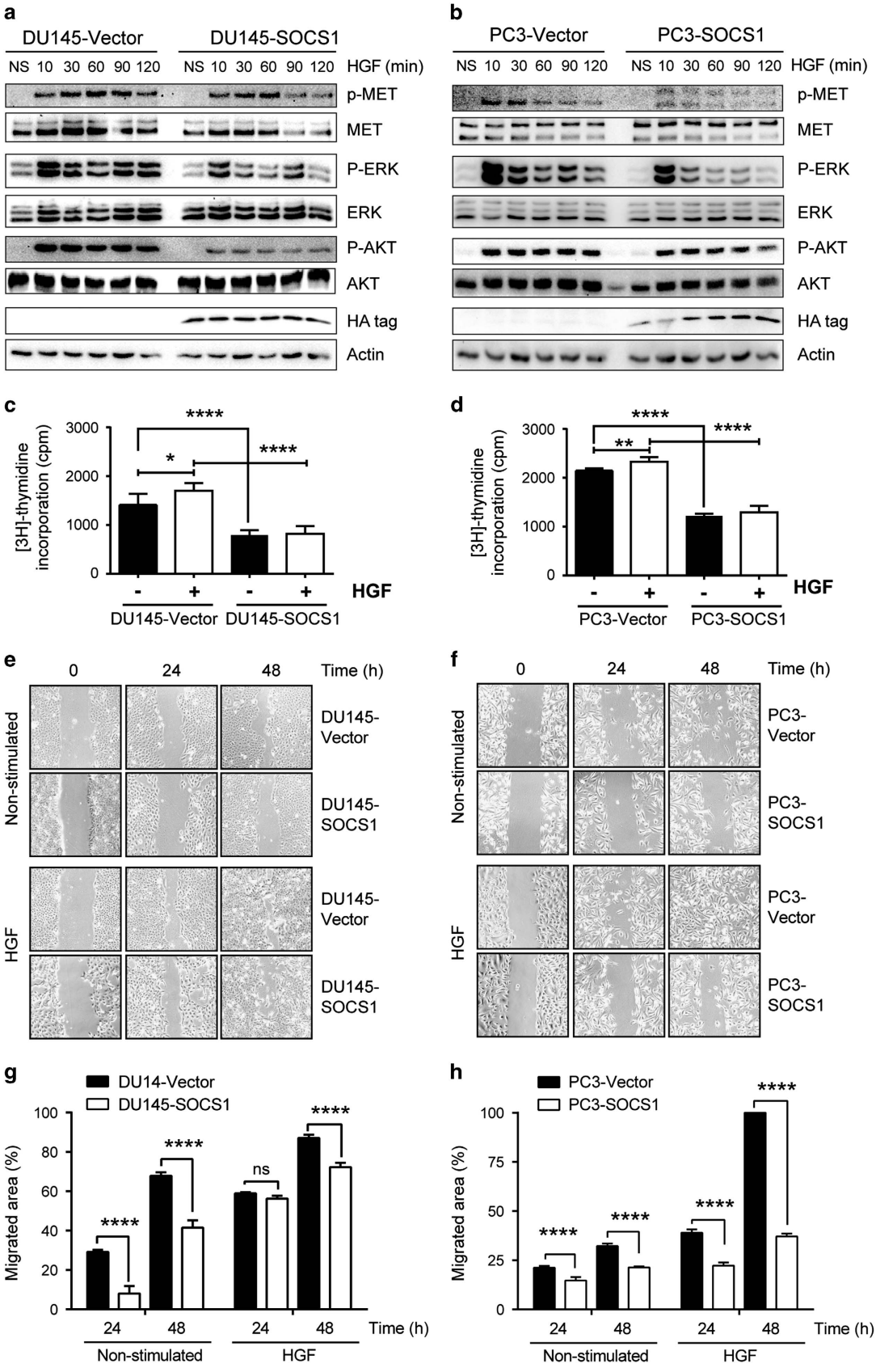
Following lentiviral transduction, SOCS1-expressing PC3 and DU145 cells were enriched based on the expression of CD8 marker, and stable SOCS1-expressing clones were established by limiting dilution (Figures 1e and f). After verifying SOCS1 expression by its HA tag, we tested its ability at inhibiting IFNγ-induced STAT phosphorylation, which was attenuated to a variable extent in different SOCS1-expresing clones of PC3 and DU145 (Figures 1g and h). On the other hand, PC3 and DU145 expressing the control vector showed constitutive STAT3 phosphorylation, which was completely abrogated by SOCS1 in all PC3 clones, but to a variable extent in DU145 clones. SOCS1-expressing clones of DU145 (DU145-S26) and PC3 (PC3-S14), which showed a strong capacity to attenuate IFNγ-induced STAT1 phosphorylation and significant inhibition of constitutive STAT3 phosphorylation, were selected to investigate the role of SOCS1 in regulating MET signaling in PCa cells.

SOCS1 expression in PCa cells attenuates HGF-induced MET signaling, cell proliferation and migration

We stimulated DU145 and PC3 cells expressing SOCS1 (DU145-SOCS1 and PC3-SOCS1) or the control vector (DU145-Vector and PC3-Vector) with HGF and assessed activation of the MET receptor and downstream signaling molecules. Although SOCS1 did not abrogate HGF-induced MET phosphorylation in DU145-Vector or PC3-Vector cells, DU145-SOCS1 cells showed a discernible reduction in phospho- and total MET levels after prolonged HGF stimulation (Figures 2a and b), suggesting SOCS1-mediated MET downmodulation.[34] Both DU145-SOCS1 and PC3-SOCS1 cells showed marked reduction in HGF-induced ERK phosphorylation. SOCS1 also caused a pronounced reduction in AKT phosphorylation in DU145 cells, although this was only minimally affected in PC3-SOCS1 cells. These results indicated that SOCS1 attenuates different components of the HGF-MET signaling pathway to a variable extent in PCa cell lines.

