JAK/STAT3 signaling is required for TGF-β-induced epithelial-mesenchymal transition in lung cancer cells

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Received December 17, 2013; Accepted January 29, 2014

DOI: 10.3892/ijo.2014.2310

Abstract. Epithelial-mesenchymal transition (EMT), a key step in the early stages of cancer metastasis, is orchestrated by several signaling pathways, including IL-6/JAK/STAT3 and TGF-β/Smad signaling. However, an association between the two signaling pathways during the EMT process is largely unknown. Here, we focused on lung cancer and demonstrated that TGF-β1 induced the phosphorylation of Smad3 (p-Smad3), upregulation of Snail, a fibroblast-like morphology, and downregulation of E-cadherin as well as upregulation of vimentin in lung cancer cell lines. SIS3 (an inhibitor of Smad3) suppressed TGF-β1-induced activation of Smad3, upregulation of Snail and the EMT process. Importantly, the JAK2/STAT3-specific inhibitor AG490 blocked Stat3 phosphorylation, resulting in attenuated levels of TGF-β1-induced p-Smad3, Snail, MMP2, and Smad-mediated PAI-1 promoter reporter gene activity in A549 and H1650 cells. Subsequently, AG490 inhibited TGF-β-induced cell migration and invasion. Moreover, exogenous IL-6 treatment stimulated Stat3 activation, enhanced TGF-β-induced expression of p-Smad3 and Snail, aggravated the EMT process, and increased lung cancer cell migration and invasion induced by TGF-β1. Our findings show that the JAK/STAT3 pathway is required for TGF-β-induced EMT and cancer cell migration and invasion via upregulation of the expression of p-Smad3 and Snail, and the IL-6/JAK/STAT3 and TGF-β/Smad signaling synergistically enhance EMT in lung carcinomas. The present study suggests a novel rationale for inhibiting cancer metastasis using anti-IL-6/JAK/STAT3 and anti-TGF-β/Smad therapeutic strategies.

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Key words: TGF-β, IL-6, Stat3, Smad3, epithelial-mesenchymal transition

Introduction

Lung cancer is the leading cause of cancer death worldwide. In fact, over 90% of deaths from solid tumors, including lung cancer, is mainly due to cancer metastasis (1). Metastasis of tumor cells is associated with epithelial-mesenchymal transition (EMT), which is a process whereby epithelial cells acquire new features of mesenchyme (2).

EMT is vital for the conversion of early-stage tumors into invasive malignancies (3). Alterations in morphology, cellular architecture, adhesion, and migration capacity are required for EMT (4). As one of the hallmarks of EMT, the functional loss of E-cadherin is currently thought to promote invasion during carcinoma progression (5). The E-cadherin repressors, in particular, Snail can be induced by a number of distinct signaling pathways, such as TGF-β, Wnt and Notch (6-8).

TGF-β signaling is a primary inducer of EMT in various cancers, including lung cancer (9-11). TGF-β binding to its receptors leads to phosphorylation of Smad2 and Smad3, which partner with Smad4 and then translocate into the nucleus where Smad transcriptional complexes control the expression of target genes, including Snail (8,12). Importantly, Smad3 and Smad4 rather than Smad2 played an essential role in TGF-β-induced Snail expression and EMT (9,13-15).

Interleukin-6 (IL-6)-mediated aberrant activation of Janus kinases/signal transducer and activator of transcription 3 (JAK/STAT3) signaling is frequently presented in human cancer including lung cancer and implicated in transformation, tumorigenicity, EMT and metastasis (16-20). Additionally, inhibition of JAK2 tyrosine kinase or blockade of activated Stat3 significantly suppressed the EMT process, cancer cell migration and invasion *in vitro*, and attenuated cancer cell metastasis *in vivo* (21-23). Although no association of JAK/STAT3 signaling with EMT of lung cancer is so far well established, elevated level of serum IL-6 has been observed in lung cancer patients when compared with normal donors and correlates with advanced lung cancer stage and an overall poor prognosis (24,25), suggesting that the IL-6/JAK/STAT3

pathway may participate in the progression of lung cancer via EMT.

Recent studies have demonstrated that TGF-β-mediated cancer metastasis initiation was associated with the activation of JAK/STAT3 pathway in colorectal cancer (26). Taken together, we hypothesized that IL-6/JAK/STAT3 signaling is required for TGF-β-mediated EMT in lung cancer.

Materials and methods

Cell culture. Human lung cell lines A549 and H1650 were purchased from Cell Bank of Chinese Academy of Science. Cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Hyclone) with 50 U/ml each of penicillin and streptomycin and 10% heat-inactivated fetal bovine serum (FBS, Invitrogen) in humidified incubators at the condition of 37˚C and 5% CO_2.

Reagents and antibodies. Human recombinant TGF-β1 and IL-6 were obtained from R&D Systems Inc. (Minneapolis, MN, USA). TGF-β1 was diluted in sterile 4 mM HCl containing 1 mg/ml human serum albumin (HSA); IL-6 was reconstituted in sterile PBS containing 0.1% HSA. JAK2 inhibitor AG490 and Smad3 phosphorylation inhibitor SIS3 were purchased from Merck KGaA (Darmstadt, Germany) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Antibodies used for western blotting were mouse anti-E-cadherin (1:3,000, BD Biosciences), mouse anti-vimentin (1:4,000, BD Biosciences), rabbit anti-Snail (1:1,000, Cell Signaling), rabbit anti-Smad3 (1:1,000, Cell Signaling), rabbit anti-phospho-Smad3 (Ser423/425, 1:1,000, Cell Signaling), rabbit anti-Stat3 (1:1,000, Cell Signaling), rabbit anti-phospho-Stat3 (Tyr705, 1:1,000, Cell Signaling), mouse anti-GAPDH (1:3,000, Abcam).

