Thyroid Transcription Factor-1 Inhibits Transforming Growth Factor-β–Mediated Epithelial-to-Mesenchymal Transition in Lung Adenocarcinoma Cells

Roy-Akira Saito,¹,² Tetsuro Watabe,¹ Kana Horiguchi,¹ Tadashi Kohyama,² Masao Saitoh,¹ Takahide Nagase,² and Kohei Miyazono¹

¹Departments of Molecular Pathology and Respiratory Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Abstract

Thyroid transcription factor-1 (TTF-1) is expressed in lung cancer, but its functional roles remain unexplored. TTF-1 gene amplification has been discovered in a part of lung adenocarcinomas, and its action as a lineage-specific oncogene is highlighted. Epithelial-to-mesenchymal transition (EMT) is a crucial event for cancer cells to acquire invasive and metastatic phenotypes and can be elicited by transforming growth factor-β (TGF-β). Mesenchymal-to-epithelial transition (MET) is the inverse process of EMT; however, signals that induce MET are largely unknown. Here, we report a novel functional aspect of TTF-1 that inhibits TGF-β–mediated EMT and restores epithelial phenotype in lung adenocarcinoma cells. This effect was accompanied by down-regulation of TGF-β target genes, including presumed regulators of EMT, such as Snail and Slug. Moreover, silencing of TTF-1 enhanced TGF-β–mediated EMT. Thus, TTF-1 can exert a tumor-suppressive effect with abrogation of cellular response to TGF-β and attenuated invasive capacity. We further revealed that TTF-1 down-regulates TGF-β2 production in A549 cells and that TGF-β conversely decreases endogenous TTF-1 expression, suggesting that enhancement of autocrine TGF-β signaling accelerates the decrease of TTF-1 expression and vice versa. These findings delineate potential links between TTF-1 and TGF-β signaling in lung cancer progression through regulation of EMT and MET and suggest that modulation of TTF-1 expression can be a novel therapeutic strategy for treatment of lung adenocarcinoma.

Introduction

Thyroid transcription factor-1 (TTF-1; the product of NKX2.1 gene), a homeodomain-containing transcription factor, is a master regulator for lung morphogenesis, and TTF-1 null mice die immediately at birth, resulting from profoundly hypoplastic lungs (1). The importance of TTF-1 in human lung homeostasis is also highlighted by the findings that individuals with TTF-1/NKX2.1 haploinsufficiency exhibit congenital pulmonary disease (2). TTF-1 is mainly expressed in type II pneumocytes and Clara cells and regulates the expression of markers of these cells, surfactant protein C (SPC) and Clara cell secretory protein (CCSP), respectively (3).

Lung cancer is the most frequent type of cancers and causes death of more than one million people annually. The prognosis remains poor despite the recent advances in chemotherapies and molecular-targeted therapies. Expression of TTF-1 has been shown in all types of lung cancers, but its frequent expression is reported in adenocarcinoma (72.1%) and small cell carcinoma (90.5%; ref. 4).

Epithelial-to-mesenchymal transition (EMT) is the differentiation switch directing polarized epithelial cells into mesenchymal cells, which plays key roles during embryonic development (5, 6). Mesenchymal cells arising through EMT significantly contribute to various fibrotic conditions, and the process of tumor cell invasion is also associated with EMT. In addition to the loss of cell-cell adhesions, EMT is characterized by the up-regulation of mesenchymal markers, including fibronectin and N-cadherin, and acquisition of fibroblast-like migratory and invasive phenotypes.

Recent studies revealed that several transcription factors, including Snail, Slug, ßEF-1 (ZEB1), and SIP1, are involved in the induction of EMT (7–9). These transcription factors repress expression of E-cadherin and induce EMT when overexpressed in epithelial cells. The inverse process, mesenchymal-to-epithelial transition (MET), has been shown to occur during development and to be perturbed in fibrotic disorders and cancer. In contrast to EMT, however, it is largely unknown as to which signals induce MET.