Next we evaluated the effect of SOCS1 on HGF-induced proliferation and cell migration. In both DU145-Vector and PC3-Vector cells, HGF caused a small, but significant increase in proliferation (Figures 2c and d). SOCS1 markedly reduced both

Figure 1. *SOCS1* gene methylation status in PCa cell lines and selection of stable SOCS1-expressing clones. (**a**) Genomic DNA from PC3, LNCaP and DU145 cell lines were treated with sodium bisulfite and analyzed by methylation-specific PCR for the *SOCS1* gene. (**b** and **c**) DU145 and PC3 cell lines were stimulated with IFNγ (1 ng ml^{-1}), IL-6 (5 ng ml^{-1}) or HGF (10 ng ml^{-1}), and SOCS1 gene expression was analyzed by real-time RT–PCR. Cumulative data from three experiments are shown. Data were compared by one-way ANOVA with Dunnett's multiple comparisons test: *$P < 0.05$, ****$P < 0.0001$. (**d**) DU145 and PC3 cell lines were transduced by a lentiviral SOCS1 expression construct that also expressed mouse CD8 from an internal ribosome entry site or the control vector expressing GFP. Transduced cells were analyzed for SOCS1 expression by evaluating mCD8 expression by flow cytometry. SOCS1-transduced cells were purified using anti-CD8 antibody-coated magnetic beads. (**e**) Purified cells were cloned by limiting dilution and CD8 expression in three clones for each cell line is shown. (**f** and **g**) Representative clones of DU145 (**l**) and PC3 (**g**) were stimulated with IFNγ for the indicated periods of time and phosphorylation of the indicated STAT proteins was assessed by western blot. SOCS1 expression was verified with an antibody to the HA epitope tag. Actin was used as protein loading control. Representative data from two similar experiments are shown. ANOVA, analysis of variance; GFP, green fluorescent protein; IL-6, interleukin-6; NTC, non-template controls; PCa, prostate cancer; RT–PCR, PCR with reverse transcription; SOCS1, suppressor of cytokine signaling 1.



basal and HGF-induced proliferation of DU145 and PC3 cells. In wound-healing assays, addition of HGF accelerated the migration of both DU145 and PC3 vector control cells (Figures 2e–h). Stable SOCS1 expression caused a significant reduction in wound-closure in both non-stimulated and HGF-stimulated cells. These results indicated that SOCS1 inhibits HGF-induced cell proliferation and migration in PCa cells.

SOCS1 blocks PCa cell invasion

HGF-dependent MET activation in tumor cells not only promotes cell proliferation and motility but also enhances cell, invasion of the extracellular matrix (ECM) and subsequent metastasis.[35] Therefore, we tested whether SOCS1 inhibited HGF/MET-induced invasion of PCa cells through the collagen matrix. After HGF stimulation, vector control cells showed increased matrix invasion (Figures 3a-f). SOCS1 expression almost completely abrogated HGF-induced invasive behavior in both DU145 and PC3 cells, bringing it to the basal level (Figures 3e and f).

SOCS1 has been reported to inhibit matrix metalloproteinases (MMP) in PCa cells.[20] As MMPs are involved in ECM degradation,[36] we determined whether HGF induced the expression of *MMP* genes and assessed the effect of SOCS1 on this induction. HGF did not upregulate *MMP1*, *MMP2*, *MMP3* and *MMP9* genes in either DU145-Vector or PC3-Vector cells (Figure 3g). However, SOCS1 markedly reduced the expression of all these genes in DU145 cells but not in PC3 cells (Figure 3g), even though the SOCS1-mediated inhibition of HGF-induced invasion was more pronounced in the latter (Figure 3d). These observations suggested that the HGF-induced invasion of ECM is likely mediated by diverse mechanisms in DU145 and PC3 cells, and that SOCS1 attenuates all these pathways.

SOCS1 expression in PCa cells decreases tumorigenesis

We implanted SOCS1-expressing and vector control DU145 and PC3 cells into the flanks of NOD.*scid.gamma* mice and assessed the growth of xenografts. SOCS1-expressing DU145 and PC3 cells showed a significantly decreased tumor growth compared with vector control cells (Figures 4a and b). The SOCS1-expressing tumors contained fewer and more dispersed cells than the control tumors (Figures 4c and d), and displayed significantly reduced number of PCNA-positive cells (Figures 4e and f), supporting the anti-proliferative function of SOCS1 in PCa growth.

Next, we assessed phosphorylation of MET and downstream signaling molecules in the tumors. The SOCS1-expressing tumors did not show appreciable change in MET phosphorylation or expression (Figures 4g and h). However, SOCS1-expressing PC3 tumors showed a discernible reduction in STAT3 and ERK phosphorylation, although DU145-SOCS1 tumors did not show any appreciable change. The results suggested that the reduced growth of SOCS1-expressing tumors result not only from attenuation of growth factor-induced signaling but also from other tumor suppressor mechanisms of SOCS1.

SOCS1 inhibits orthotopic tumor growth and metastasis by PCa cells

Metastasis is the main cause of PCa patient morbidity and mortality. Repression of the *SOCS1* gene positively correlates with disease progression in prostate and other cancers.[20] Given that SOCS1 attenuates the ability of PCa cell lines to penetrate collagen matrix (Figure 3), we evaluated its role in attenuating PCa metastasis in an orthotopic model.[37] SOCS1-expressing DU145 and PC3 cells developed significantly smaller tumors at the primary site when compared with vector control cells (Figures 5a and b) and showed reduced numbers of PCNA-positive cells (Figures 5c and d). Macroscopic metastatic nodules in the viscera, adrenal gland, pancreas and pleura were observed in 50 and 75% of control vector-transduced DU145 and PC3 cells, respectively, while none of the mice bearing SOCS1-expressing tumors showed macro-metastasis (Figures 5e and f). These results indicated that SOCS1 suppressed the metastatic potential of PCa cells.

SOCS1 expression increases collagen deposition and myofibroblast content in PCa xenografts

Cells in SOCS1-expressing DU145 and PC3 xenografts appeared to be separated by amorphous material (Figures 4c and d). To determine if this amorphous material was ECM, we stained the tumor sections with Sirius Red and Masson's trichrome, which detect type-I and type-III collagen fibrils, respectively.[38] SOCS1-expressing tumors showed significantly higher collagen content compared with control tumors (Figures 6a-d). As ECM in the cancer stroma is produced by cancer-associated fibroblasts (CAFs),[39] we stained the tumor sections for the myofibroblast marker α-smooth muscle actin (α-SMA), which revealed significantly more α-SMA-positive cells in SOCS1-expressing tumors (Figures 6e and f).

As the accumulation of ECM in tumors is influenced by not only its production but also its dynamic modulation by MMPs and tissue inhibitors of MMPs (TIMP),[36] we examined the expression of candidate *MMP* and *TIMP* genes. As shown in Figure 6g, DU145-SOCS1 tumors showed reduced expression of *MMP1*, *MMP3* and *TIMP1* and increased expression of *MMP2,* whereas PC3-SOCS1 tumors showed reduced expression of *MMP9, TIMP1* and *TIMP3*. These results indicated that SOCS1 differentially affected the ECM remodeling enzymes, and that this modulation occurred in a cell-specific manner.