Western blot analysis. Western blot analysis was performed according to the protocol described by us (27) with some modifications. Briefly, cells were lysed in a RIPA buffer with protease inhibitor cocktail (Sigma-Aldrich) and phosphatase inhibitor cocktail (Sigma-Aldrich), and then clarified by centrifugation. Total cell lysates were resuspended in SDS sample buffer and resolved by SDS-PAGE. Proteins were transferred to nitrocellulose membrane (Millipore, Bedford, MA, USA) and then blocked with 5% BSA/TBST buffer for 1 h at room temperature. Membranes were incubated with primary antibodies overnight before incubation with the corresponding HRP-conjugated secondary antibodies and detected using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific Inc.). All experiments were performed in triplicate.

RNA extraction and quantitative real-time PCR (qRT-PCR). Cells were grown to 80% confluence on 6-well plates and subjected to RNA extraction using 1.0 ml RNAiso Plus (Takara) according to the manufacturer's instructions. Synthesis of cDNA with reverse transcriptase was performed by M-MLV First Strand kit (Invitrogen). cDNA aliquots were subjected to qRT-PCR reactions using the Platinum®SYBR®Green qPCRsupermix-UDG with ROX (Invitrogen) on ABI PRISM 7500 Sequence Detection System (Applied Biosystems). Primers used for qRT-PCR were as follows: 5'-CGAAAGGCCTTCAACTGCAAAT-3' (forward) and 5'-ACTGGTACTTCTTGACATCTG-3' (reverse) for Snail; and 5'-TGCACCACCAACTGCTTAGC-3' (forward) and 5'-GGCATGGACTGTGGTCATGAG-3' (reverse) for GAPDH. The PCR program was 50˚C for 2 min, 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. A standard melting-curve analysis was carried out at the end of the amplification. Each qRT-PCR experiment was done in triplicates. All the mRNA expression values were normalized to an internal control GAPDH.

Luciferase reporter gene construct. The promoter region of PAI-1, -960 to +124, was amplified by PCR with primers (forward: CGGGGTACCGCACACCCTGCAAACCTGCC and reverse: CCGCTCGAGCGATTGGCGGTTCGTCCTG, containing a *Kpn*I site and a *Xho*I site, respectively). After digestion with *Kpn*I and *Xho*I, the resultant fragments were directly ligated into the pGL3-basic luciferase vector (Promega). Before transfection, the sequence of plasmid construct was confirmed by direct sequencing.

Transient transfection and reporter gene assays. Cells were co-transfected with 800 ng pgl3-basic constructs with PAI-1 promoter and 16 ng SV-40 plasmid (as a normalizing control) using Lipofectamine 2000 (Invitrogen). Four hours later, the cells were treated with or without 50 μM AG490 and then incubated for 18 h in the presence or absence of 5 ng/ml TGF-β1. Finally, luciferase activity of the transfected cells was determined using the Dual-Luciferase Reporter Assay System (Promega) on a TD20/20 Luminometer (Turner Designs, Sunnyvale, CA, USA). Three independent transfection experiments were carried out, and each was done in triplicate. Results are reported as relative luciferase activities, which are obtained by dividing firefly luciferase activity with SV-40 luciferase activity.

Cell invasion and migration assays. For invasion assay, BioCoat Matrigel invasion chambers (BD Biosciences) with 8-μm pore polycarbonate membranes were pre-treated with serum-free RPMI-1640 (Gibco-Invitrogen) medium at 37˚C for two hours. After removing the medium, we added 750-μl medium with 10% FBS as chemoattractant to each lower chamber, and then added $5x10^4$ cells with 1% FBS medium to each upper chamber. Two hours later, the regents (5 ng/ml TGF-β1, 50 μM AG490 or 50 ng/ml IL-6) were added to the upper chambers, and further incubated at 37˚C for 12 h. The inserts were removed and non-invasive cells on the upper surface were removed by a cotton swab. The invasive cells on the lower surface of the membrane were then fixed in 100% methanol for 15 min, air-dried, and stained with 1% crystal violet. Cells from three microscopic fields were photographed and counted. For migration assay, similar procedure to the invasion assay was conducted but omitting addition of Matrigel. Each experiment was done in triplicate.

Statistical analysis. Data are presented as the mean ± standard deviations (SD) and Student's t-test was performed to determine statistically significant differences between two groups. Statistical differences were considered to be significant at P<0.05. All the statistical analysis was performed