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that regulates a broad range of cellular responses (10). Three isoforms of TGF-β ligands, i.e., TGF-β1, TGF-β2, and TGF-β3, show different expression profiles in various tissues, including the lung. TGF-β binds to type II and type I serine/threonine kinase receptors and transmits intracellular signals. Smads are the major transducer of TGF-β signaling; Smad2 and Smad3 are phosphorylated by the TGF-β type I receptor and form complexes with Smad4. These complexes accumulate in the nucleus and regulate transcription of target genes. TGF-β suppresses growth of epithelial cells, whereas tumor cells frequently lose the responsiveness to growth inhibitory activity of TGF-β. Moreover, TGF-β is known to promote tumor progression through a diverse repertoire of tumor cell autonomous and host-tumor interactions. TGF-β is the major mediator of EMT and is critically involved in epithelial-mesenchymal interactions during lung morphogenesis (11).

In a model of chronic renal injury, bone morphogenetic protein-7 (BMP-7) has been shown to reverse TGF-β–induced EMT (12), and this finding encouraged us to explore the therapeutic strategy to induce MET in cancer cells, most of which exist in an intermediary phenotypic state of “partial EMT” with the potential to undergo “full EMT.” Here, we studied the function of TTF-1 in...
lungs cancer. Because TTF-1 positivity has been reported to be a
good prognostic marker in patients with non–small cell lung cancer (13), we focused on lung adenocarcinoma in the present
study. Our results suggest that depletion of TTF-1 in lung adenocarcinoma accelerates the process of EMT, leading to
progression of cancer.

Materials and Methods

Reagents and antibodies. TGF-β1 was purchased from R&D Systems
and used at the concentration of 1 ng/mL. Anti–phosphorylated Smad2,
phosphorylated Smad1/Smad3, fibronectin, and Snail antibodies were from
Cell Signaling. Anti–total Smad2/3, N-cadherin, E-cadherin, ZO-1, and CD31
antibodies were from BD Pharmingen (Transduction Laboratories). Anti–
TTF-1 antibody was from Lab Vision Corporation. Anti–α-tubulin and pan-
cytokeratin antibodies were from Sigma-Aldrich. LY364947 was from
Calbiochem and used at the concentration of 3 μM/L.

Cell lines. A549 and Lewis lung cancer (LLC) cells were from Cell
Resource Center for Biomedical Research, Institute of Development, Aging
and Cancer, Tohoku University. NCI-H441 (H441) cells were from American
Type Culture Collection. LC-2/ad cells were from RIKEN BRC.

Cloning of the human TTF-1 cDNA. There are two alternative
transcripts of TTF-1 gene, and the short form consists of over 90% of
total transcripts (14). We cloned open reading frame of the short form from
the cDNAs of L129 cells.

Phase contrast and fluorescence microscopy. Phalloidin staining and
immunocytochemical analyses were carried out, as described previously
(15). Fluorescence was examined by a confocal laser scanning microscope
(Carl Zeiss). Cells were also photographed using a phase-contrast
microscope (Olympus).

Luciferase reporter assay. Human E-cadherin promoter construct was
kindly provided by Dr. F. van Roy (Ghent University). Luciferase activity
was determined as described previously (15).

Immunoblot analysis. Radioimmunoprecipitation assay buffer and lysis
buffer were used for immunoblotting of TTF-1 and other proteins,
respectively. Detailed procedures were described previously (16).

RNA isolation and reverse transcription–PCR. Total RNA was isolated
with RNeasy (Qiagen), and first-strand cDNA was synthesized using the
SuperScript First-Strand Synthesis System (Invitrogen). Quantitative reverse
transcription–PCR (RT-PCR) analysis was performed using the ABI PRISM
7500 Fast Real-Time PCR System (Applied Biosystems) and Power SYBR
Green. The expression level was normalized to that of glyceraldehyde-3-
phosphate dehydrogenase. PCR primers are listed in Supplementary
Table S1.

Gelatin zymography. The cells infected with Ad-LacZ or Ad-TTF-1 were
cultured with serum-free media for 48 h, and the conditioned media were
collected. Equal amounts of samples were applied to a 10% (w/v)
polyacrylamide gel impregnated with 1 mg/mL gelatin. After electropho-
resis, the gel was stained with 0.5% Coomasie blue.