Expression of SOCS1 and MET genes in human PCa specimens

Induction of the *SOCS1* gene by HGF in PC3 and DU145 cells (Figures 1c and d) suggested a negative feedback regulation of MET signaling by SOCS1. The higher level of *SOCS1* gene induction in DU145 cells than in PC3 cells is consistent with elevated MET expression in the former.[40] Therefore, we analyzed the association between *SOCS1* and *MET* gene expression in the human PCa databases available from Memorial Sloan Kettering Cancer Center

Figure 2. SOCS1 attenuates HGF-induced signaling, proliferation and migration of PCa cells. (**a** and **b**) DU145 (**a**) and PC3 (**b**) cells expressing the control vector or SOCS1 were stimulated with HGF (10 ng ml^{-1}) for the indicated periods, lysed and evaluated by western blot for phosphorylation of the MET receptor and downstream effectors AKT and ERK. The blots were stripped and reprobed for the total proteins. SOCS1 expression was verified by antibody to the HA epitope tag. Actin was used as protein loading control. Representative results from three experiments with similar results are shown. (**c**, **d**) Control and SOCS1-expressing DU145 (**c**) and PC3 (**d**) cells were seeded in 96-well-plates (5000 cells per well) in triplicates in serum-deprived medium for 18 h, in the presence or absence of human HGF (10 ng ml^{-1}). After adding [^{3}H]-thymidine for 6 h, the cells were harvested to evaluate thymidine incorporation. Representative results of three independent experiments are shown. Data represent mean ± s.d.. Data were compared by one-way ANOVA with Tukey's multiple comparisons: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (**e**–**h**) Confluent cultures of Control and SOCS1-expressing DU145 (**c**) and PC3 (**d**) cells were tested for wound-healing ability in the presence or absence of HGF (30 ng ml^{-1}) at the indicated time points. Representative images (**e** and **f**) and cumulative data (**g** and **h**; mean ± s.d.) from results from three experiments are shown. Data were compared by two-way ANOVA with Sidak's multiple comparisons test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ANOVA, analysis of variance; PCa, prostate cancer; SOCS1, suppressor of cytokine signaling 1.