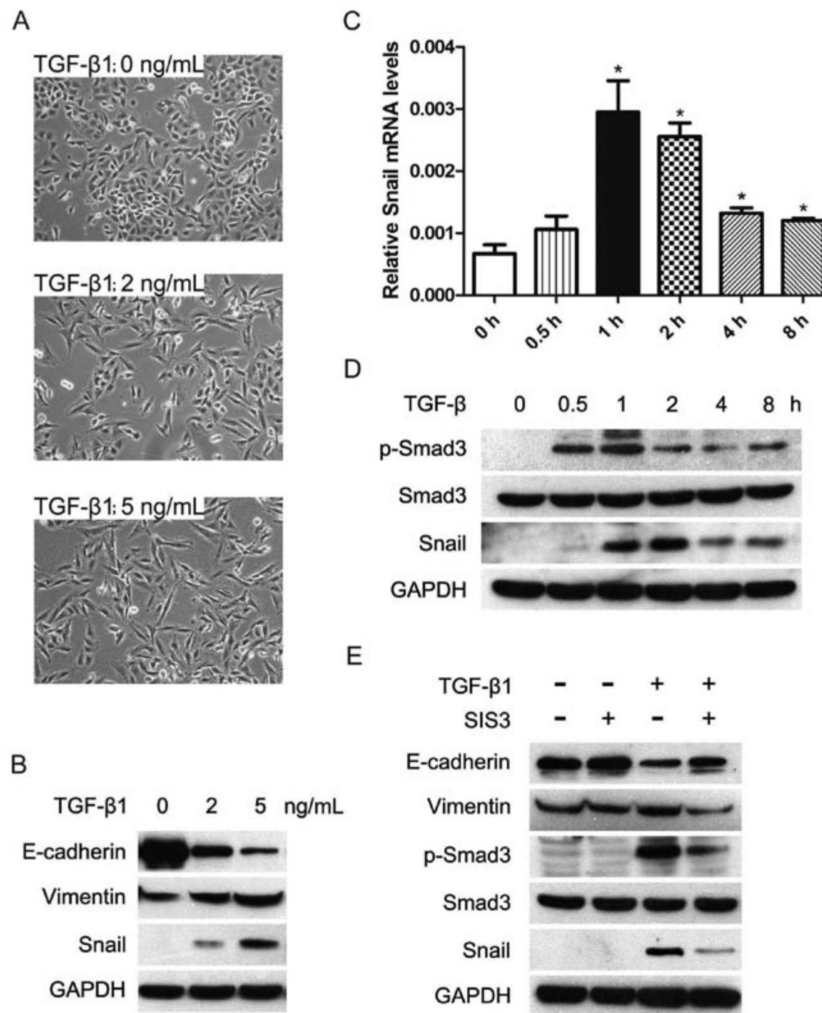

Figure 1. TGF-β-induced EMT is dependent on p-Smad3 and Snail in human lung cancer cells A549. Cells were serum-starved for 24 h before treatments. (A) TGF-β1 exposure induced transition of the epithelial to the mesenchymal-like phenotype in A549 cells. Cells were treated with 1% FBS without TGF-β1 (upper), 1% FBS with 2 ng/ml TGF-β1 (middle), and 1% FBS with 5 ng/ml TGF-β1 (lower). Cell morphology was examined at 24 h after TGF-β1 treatment and photographed using a phase-contrast microscope. (B) TGF-β1 altered expression of EMT molecular markers. Cells were treated with or without TGF-β1 for 24 h; total cell lysates were extracted and subjected to western blot analyses for expression of E-cadherin, vimentin and Snail. GAPDH was used as an internal control. (C) TGF-β1 upregulated mRNA expression of Snail. Cells were exposed to 5 ng/ml TGF-β1 for the indicated times, and mRNA level of Snail was determined by quantitative RT-PCR. (D) TGF-β1 phosphorylated Smad3 and upregulated protein level of Snail expression. Cells were treated with or without 5 ng/ml TGF-β1 for the indicated times, and p-Smad3, Smad3 and Snail protein expressions were detected by western blotting. (E) SIS3 inhibited TGF-β-induced phosphorylation of Smad3 and restored expression of EMT molecular markers in the presence of TGF-β. Cells were pretreated with 3 μM SIS3 (Smad3 phosphorylation inhibitor) for 4 h, and then exposed to 5 ng/ml TGF-β1 for 24 h, the levels of E-cadherin, vimentin, p-Smad3, Smad3 and Snail were determined by western blotting.

using GraphPad Prism 5.01 software (GraphPad Software, Inc.) and STATA 10.1 software (Stata Corp, College Station).

Results

Snail is involved in TGF-β-induced EMT. The A549 cell line has been widely used as a model system to study the mechanisms of carcinogenesis and tumor progression in lung cancer. First, we observed the morphological changes in A549 cells in the presence of TGF-β1. In the absence of TGF-β1, A549 cells maintained a classic epithelial morphology; however, after treatment with TGF-β1 for 24 h, A549 cells displayed a spindle-shape, fibroblast-like morphology (Fig. 1A). Next, we used western blot assay to investigate the expression of epithelial marker, E-cadherin, and the mesenchymal marker vimentin. The results showed that the expression of E-cadherin was significantly decreased and the expression of vimentin was increased with TGF-β1 treatment for 24 h in A549 cells (Fig. 1B).

Snail plays a vital role in regulating EMT during tumor progression (7,8). Therefore, we investigated whether expression of Snail was involved in TGF-β-induced EMT in A549 cells. As a result, TGF-β1 treatment increased Snail mRNA expression, which showed the highest level at 1 h (Fig. 1C). The inducing effects of TGF-β on Snail were also seen at protein level in A549 cells (Fig. 1B and D). Taken together, these data indicated that TGF-β was able to upregulate the expression of Snail and subsequently induce EMT in lung cancer cells.