Wound healing and invasion assays. Wound healing assay was
performed as described previously (16). Video time-lapse imaging was
performed as described in the supplementary information. Images were
analyzed using the Image J software (NIH).

Cell invasion assay was performed using a Cell Culture Insert (BD
Biociences). Collagen IC was coated on the upper side of the chamber.
Cells were trypsinized and resuspended in each well at a concentration of 5 × 10⁵
per well. After 8 h, the cells on the upper side of the filters were
removed, and the cells on the lower surface were fixed in methanol and
stained with 0.2% crystal violet and 20% methanol.

RNA interference and oligonucleotides. Transfection of small
interfering RNA (siRNA) was performed using HiPerFect reagent (QIAGEN).
Human TTF-1 siRNA ( Stealth RNAi HSS144278) and negative control
(Stealth RNAi 12935-200) were purchased from Invitrogen.

ELISA assay. The culture supernatants were acidified with 1 N HCl for
10 min, followed by neutralization with 1.2 N NaOH/0.5 mol/L HEPES. The
samples were then subjected to ELISA for TGF-β2 (R&D Systems).

Results

Ectopic expression of TTF-1 in lung adenocarcinoma cells. A549 lung adenocarcinoma cells lack TGF-1 expression, whereas
H441 cells endogenously express it (17). Adenoviral transduction of TTF-1 (Ad-TTF-1) yielded similar levels of TTF-1 transcripts in
A549 cells compared with those in H441 cells infected with control
adenoviruses encoding LacZ (Ad-LacZ; Supplementary Fig. S1A).
TTF-1 was located in the nucleus in A549 cells infected with Ad-
TTF-1 (Supplementary Fig. S1B), and the known targets of TTF-1,
including CCSP and SPC, were induced 96 h after adenoviral
transduction (Supplementary Fig. S1C).

TTF-1 inhibits EMT in lung adenocarcinoma cells. To study the
effects of TTF-1 in lung adenocarcinoma cells, we first examined morphologic changes of A549 cells. TTF-1 caused
apparent changes from an elongated shape to a polygonal or round
appearance (Fig. 1A). Because formation of cell–cell adhesions is mainly dependent on E-cadherin system in epithelial
cells, we further explored whether TTF-1 influences E-cadherin
expression. Luciferase assay showed that TTF-1 enhances the human E-cadherin promoter activity in a dose-dependent fashion
(Fig. 1B). Untreated A549 cells lacked E-cadherin expression at low
cell density as confirmed by immunocytochemistry. When the cells
proliferate to higher cell density, diffuse and weak E-cadherin
staining was observed (Fig. 1C). Forced expression of TTF-1 resulted in stronger staining of E-cadherin on the cell
membrane or in the cytoplasm (Fig. 1C, bottom left). These findings suggested that TTF-1 might restore the epithelial
property, at least partially, and prompted us to explore the effect of TTF-1 on EMT in lung adenocarcinoma cells.

Because TGF-β has been shown to elicit EMT in A549 cells (18),
we further investigated the effects of TTF-1 in the presence or
absence of TGF-β stimulation. In contrast to untreated A549 cells,
TGF-β triggered drastic morphologic changes to a spindle-like or
fibroblast-like appearance (Fig. 1C and D). E-cadherin staining was
completely lost in TGF-β–treated cells, regardless of cell density, and
actin reorganization was apparent by phalloidin staining, showing
the induction of EMT by TGF-β. Interestingly, EMT, induced by TGF-β, was clearly inhibited by ectopic TTF-1 (Fig. 1C and
D).