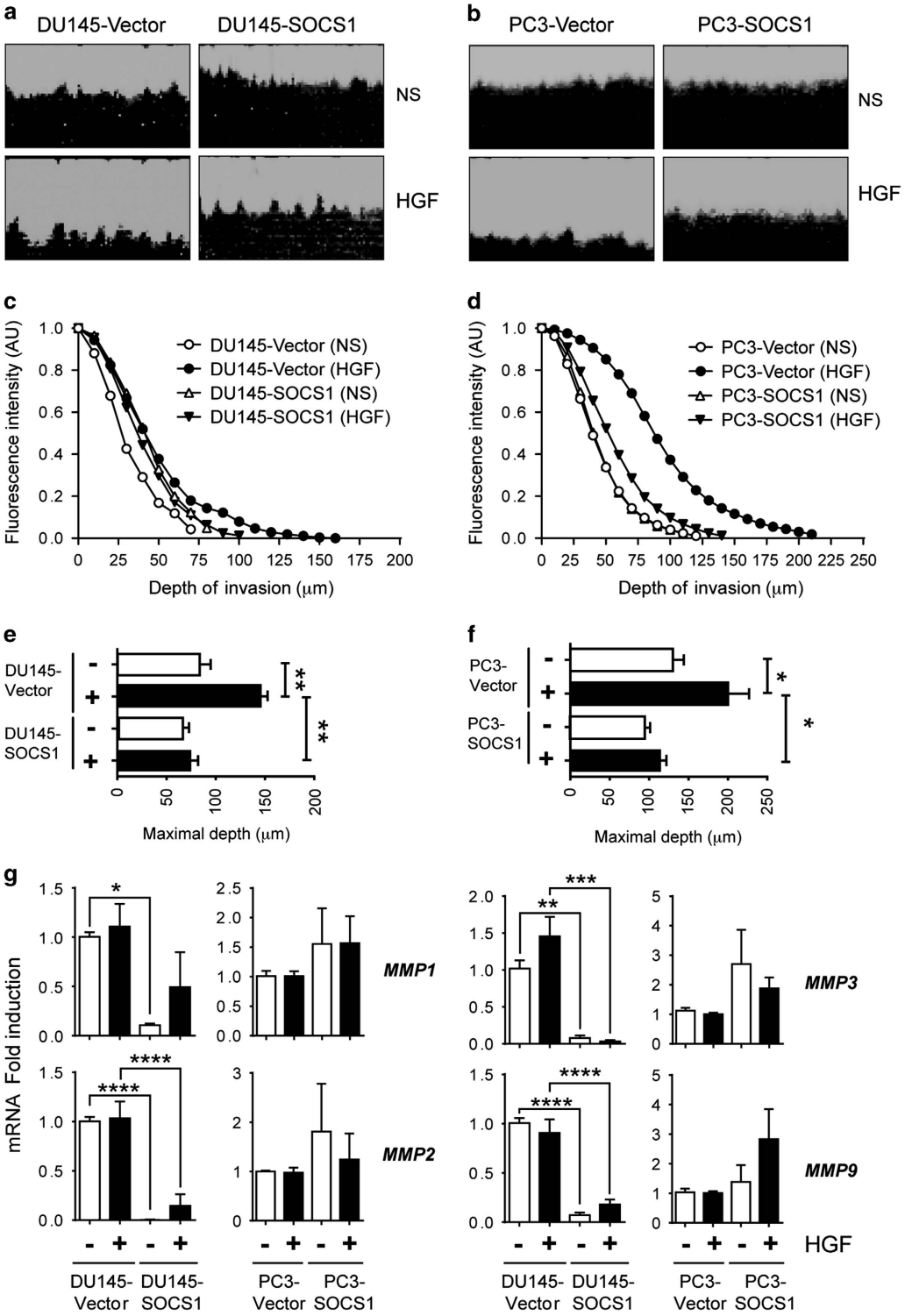


Figure 3. SOCS1 expression in PCa cells inhibits HGF-induced invasion of collagen matrix, but differentially affects the expression of genes involved in matrix degradation. (**a**–**f**) DU145 (**a**,**c** and **e**) and PC3 (**b**,**d** and **f**) cells expressing the control vector or SOCS1 were allowed to invade a collagen gel matrix layered over semi-solid agar in the presence or absence of HGF (20 ng ml^{-1}) in 96-well microtiter plates. After 24 hours, the cells were stained with calcein-AM and examined by confocal microscopy and the *z*-stage images were acquired at incremental 5 μM depth from the top. The images were reconstructed to measure the depth of invasion as assessed by green fluorescence. Cross-sectional images (**a** and **b**) and fluorescence intensity at different depths (**c** and **d**) from a representative experiment are shown. Quantification of the maximal depth of invasion (mean ± s.d.) from three independent experiments is shown in (**e** and **f**). Data were compared by Kruskal–Wallis test: *$P < 0.05$; **$P < 0.01$. (**g**) Control and SOCS1-expressing DU145 and PC3 cells were cultured to 80% confluence, stimulated with HGF (10 ng ml^{-1}) for 2 h and the expression of the indicated matrix metalloproteinase genes was assessed by real-time RT–PCR. Expression level of the indicated genes over the housekeeping gene *GAPDH* in non-stimulated vector control cells was used as reference to calculate the fold induction in HGF-stimulated and SOCS1-expressing cells. Mean ± s.d. values from three independent experiments are shown. Data (mean ± s.d.) were compared by one-way ANOVA with Tukey's multiple comparisons: *$P <$ 0.05, ***$P <$ 0.001, ****$P <$ 0.0001. MMP, matrix metalloproteinases; PCa, prostate cancer; RT–PCR, PCR with reverse transcription; SOCS1, suppressor of cytokine signaling 1.

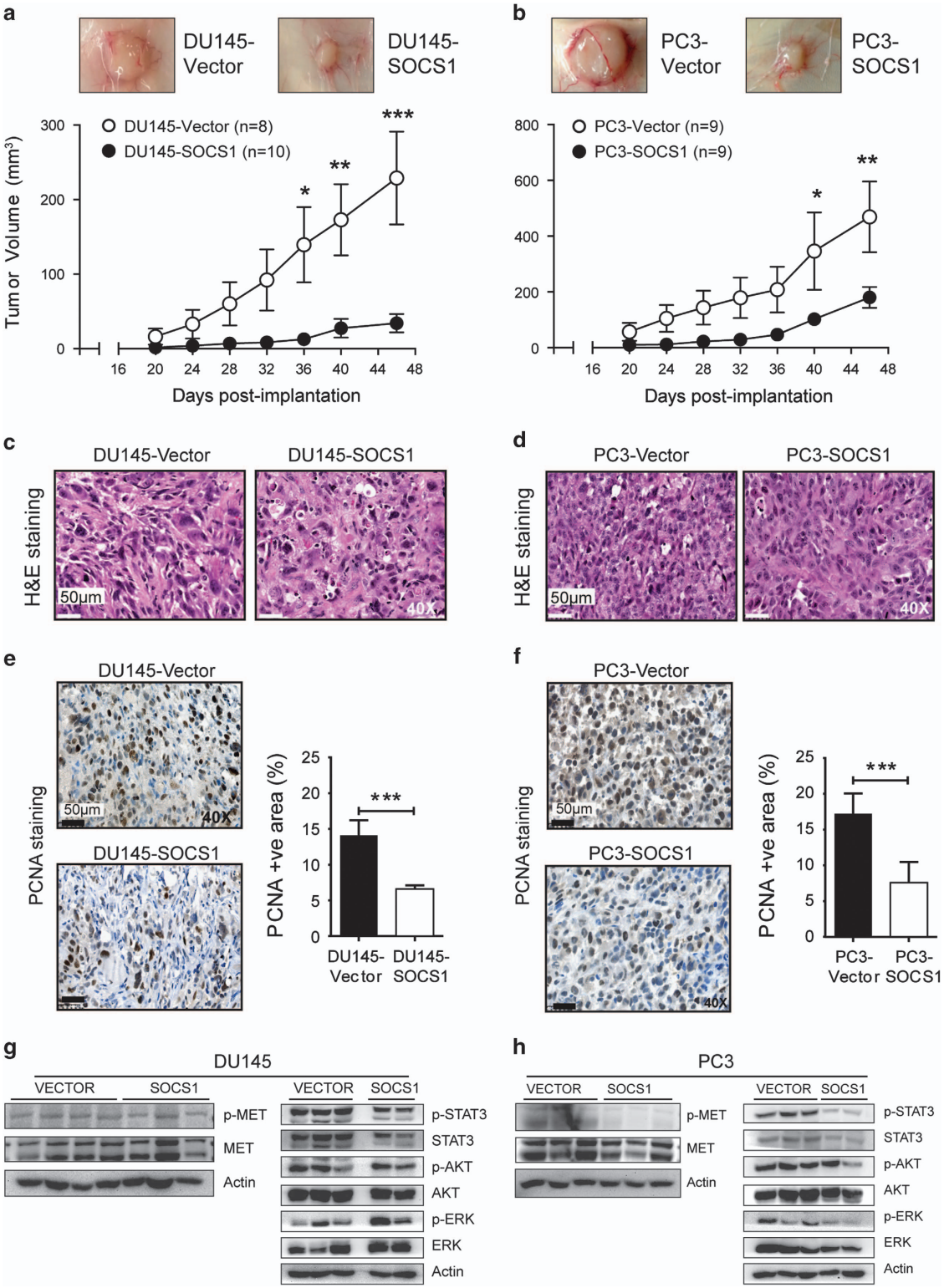


Figure 4. SOCS1 inhibits tumor growth of PCa cells in a xenograft model. (**a** and **b**) DU145 and PC3 cells expressing SOCS1or control vector were injected subcutaneously into the flanks (10^6 cells) of NSG mice, and tumor growth was evaluated at day 46 post-inoculation. Representative tumors and cumulative data on tumor volume (mean ± s.d.) from three independent experiments are shown. Data were compared by unpaired *t*-test: $^{***}P < 0.001$. (**c** and **d**) Hematoxylin and eosin-stained (H&E) sections of the above tumors. SOCS1-expressing tumors showed fewer cells than control tumors that are separated by amorphous extracellular material. (**e** and **f**) Immunohistochemical evaluation of PCNA in the tumor sections shown in (**a** and **b**). The PCNA-staining areas were quantified in at least 10 random fields of tumor sections from at least three mice per group. Data represent the mean ± s.d., which was compared by unpaired *t*-test: $^{***}P < 0.001$. (**g** and **h**) Western blot evaluation of phosphor-MET, MET and signaling proteins from representative tumors of the indicated cell types. NSG, NOD.*scid.gamma*; PCa, prostate cancer; SOCS1, suppressor of cytokine signaling 1.