Phosphorylated Smad3 is required for TGF-β-induced EMT. Although Smad3 is the key to TGF-β-induced EMT (13,15), the

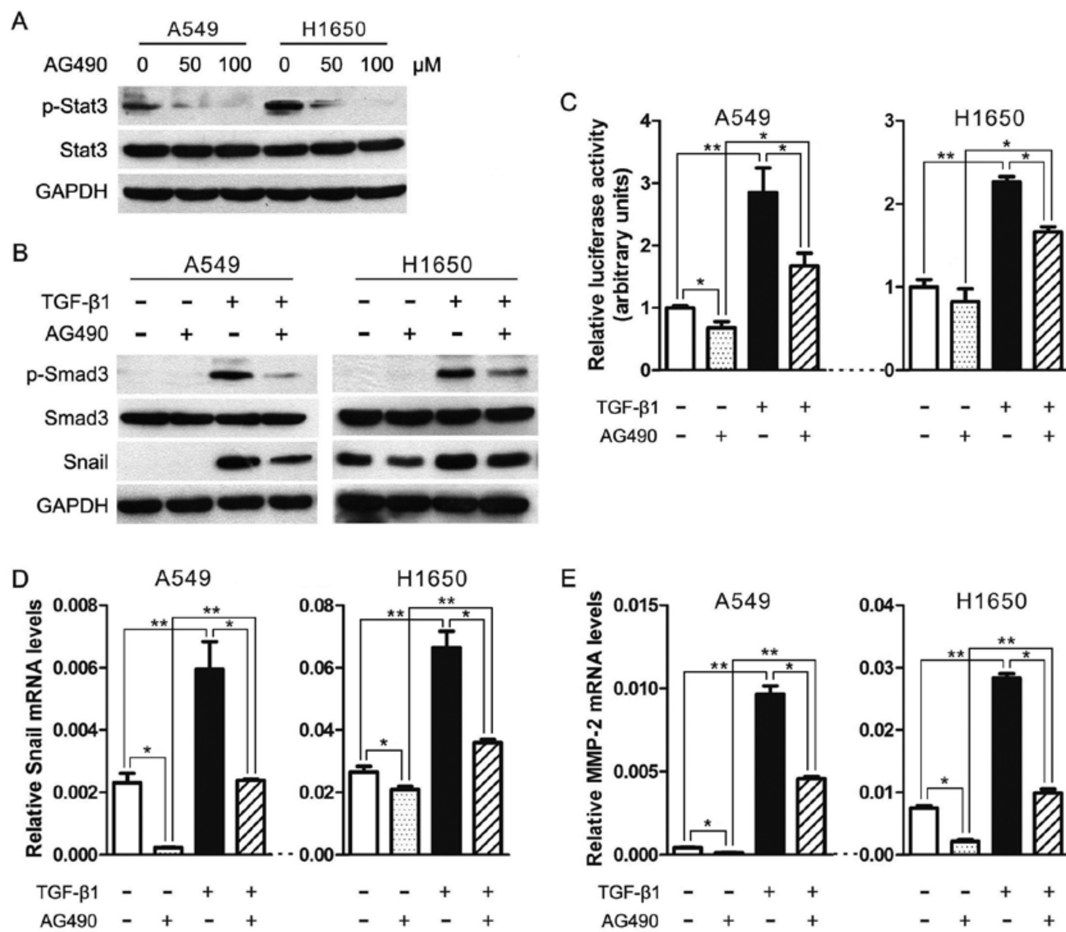

Figure 2. AG490 inhibits Smad3 activation and transcriptional responses in lung cancer A549 and H1650 cells. (A) AG490 inhibited phosphorylation of Stat3. After serum starvation overnight, cells were subjected to AG490 treatment (JAK2/STAT3 inhibitor, 50 or 100 μM) for 4 h, and the expression of p-Stat3, total Stat3 was analyzed using western blotting. (B) AG490 suppressed TGF-β-induced Smad3 activation and Snail upregulation. Cells were pretreated with 50 μM AG490 for 4 h and then treated with 5 ng/ml TGF-β1 for 4 h as indicated. Levels of p-Samd3, Smad3 and Snail were monitored by western blotting. (C) AG490 attenuated TGF-β-induced PAI-1 promoter activation. After transient transfection with PAI-1 promoter construct, cells were treated with 50 μM AG490 for 4 h and then incubated for 18 h in the absence or presence of 5 ng/ml TGF-β1. Relative luciferase activity was expressed as the mean fold change from basal level ± SD of three independent experiments. (D and E) AG490 diminished TGF-β-induced increase in Snail and MMP2. Cells were treated with 50 μM AG490 for 4 h and then exposed to 5 ng/ml TGF-β1, and the mRNA levels of Snail (treated with TGF-β1 for 1 h) and MMP2 (24 h) were determined by quantitative RT-PCR. *P<0.05; **P<0.01.

role of phosphorylated Smad3 (p-Smad3) in TGF-β-induced EMT has not been highlighted. To address this, we inhibited the activation of Smad3 by a specific inhibitor SIS3 (28) and found that SIS3 could remarkably decrease TGF-β-induced phosphorylation of Smad3 (Fig. 1E). SIS3 significantly restored E-cadherin expression and impaired vimentin and Snail expression in the presence of TGF-β1 (Fig. 1E). These results suggested that TGF-β-induced EMT depends on p-Smad3 and Snail in lung cancer cells.