E-cadherin expression was enhanced by the TGF-β type I
receptor inhibitor LY364947 (Supplementary Fig. S2A), suggesting
that blockade of endogenous TGF-β signaling induces E-cadherin
up-regulation. TTF-1 further enhanced E-cadherin expression,
in addition to the effect of LY364947 (Supplementary Fig. S2C).
TTF-1-mediated E-cadherin up-regulation antagonism to
TGF-β–mediated EMT were further confirmed by immunoblotting
(Supplementary Fig. S2B). Besides loss of E-cadherin, EMT is
caracterized by up-regulation of mesenchymal markers. TGF-β–
mediated up-regulation of fibronectin was antagonized by TTF-1,
whereas that of N-cadherin was not significantly affected

Animal models and statistical analyses. C57/BL6 mice, 5 to 6 wk of
age, were obtained from Sankyo Laboratory. A total of 1 × 10⁶ cells in
100 μL PBS were injected s.c. into mice. Tumor volume was
approximated by using the equation, vol = (a × b²) / 2, wherein vol is
volume, a is the length of the major axis, and b is the length of the minor
axis. The results were analyzed statistically by the multivariate ANOVA
method, and P value was calculated by log-rank test. The excised samples
were put into OCT compound, frozen in dry-iced acetone, and further
sectored for immunohistochemistry.

Animal models and statistical analyses. C57/BL6 mice, 5 to 6 wk of
age, were obtained from Sankyo Laboratory. A total of 1 × 10⁶ cells in
100 μL PBS were injected s.c. into mice. Tumor volume was
approximated by using the equation, vol = (a × b²) / 2, wherein vol is
volume, a is the length of the major axis, and b is the length of the minor
axis. The results were analyzed statistically by the multivariate ANOVA
method, and P value was calculated by log-rank test. The excised samples
were put into OCT compound, frozen in dry-iced acetone, and further
sectored for immunohistochemistry.
LY364947 suppressed the induction of fibronectin and N-cadherin by TGF-β and up-regulated E-cadherin expression (Supplementary Fig. S2). In addition to E-cadherin, A549 cells were further immunostained for other epithelial markers, i.e., ZO-1 and pan-cytokeratin (Supplementary Fig. S3). ZO-1 expression was observed in both LacZ-expressing and TTF-1–expressing cells. In LacZ-transduced cells, TGF-β treatment led to the reduction of its staining on the cell membrane, whereas this effect was clearly antagonized by TTF-1. Pan-cytokeratin expression was decreased but sustained even after TGF-β treatment.

**TTF-1 attenuates matrix metalloproteinase-2 activity, cell migration, and invasive capacity of lung adenocarcinoma cells.** EMT is accompanied with enhancement of matrix metalloproteinase (MMP) activities that facilitate degradation of extracellular matrices surrounding tumor cells. TGF-β treatment enhanced the expression of MMP-2, as determined by quantitative RT-PCR, and this effect was inhibited by TTF-1 (Fig. 2A). LY364947 effectively blocked the effect of TGF-β to induce MMP-2 in both of the control and TTF-1–expressing cells (Supplementary Fig. S4A). Gelatin zymography further showed that MMP-2 activity was enhanced by TGF-β, and this effect was inhibited by TTF-1 (Fig. 2B).

To analyze functional aspects of TGF-β–induced EMT and antagonistic action of TTF-1, we performed wound healing and invasion assays. TGF-β treatment led to highly migratory behavior of cells and earlier closure of wounds after 72 hours, despite of its growth inhibitory action (Fig. 2C, top left). Expression of TTF-1 resulted in retardation of wound closure reflecting attenuated migratory property, and TGF-β treatment failed to enhance cell migration in TTF-1–transduced cells in contrast to LacZ-transduced cells (Fig. 2C, bottom left). These effects were quantitated by time-lapse movies (Fig. 2C, right and Supplementary Videos).