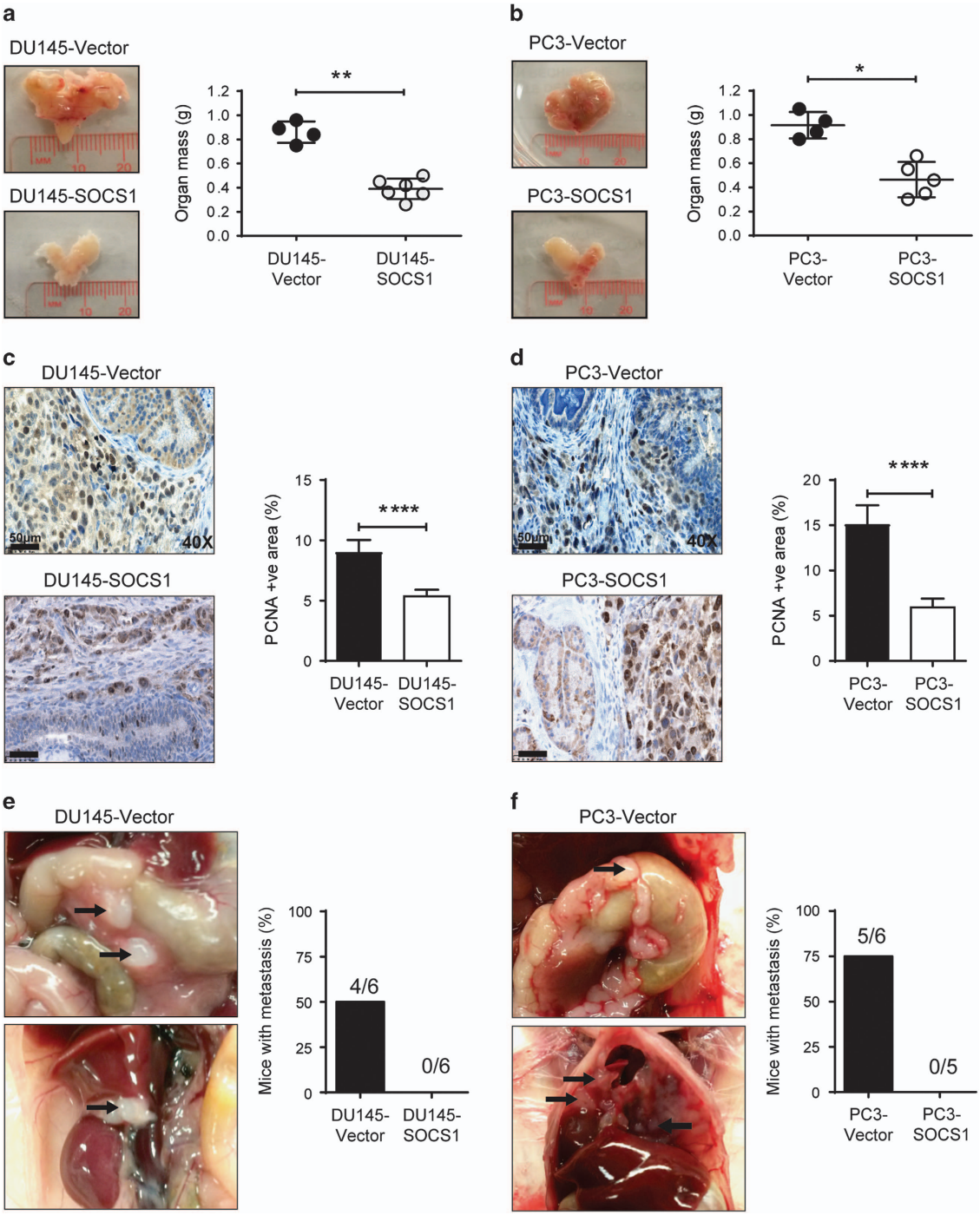


Figure 5. SOCS1 inhibits orthotopic tumor growth of human PCa cells and metastasis. (**a** and **b**) DU145 and PC3 PCa cells transduced with SOCS1 or an empty vector were injected into the prostate glands of NSG mice. After 6 weeks, prostate glands were resected and weighed. Representative images of the prostate glands and cumulative data from two experiments are shown. Data represent mean ± s.d. and were compared by Mann–Whitney test: $*P < 0.05$, $**P < 0.01$. (**c** and **d**) IHC staining of PCNA in formalin-fixed, paraffin-embedded sections of the orthotopic tumors formed by SOCS1-expressing and control DU145 and PC3 cells. Representative images and quantification of the PCNA-staining areas are shown. Data represent mean ± s.d. and were compared by unpaired *t*-test: $****P < 0.0001$. (**e** and **f**) The formation of distant metastasis in mice bearing orthotopic tumors of control and SOCS1-expressing DU145 and PC3 cells was assessed by macroscopic examination for metastatic nodules in peripheral organs at 6 weeks of post inoculation. Representative images and cumulative data from two separate experiments are shown. IHC, immunohistochemical; NSG, NOD.*scid.gamma*; PCa, prostate cancer; SOCS1, suppressor of cytokine signaling 1.

(MKSCC),[41] Fred Hutchinson Cancer Research Center (FHCRC)[42] and The Cancer Genome Atlas (TCGA)[43] studies. The expression of *SOCS1* and *MET*, and that of *KLK3* (coding for PSA) and *EGFR* (another member of the RPTK family) were not significantly altered across the patient groups with different Gleason scores in all three PCa data sets (Figure 7a). However, metastatic PCa showed significantly reduced expression of all four genes compared with localized or primary PCa in the MKSCC study but