JAK/STAT3 signaling is required for TGF-β-mediated transcriptional responses and EMT. Based on the notion that TGF-β-induced metastasis initiation requires the participation of JAK/STAT3 signaling pathway in colorectal cancer (26), we hypothesize that the JAK/STAT3 signaling is necessary for TGF-β-mediated EMT. To test this hypothesis, we first used a JAK2-specific inhibitor AG490 to expectably suppress the JAK/STAT3 signaling activity and found that AG490 can significantly depress the phosphorylation of Stat3 (p-Stat3) in lung cancer cell lines A549 and H1650 (Fig. 2A). Secondly and importantly, AG490 markedly reduced Smad3 phosphorylation induced by TGF-β1 (Fig. 2B). Thirdly, we performed luciferase reporter assay to assess whether JAK/STAT3 pathway could regulate TGF-β-induced Smad transcriptional activity in A549 and H1650 cells. Cells transfected with Smad-mediated PAI-1 reporter plasmid were stimulated with TGF-β in the presence and absence of AG490. As illustrated in Fig. 2C, TGF-β1 can significantly increase the luciferase reporter activity, and AG490 treatment significantly attenuated both the basal and TGF-β-induced PAI-1 promoter activation. Fourthly, AG490 can diminish TGF-β-induced increase in Snail and MMP2 (Fig. 2B, D and E), which have been widely recognized to promote cancer metastasis. These data demonstrate that JAK/STAT3 signaling is required for TGF-β-induced phosphorylation of Smad3, Smad-mediated transcriptional responses and EMT in lung cancer.

AG490 blocks TGF-β-induced cell migration and invasion. Given the facts that TGF-β signaling can stimulate the migra-

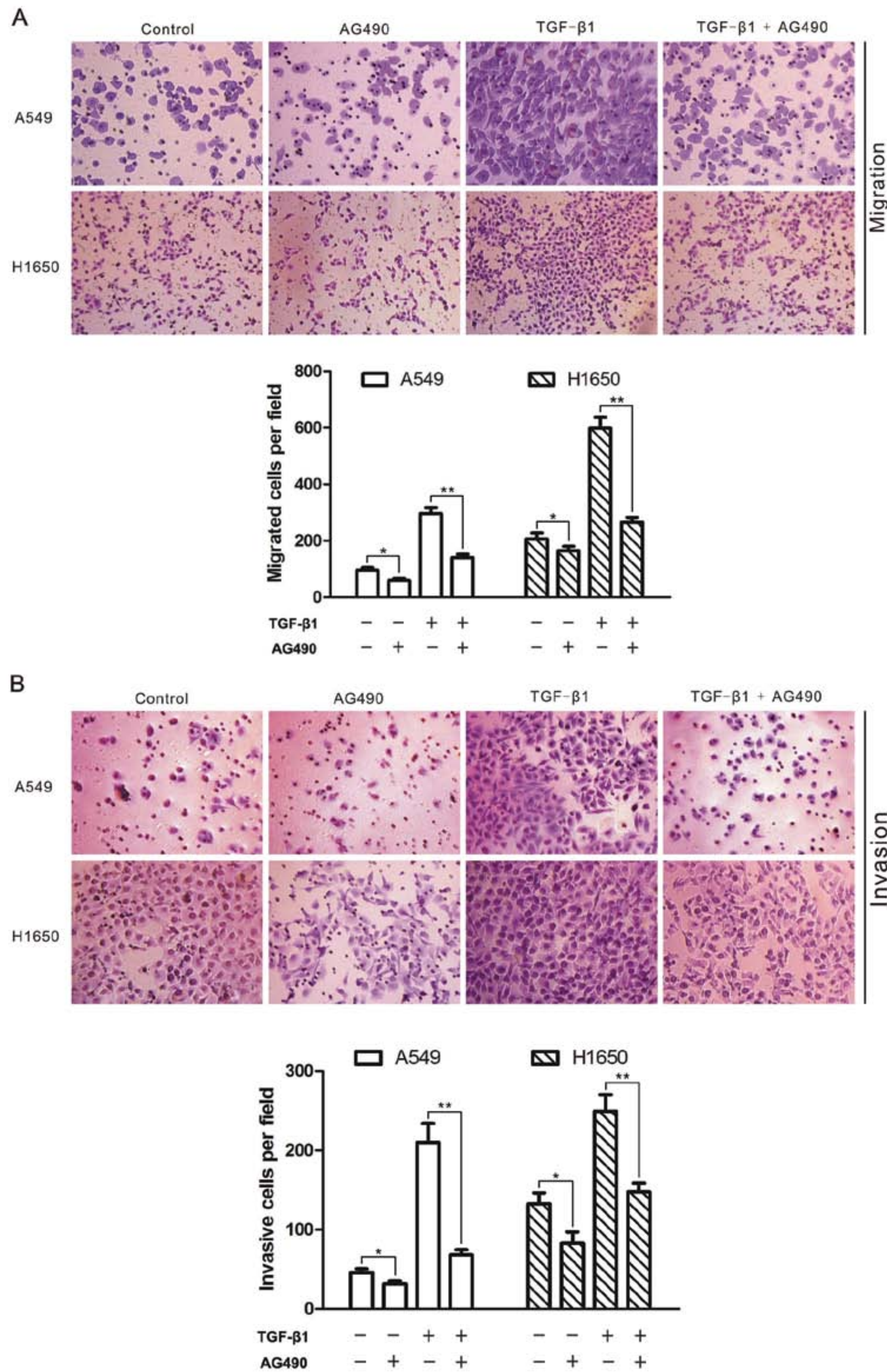

Figure 3. AG490 abrogates TGF-β-induced migration and invasion in A549 and H1650 cells. (A) AG490 inhibited TGF-β-induced cell migration. Cells were treated with 50 μM AG490 for 4 h followed by 5 ng/ml TGF-β1 for 12 h, and allowed to migrate through an 8-μM pore in transwells. Migrated cells through the pores were stained with 1% crystal violet and counted under a light microscope (magnification, x200). (B) AG490 suppressed TGF-β-induced cell invasion. Cells were treated as above and allowed to pass through Matrigel-coated membrane in transwells. Invaded cells through the filter were stained and counted. The data summarized in the bar charts are presented as mean ± SD of three independent fields. *P<0.05; **P<0.01.