The process of cancer invasion involves the degradation of basement membrane and extracellular matrices that are mainly composed of collagen. To determine the invasive capacity of lung

---

**Supplementary Figure S2**

A, phase contrast microscopy of A549 cells infected with Ad-LacZ or Ad-TTF-1. B, luciferase reporter assay of human E-cadherin in A549 cells. Bars, SD. C, immunocytochemistry for E-cadherin (green). Red, TRITC-phalloidin; blue, TOTO3 (nuclei). A549 cells infected with Ad-LacZ or Ad-TTF-1 for 48 h were incubated with or without TGF-β1 for additional 48 h. D, high magnification of the cells treated as in C. Red, TRITC-phalloidin; blue, TOTO3 (nuclei).
TTF-1 negatively regulates the expression of molecules involved in EMT. In A549 cells, ectopic TTF-1 inhibited the induction of TGF-β target genes, Smad7 and PAI-1, which are regulated by Smad pathway (Fig. 3A). Despite of these striking differences, phosphorylation of Smad2 or Smad3 after TGF-β treatment displayed no significant difference between the control and TTF-1-expressing cells (Supplementary Figs. S5A and S5B). Next, we knocked down the expression of endogenous TTF-1 in H441 cells. Transfection of TTF-1 siRNA effectively suppressed the expression of TTF-1 (Fig. 3B, left). TTF-1 knockdown resulted in enhanced induction of Smad7 and PAI-1 after TGF-β stimulation in H441 cells (Fig. 3B, right), consistent with the results in A549 cells.

Recent data have shown that Smad3 physically interacts with TTF-1 and regulates the transcription of the TTF-1 target gene SPB (19, 20). Taken together, it is suggested that TTF-1 suppresses Smad-mediated transcription of a subset of TGF-β target genes in the nucleus and, thereby, inhibits TGF-β-mediated EMT in lung adenocarcinoma cells.

We further explored the antagonistic effects of TTF-1 against TGF-β–induced EMT. Expression of E-cadherin is regulated by multiple transcription factors, including zinc finger transcriptional repressors Snail and Slug (8). TTF-1 suppressed the basal expression level of Snail and Slug, and their rapid induction after TGF-β treatment was also inhibited by TTF-1 (Fig. 3C, left). Suppressed expression of Snail was also shown by immunoblotting (Fig. 3C, right). Although LY364947 treatment suppressed the expression of Snail and Slug after 24 h in LacZ-transduced cells, it did not induce further decrease in TTF-1–transduced cells (Supplementary Figs. S4B and C).

Luciferase assay showed that Snail or Slug suppresses the human E-cadherin promoter activity, antagonizing the action of TTF-1 to enhance it (Supplementary Fig. S6A). Furthermore, adenoviral
transduction of human Snail resulted in down-regulation of E-cadherin and up-regulation of N-cadherin and fibronectin, mimicking the effect of TGF-β (Supplementary Fig. S6B). These results support the idea that Snail and Slug are involved in the regulation of EMT in A549 cells, as previously described in other cell types.

Recently, platelet-derived growth factor (PDGF) signaling (21) and collagen I (22) have been reported to be involved in TGF-β-induced EMT. In A549 cells, TGF-β stimulation resulted in the induction of PDGF-B and α1(I) collagen, whereas this effect was blocked by ectopic TTF-1 (Supplementary Figs. S7A and B). These results suggest that TTF-1 blocks EMT and induces epithelial differentiation by suppression of an array of events leading to EMT. In addition, induction of CTGF after TGF-β treatment was also inhibited by TTF-1 (Supplementary Fig. S7C). Thus, it is also suggested that TTF-1 can act as an antifibrotic factor in cancer, as well as in fibrotic disorders, through down-regulation of fibrotic factors.

Silencing of TTF-1 modulates epithelial phenotypes and enhances TGF-β-mediated EMT. To further address the effect of TTF-1 on TGF-β-induced EMT, we knocked down endogenous TTF-1 in H441 cells. Control or TTF-1 siRNA was transfected at 0 and 72 hours in the presence or absence of continuous TGF-β stimulation, and the cell morphology was examined at 144 hours (Fig. 4A), because it was previously reported that alveolar epithelial cells undergo EMT when chronically treated with TGF-β for >144 hours (23). Silencing of TTF-1 in H441 cells resulted in morphologic changes to a flattened or elongated shape with decreased cell-cell attachment (Fig. 4A). TGF-β treatment led to the reorganization of actin stress fibers, whereas cell-cell adhesions were sustained (Fig. 4A and Supplementary Fig. S8A). The cells with combined treatment of TTF-1 knockdown and TGF-β showed impaired cell-cell attachment, and fibroblast-like cells were frequently found when cultured at low cell density (Fig. 4A, bottom).