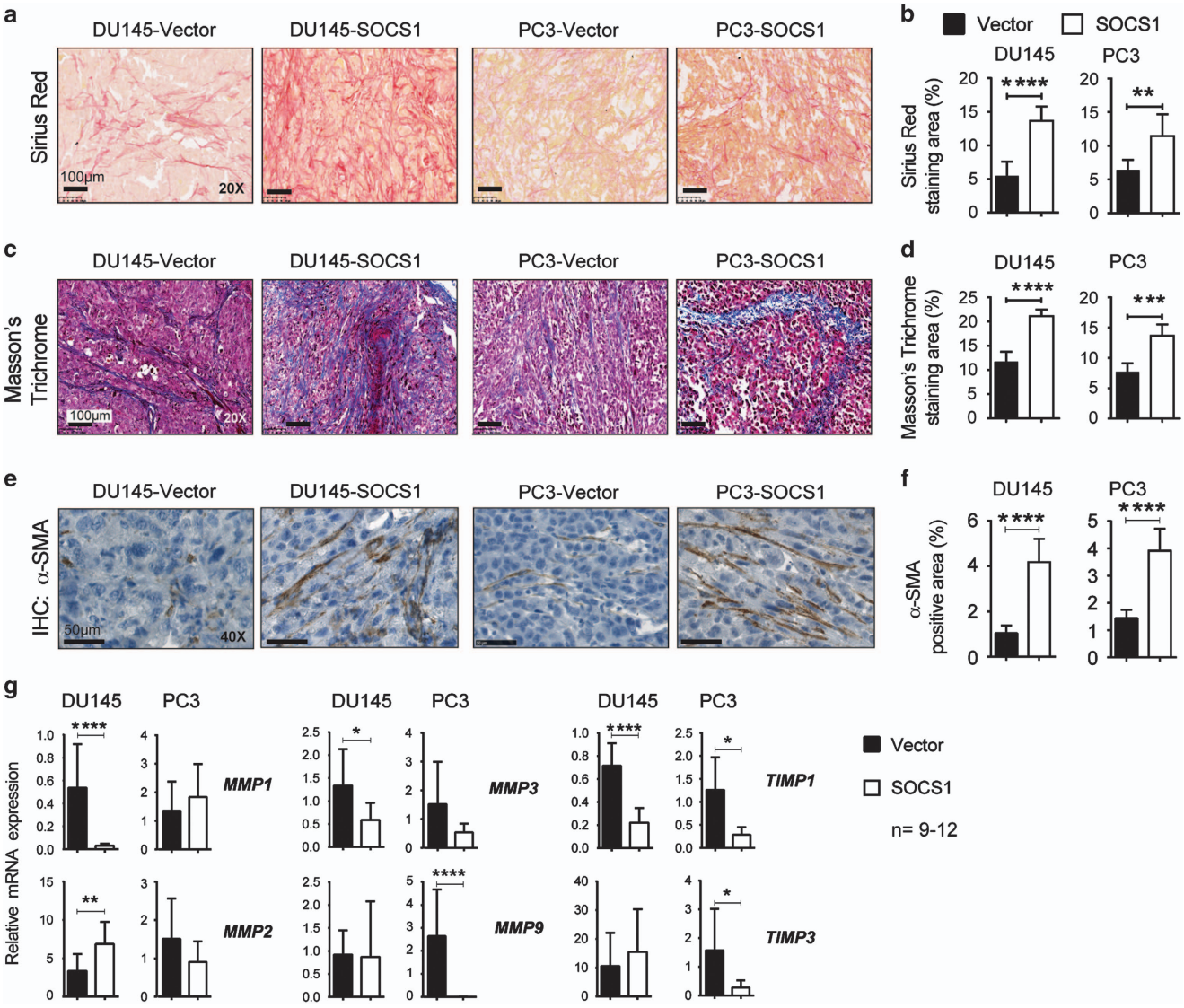


Figure 6. SOCS1 expression in PCa cells induces collagen deposition in xenograft models. (**a**–**d**) formalin-fixed, paraffin-embedded tumor sections of SOCS1-expressing and control tumors formed by DU145 and PC3 cells were stained by Sirius Red (**a** and **b**) or Masson's trichrome (**c** and **d**) to reveal type-I collagen fibers. Representative images (**a** and **c**) and quantification of collagen staining from at least 10 random fields of tumor sections from three mice per group (**b** and **d**) are shown. (**e** and **f**) Immunohistochemical (IHC) evaluation of α-smooth muscle actin (SMA) to reveal myofibroblasts. Representative images (**e**) and quantification of SMA-positive cells (**f**) are shown. Data represent the mean ± s.d. (**b**, **d** and **f**) and statistical significance was determined by unpaired *t*-test: $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (**g**) Expression of the indicated MMP and TIMP genes was assessed by real-time RT–PCR. Expression level of the indicated genes over the housekeeping gene *GAPDH* in each tumor sample is shown. Mean ± s.d. values from 9 to 12 tumors per group are shown. Statistical comparisons were performed using one-way ANOVA with Tukey's multiple comparisons test: $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$. ANOVA, analysis of variance; MMP, matrix metalloproteinases; PCa, prostate cancer; RT–PCR, PCR with reverse transcription; SOCS1, suppressor of cytokine signaling 1; TIMP, tissue inhibitors of MMPs.

not in the FHCRC data set (Figure 7b). *SOCS1* gene expression showed a negative correlation with that of KLK3, while the inverse relationship between SOCS1 and EGFR was significant only in the provisional TCGA data set (Figure 8). *SOCS1* and *MET* gene expression showed a significant positive correlation in the TCGA data set and a similar tendency in the FHCRC data set, whereas an inverse relationship was observed in the MKSCC data set (Figure 8).

DISCUSSION

In several human neoplasms including PCa, the *SOCS1* gene is repressed by CpG methylation and microRNA.[9–13,17–20] The tumor suppressor mechanism of SOCS1 in PCa is not yet well understood. The feedback inhibition of the JAK-STAT pathway by SOCS1 could contribute to tumor suppression in a cancer cell-intrinsic manner as well as by reducing inflammation within the tumor microenvironment. In PCa cells, SOCS1 has been shown to block IL-6-induced STAT3 activation and downmodulate cyclins and cyclin-dependent kinases and thereby inhibit cell growth.[7,8] Accruing evidence indicates that SOCS1 also regulates RTK signaling pathways.[44] SOCS1 inhibits MET signaling by promoting its ubiquitination and proteasomal degradation.[21,34] In this study, we have shown that SOCS1 attenuates MET signaling in PCa cells and inhibits HGF-induced cell proliferation, migration and invasion. We also show that SOCS1 attenuates tumor growth *in vivo*, accompanied by increased deposition of ECM.