tion and invasion of tumor cells (29), and the JAK/STAT3 signaling is implicated in the regulation of cell migration and invasion (30), we adopted transwell assays to examine whether JAK/STAT3 signaling is involved in TGF-β-induced migration and invasion in A549 and H1650 cells. As a result, TGF-β1 enhanced the migratory ability of the two cell lines, and AG490 blocked both basal and TGF-β-stimulated cell migration (Fig. 3A). Moreover, AG490 also blocked tumor cell

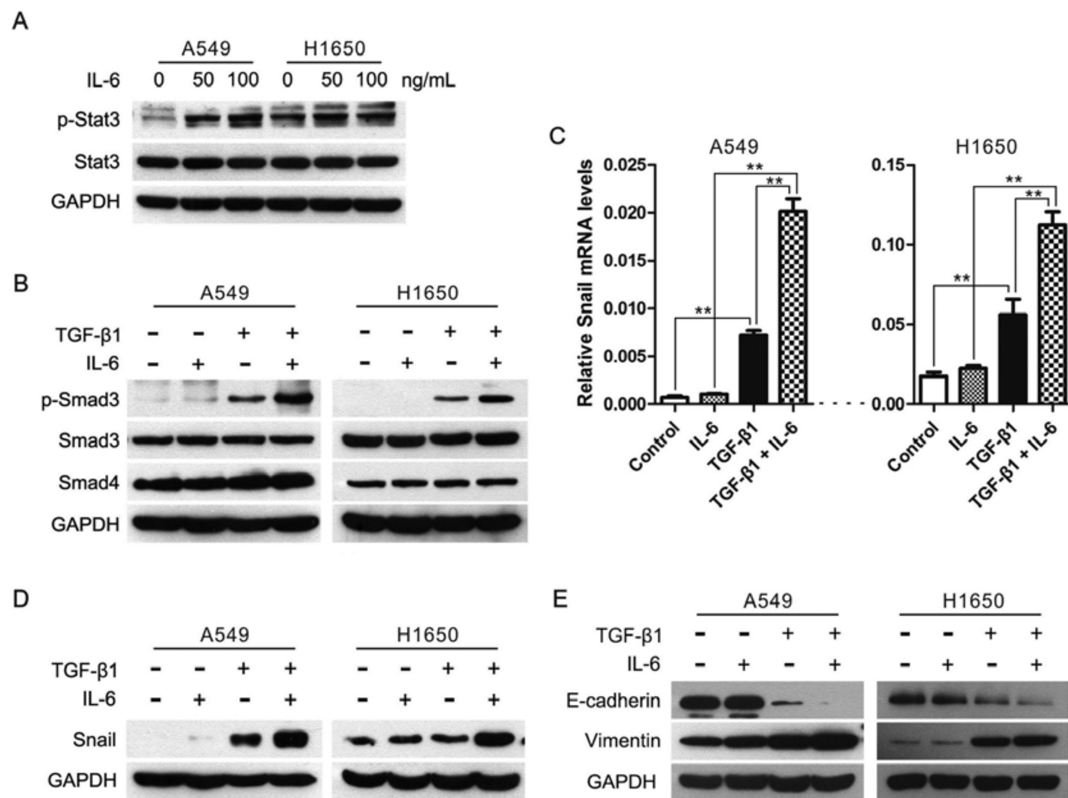

Figure 4. IL-6 enhances TGF-β/Smad pathway and TGF-β-induced EMT in A549 and H1650 cells. (A) IL-6 enhanced phosphorylation of Stat3. Cells were subjected to IL-6 treatment (50 or 100 ng/ml) for 1 h, and p-Stat3 and Stat3 expressions were analyzed by western blotting. (B) IL-6 enhanced TGF-β-induced phosphorylation of Smad3. Cells were incubated for 1 h with 5 ng/ml TGF-β1 and/or 50 ng/ml IL-6 as indicated, the expression levels of protein for p-Smad3, Smad3 and Smad4 were assessed by western blotting. (C and D) IL-6 increased TGF-β-induced Snail expression. Cells were treated as above, and the mRNA and protein levels of Snail were detected by qRT-PCR and western blotting. (E) IL-6 promoted TGF-β-induced EMT. Western blotting was used to evaluate expression levels of E-cadherin and vimentin in cells treated with 5 ng/ml TGF-β1 or 50 ng/ml IL-6 for 24 h in A549 cells or 48 h in H1650 cells. **P<0.01.

invasion induced by TGF-β1 (Fig. 3B). These results suggested that the basal JAK/STAT3 signaling was required for TGF-β-induced migration and invasion of lung cancer cells.

IL-6 enhances TGF-β-induced EMT in lung cancer cells. According to our findings described above, we speculated that enlargement of the JAK/STAT3 signaling might enhance the TGF-β/Smad signaling and TGF-β-induced EMT. Therefore, we used human recombinant IL-6 to enhance the JAK/STAT3 signaling pathway (Fig. 4A), and found that IL-6 stimulation increased phosphorylation levels of Smad3 in cells treated with TGF-β1, albeit total Smad3 and Smad4 levels did not alter in cells treated with IL-6 and/or TGF-β1 (Fig. 4B). Next, we examined the effect of IL-6 and/or TGF-β1 treatment on the expression of Snail, the important EMT inducer, in A549 and H1650 cells. After serum starvation for 24 h, Snail mRNA levels were rapidly induced upon TGF-β1 treatment in A549 and H1650 cells, but not in IL-6 treated cells (Fig. 4C). More importantly, Snail expression was higher in cells treated with TGF-β1 and IL-6 than TGF-β1 alone (Fig. 4C and D).

Subsequently, we evaluated the synergistic effect of IL-6 and TGF-β on the induction of EMT in lung cancer cells and found that the expression of E-cadherin was almost abrogated in cells treated with TGF-β1 and IL-6, and the expression of vimentin was higher in cells treated with TGF-β1 and IL-6 than TGF-β1 alone (Fig. 4E). Taken together, these data showed that the IL-6/JAK/STAT3 signaling enhanced TGF-β/Smad pathway and then accelerated the TGF-β-induced EMT process in lung cancer cells.

IL-6 increases TGF-β-induced cell migration and invasion. Finally, we determined whether IL-6 strengthened TGF-β-induced migration and invasion in A549 and H1650 cells treated with TGF-β1 and/or IL-6, and observed that IL-6 or TGF-β1 enhanced the migration and invasion of lung cancer cells (Fig. 5). More importantly, cells treated with TGF-β1 in combination with IL-6 showed higher capability of migration and invasion than TGF-β1 alone (Fig. 5). The results indicated that IL-6 was able to enhance TGF-β-induced lung cancer cell migration and invasion.

Discussion

EMT is a critical event occurring during cancer metastasis (3). Increasing number of studies have yielded distinct signaling pathways that regulate EMT (31). In this study, we provided new insight into the contribution of an interaction between the JAK/STAT3 and TGF-β/Smad pathways to the induction of EMT in lung cancer. To the best of our knowledge, this is the first evidence of identifying that the JAK/STAT3 pathway can enhance TGF-β-induced EMT.

It has been demonstrated that the JAK/STAT3 pathway is required to sustain EGF/EGFR-induced EMT-associated phenotypes in ovarian and breast cancers (32,33). Moreover,