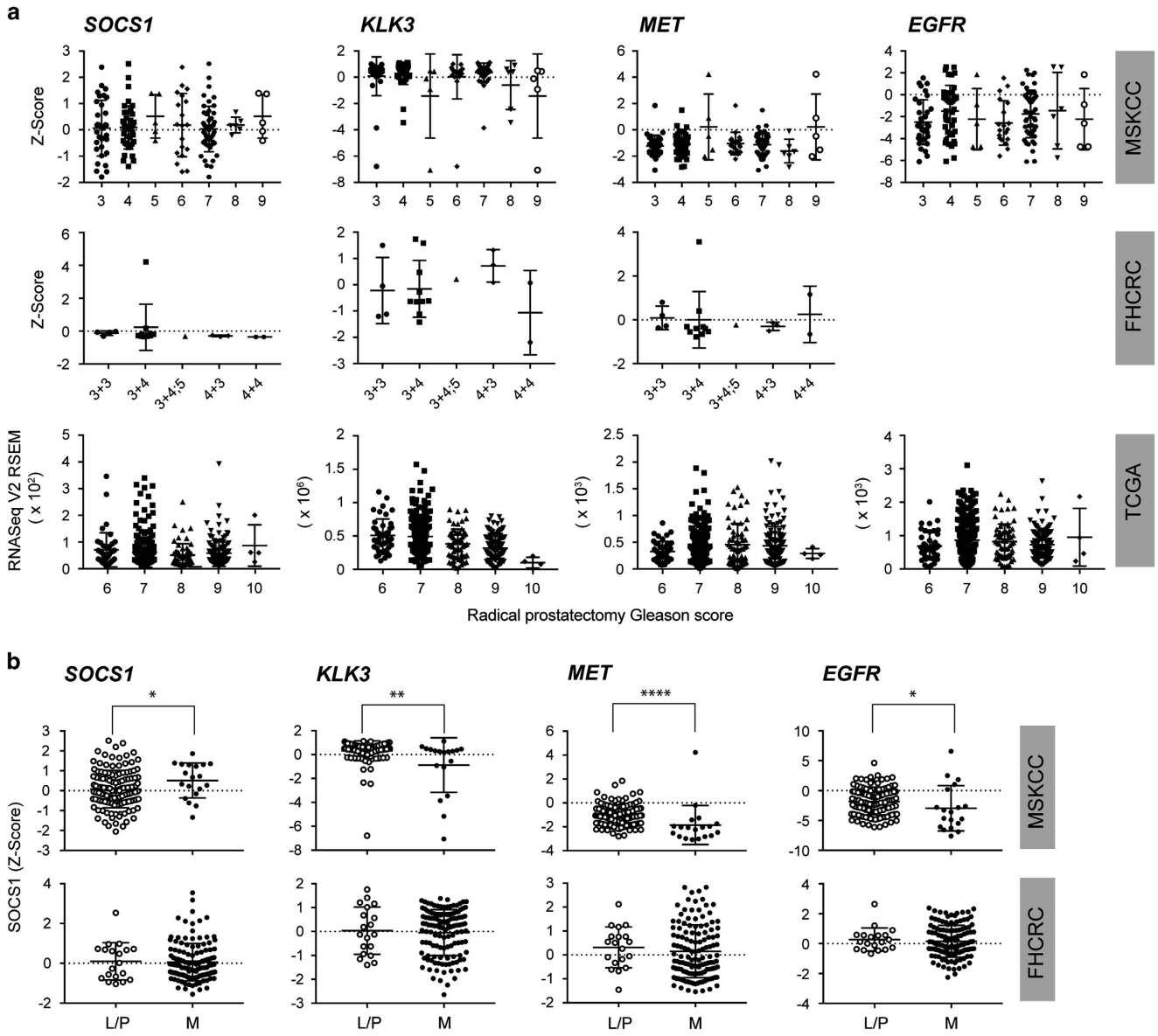


Figure 7. Expression of *SOCS1* and *MET* genes in human PCa specimens. The expression of *SOCS1*, *KLK3* (PSA), *MET* and *EGFR* genes was analyzed in **a**. PCa specimens grouped according to the Gleason score in MKSCC, FHCRC and TCGA data sets, and (**b**) in localized/primary (L/P) and metastatic (M) PCa in MKSCC and FHCRC data sets. Difference in gene expression between groups was evaluated by Mann-Whittney test: * < 0.05, ** < 0.01, **** < 0.0001. FHCRC, Fred Hutchinson Cancer Research Center; MKSCC, Memorial Sloan Kettering Cancer Center; PCa, prostate cancer; SOCS1, suppressor of cytokine signaling 1; TCGA, The Cancer Genome Atlas.

Suppression of SOCS1 in the normal prostate epithelial cell line RWPE-1 has been reported to increase their ability to invade Matrigel matrix, which contains ECM proteins and growth factors, whereas restoration of SOCS1 in PC3 and LNCaP attenuates Matrigel invasion.[20,45] Indeed, HGF-stimulated DU145 and PC3 cells displayed heightened invasion of the collagen matrix that was inhibited by SOCS1. As aberrantly high MET expression occurs in metastatic PCa, and the loss of SOCS1 expression correlates with biochemical recurrence,[20,27,28,35] our findings support the idea that SOCS1 is an important regulator of deregulated MET-mediated metastasis in PCa. Mechanistically, SOCS1 has been shown to reduce the expression of MMP2 and MMP9 in PCa cells, which has been correlated to its ability to inhibit Matrigel invasion.[20] In agreement with this report, SOCS1 attenuated the expression of *MMP1*, *MMP2*, *MMP3* and *MMP9* genes in DU145 cells.[20] However, HGF, which markedly enhanced the invasive potential in DU145 and PC3 cells, did not increase the expression of these *MMP* genes. It is possible that the *MMP* genes are highly expressed in metastatic cell lines, so that HGF stimulation does not further augment their expression. In addition, HGF-induced modulation of other cellular behavior such as increased cell motility could contribute to their highly invasive behavior. Consistent with reduced growth and invasive potential *in vitro*, SOCS1-expressing PCa cells showed decreased tumor growth and metastasis *in vivo*, which corroborated with the findings of Kobayashi *et al.*[20]

Intriguingly, SOCS1-mediated attenuation of PCa cell growth was accompanied by increased deposition of collagen and higher content of myofibroblasts. Although CAFs generally promote cancer growth and metastasis,[39] CAFs can develop plasticity and exert tumor-inhibitory effects in a highly context-dependent manner that is influenced by not only the CAF-derived factors but also the interacting malignant counterpart.[46] Our findings suggest that SOCS1 expression in PCa cells modulates the tumor stroma and attenuates the tumor-promoting potential of CAFs. Increase in collagen deposition and inhibition of tumor cell