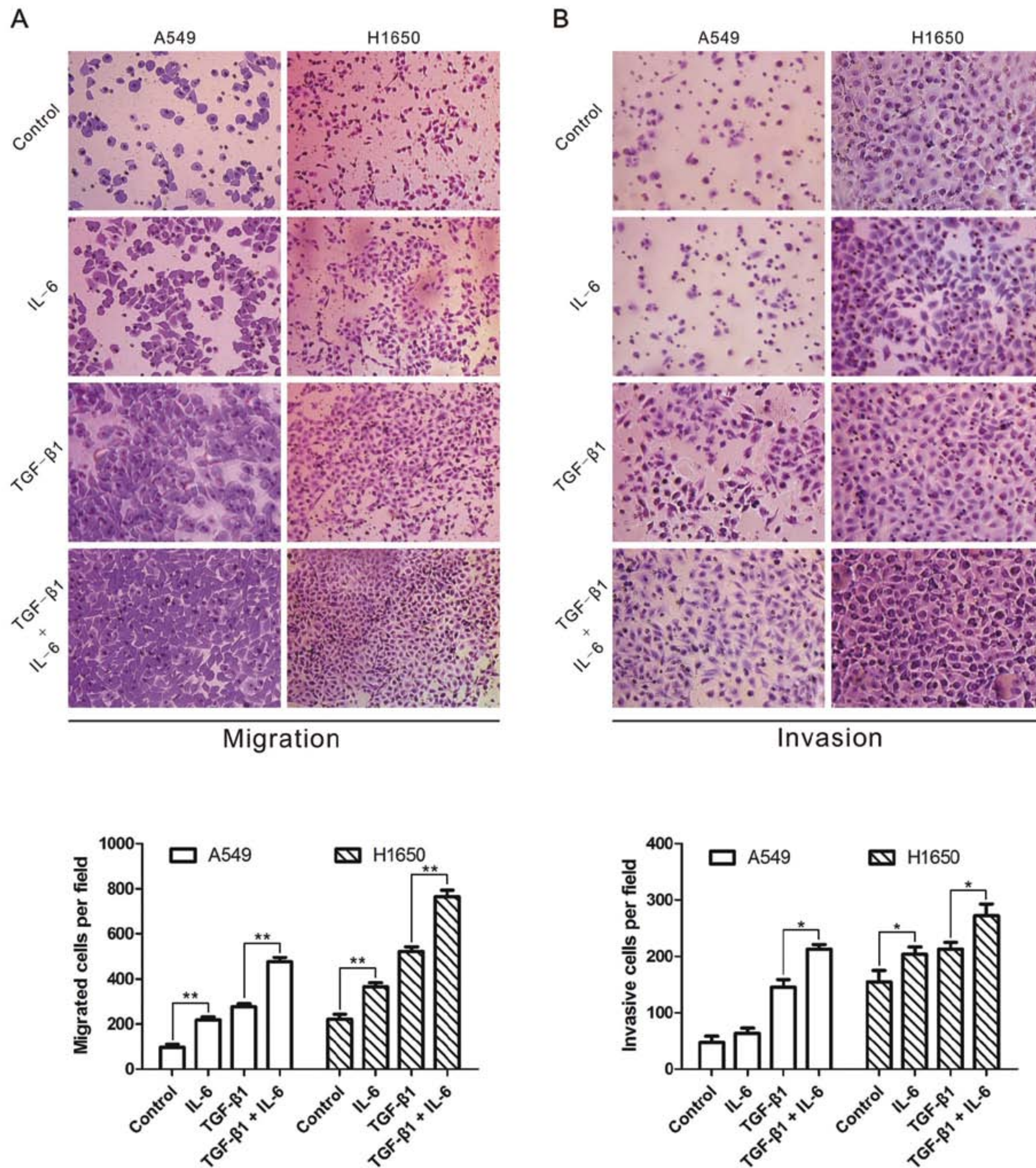

Figure 5. IL-6 improves TGF-β-induced migration and invasion in lung cancer A549 and H1650 cells. (A) IL-6 promoted TGF-β-induced cell migration. Cells were incubated with 5 ng/ml TGF-β1 and/or IL-6 50 ng/ml, and cell migration was assessed with transwell chambers. (B) IL-6 enhanced TGF-β-induced cell invasion. Cells were treated as above, and cell invasion was examined with Matrigel-coated transwell chambers. Cells were cultured for 12 h and stained with 1% crystal violet, and the number of migrated and invasive cells was counted in triplicate. $^{*}P<0.05$; $^{**}P<0.01$.

Yamashita *et al* established a molecular link between Stat3, LIV1, and Snail during the EMT of gastrula organizer cells in zebrafish embryos (34). Our results support the potential role of the JAK/STAT3 signaling in promoting EMT, which may be vital for cancer invasion and metastasis.

In the process of EMT, epithelial cells acquire expression of mesenchymal components and manifest migratory and invasive phenotypes (35). As a functional consequence of TGF-β signaling, decreased expression of E-cadherin, increased expression of vimentin and increased abilities of migration and invasion were displayed in lung cancer cells A549 and H1650 (Figs. 4 and 5). These findings are in accordance with previous studies that TGF-β can induce EMT in certain types of cancer cells (9,36-38). Specifically, we found that TGF-β can increase the expression of Snail at both mRNA and protein levels in lung cancer cells, consisting with the findings obtained in pancreatic cancer Panc-1 cells (39), Madin-Darby canine kidney cells (40), retinal pigment epithelia (41) and mouse mammary epithelial HMuMg cells (42). Therefore, upregulation of Snail can be a common mechanism underlying TGF-β-induced EMT.

Besides TGF-β, IL-6 was reported to induce EMT in breast, colorectal and prostate cancer cells (16-18). In lung cancer cells, we did not find that IL-6 directly elicited EMT. This discrep-

ancy may be explained by the following three aspects: first, here we used low dose of IL-6 to treat cells, unlike Sullivan *et al* who ectopically expressed IL-6 to enlarge its secretion (16). Second, this difference depends on the context of certain types of cancer cells. Third, IL-6 might be indirectly involved in the EMT process in lung cancer cells. In support of this, we found that IL-6 promoted the EMT process induced by TGF-β, suggesting the cooperation of IL-6/JAK/STAT3 with TGF-β/Smad signaling pathways in EMT.

Our results showed that IL-6 boosted the expression of Snail induced by TGF-β/Smad pathway, contributing greatly to EMT (8,43). It is worth to notice that TGF-β-induced Snail transcription was highly dependent on the cooperation of p-Smad with c-Myc, which was required for rapid Snail activation upon Smad (14). Significantly, IL-6 is capable of inducing c-Myc expression through activating Stat3 which binds directly to the promoter of c-Myc (44,45). Therefore, IL-6 can enhance TGF-β-induced Snail both c-Myc- and Smad-dependently.

Notably, in EGFR-overexpressing tumor cells, activated Stat3 is responsible for EGF-mediated desensitization of the TGF-β signaling (46). In contrast, we focused on A549 cells expressing lower EGFR and H1650 cells harboring mutated EGFR, and found that the IL-6-induced Stat3 activation enhanced the TGF-β/Smad signaling. Similarly, IL-6 regulation of TGF-β receptor compartmentalization and turnover enhances TGF-β1 signaling in human renal epithelial cells (47). These findings suggest that different effects of IL-6 and/or Stat3 on TGF-β signaling depends upon context of cell types, further investigations are warranted to uncover the underlying mechanisms.

In conclusion, our findings identify that the JAK/STAT3 pathway is required for TGF-β-induced EMT and lung cancer cell migration and invasion, and the IL-6/JAK/STAT3 and TGF-β/Smad signaling can synergistically enhance EMT in lung carcinomas. This study suggests a new rationale for inhibiting cancer metastasis using anti-IL-6/JAK/STAT3 and anti-TGF-β/Smad therapeutic strategies.

Acknowledgements

This study was supported in part by the grants from National Natural Science Foundation of China (81372277 and 81171894 to H.-T. Zhang; 81201575 to Z. Liu), Program for New Century Excellent Talents in University (NCET-09-0165 to H.-T. Zhang), the Jiangsu Province Key Provincial Talents Program (RC2011106 to J. Zhao), '333' Project of Jiangsu Province Government (to H.-T. Zhang), Soochow Scholar Project of Soochow University (to H.-T. Zhang), and Suzhou Key Laboratory for Molecular Cancer Genetics (SZS201209 to H.-T. Zhang).

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