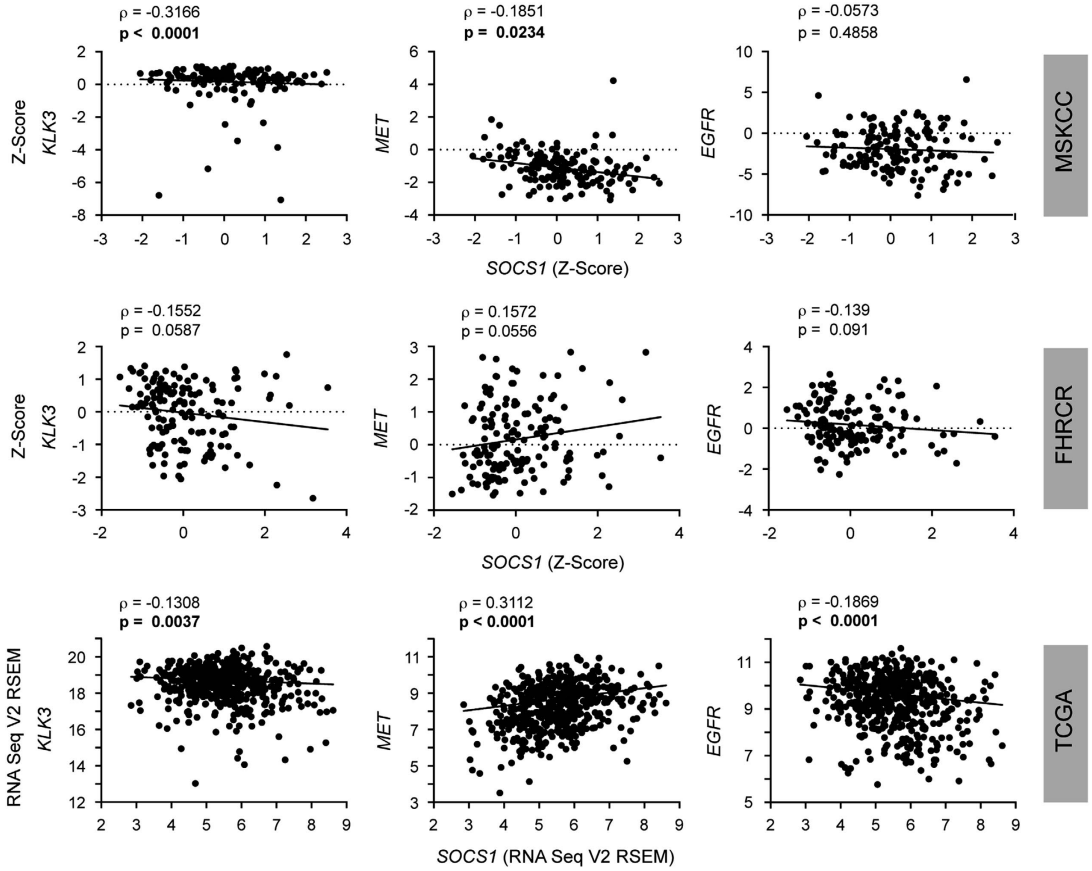


Figure 8. Correlation between *SOCS1* and *MET* gene expression in human PCa. The *SOCS1* gene expression was plotted against that of *KLK3*, *MET* or *EGFR* genes in MKSCC, FHCRC and TCGA data sets. The Spearman correlation (*ρ*) and *P*-values are given at the top of each plot. Statistically significant correlations are indicated in bold font. FHCRC, Fred Hutchinson Cancer Research Center; MKSCC, Memorial Sloan Kettering Cancer Center; PCa, prostate cancer; SOCS1, suppressor of cytokine signaling 1; TCGA, The Cancer Genome Atlas.

growth have been reported for certain other tumor suppressors.[47,48] Clearly further studies are needed to understand how SOCS1 expression in cancer cells modulates the tumor stroma and functions of CAFs in PCa.

While SOCS1 regulates oncogenic JAK-STAT signaling in hematopoietic cancers, regulation of RTK signaling, co-operation with p53 and control of the oncogenic potential of $p21^{CIP1/WAF1}$ are potential tumor suppressor mechanisms of SOCS1 in epithelial and other cancers.[16,34,49] Our findings indicate that attenuation of MET signaling and inhibiting the tumor-promoting potential of CAFs within the tumor microenvironment could be potential tumor suppression mechanisms of SOCS1 in PCa. Hence, evaluating SOCS1 expression in prostatectomy specimens could help to identify patients who would benefit from MET inhibitors. In this context, a recent phase III clinical trial of the MET/VGEFR inhibitor Cabozantinib on metastatic castration-resistant PCa failed to improve overall survival.[50] Nevertheless, this trial showed improvement of several other disease parameters such as progression-free survival, circulating tumor cell conversion and bone scan response, indicating that the drug did affect the tumor cells and the bone microenvironment.[50] Even though the failure of this phase III trial to achieve the primary objective can be attributed to many factors including the study design, this study highlighted the need for developing biomarkers to reliably identify patients, who would respond to MET inhibitor therapy.[50,51] We posit that SOCS1 could be one such potential biomarker. However, our findings suggest that the *SOCS1* gene expression may not truly reflect SOCS1 protein expression. First, there is no plausible explanation for the difference in *SOCS1* gene expression in metastatic PCa between the MSKCC and FHCRC data sets, and the discordant correlation between *SOCS1* and *MET* gene expression in MKSCC and TCGA data sets. Second, methylation analysis of specific regions of the *SOCS1* gene also may not be useful. For instance, even though both alleles of *SOCS1* are methylated in LNCaP cells for the region that we have examined, these cells have been reported to express SOCS1 protein.[8,52] As *SOCS1* gene can be methylated on multiple CpG islands in human cancers,[9] methylation of one or a few sites need not necessarily result in the loss of gene expression. Third, microRNA miR30d-mediated downmodulation SOCS1 protein expression appears to be more predominant in PCa than epigenetic repression of the *SOCS1* gene by CpG methylation.[20] Fourth, SOCS1 affects MET at the protein level by promoting its ubiquitination and proteasomal degradation.[34] Hence, developing tools such as clinical grade antibodies and immunohistochemical techniques to screen SOCS1 and MET protein expression in prostatectomy specimens would be a promising approach to identify PCa patients who would benefit from MET inhibitor therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Robert Day for providing PCa cell lines. AVH and RK are supported by Master's degree scholarship from the Faculty of Medicine, Université de Sherbrooke. DB is a recipient of a postdoctoral fellowship from FRQS. CRCHUS is an FRQS-funded

research center. This work was supported by Movember Discovery Grant from Prostate Cancer Canada (Grant number D2013-20).

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Supplementary Information accompanies the paper on the Prostate Cancer and Prostatic Diseases website (http://www.nature.com/pcan)