H441 cells were also immunostained for E-cadherin, ZO-1, and pan-cytokeratin (Supplementary Fig. S8B). E-cadherin staining on

Figure 3. TTF-1 down-regulates the molecules involved in EMT. A, quantitative RT-PCR. Kinetic expression of Smad7 and PAI-1 indicated as in Fig. 2A. B, left, immunoblotting of TTF-1 in H441 cells transfected with mock, control siRNA (si NTC), and siRNA for TTF-1 (si TTF-1). β-Tubulin was used as a loading control. Right, quantitative RT-PCR. Kinetic expression of Smad7 and PAI-1. H441 cells were transfected with si NTC or si TTF-1 and treated with TGF-β1 for the indicated time periods. C, left, quantitative RT-PCR. Kinetic expression of Snail and Slug. Right, immunoblotting of Snail in A549 cells infected with Ad-LacZ or Ad-TTF-1 and treated with or without TGF-β1 for 24 h.
the cell membrane was clearly observed in H441 cells. In contrast to A549 cells, E-cadherin expression was persistent even after TGF-β treatment. TTF-1 knockdown alone failed to significantly suppress its expression, but simultaneous treatment with TGF-β resulted in loss of cell-cell adhesions and substantially decreased E-cadherin staining. Irregular staining of ZO-1 was noted in H441 cells, and TTF-1 knockdown or TGF-β treatment led to its reduced expression. Pan-cytokeratin expression was decreased but sustained even after TGF-β treatment or TTF-1 knockdown. Together with the results in A549 cells, cytokeratins might be persistently expressed in lung cancer cells with mesenchymal phenotypes, consistent with the clinical findings that most lung cancer cells keep expressing cytokeratins.

We next examined the effect of TTF-1 knockdown on both TGF-β-mediated rapid induction of Snail or Slug and expression of EMT markers. Consistent with the observations in A549 cells (Fig. 3C), silencing of TTF-1 resulted in enhanced induction of Snail and Slug (Fig. 4B). Enhanced induction of Snail was also shown by immunoblotting (Fig. 4C, left). We also studied the effect of chronic exposure (144 hours) to TGF-β. TTF-1 knockdown resulted in enhanced expression of Snail, and the induction of fibronectin mediated by TGF-β was enhanced under the condition that TTF-1 was knocked down (Fig. 4C, right). These observations support the action of TTF-1, which inhibits EMT mediated by TGF-β. Contrary to the immunocytochemical observations (Supplementary Fig. S8B), E-cadherin expression was not significantly affected by either TGF-β treatment or TTF-1 knockdown in a bulk population of the cells cultured at high cell density (Fig. 4C, right). This result suggested that E-cadherin expression is retained by other mechanisms that might overcome the effect of TGF-β or TTF-1 in H441 cells cultured at high cell density.

Reciprocal regulation of TTF-1 expression and TGF-β signaling. To address the effect of TGF-β on the expression of TTF-1, we used two different lung adenocarcinoma cell lines, H441 and LC-2/ad, which endogenously express TTF-1. TGF-β treatment for 72 hours suppressed the expression of TTF-1 mRNA and protein

---

**Cancer Research**

Cancer Res 2009; 69: (7). April 1, 2009 2788 www.aacrjournals.org

Figure 4. Silencing of TTF-1 enhances TGF-β–mediated EMT. A, phase contrast microscopy of H441 cells transfected with si NTC or si TTF-1 and incubated with or without TGF-β1 for 144 h. B, quantitative RT-PCR. Kinetic expression of Snail and Slug indicated as in Fig. 3B. C, left, immunoblotting of Snail in H441 cells transfected with si NTC or si TTF-1 and treated with or without TGF-β1 for 24 h; right, immunoblotting of E-cadherin and mesenchymal markers (fibronectin and Snail). H441 cells were transfected with si NTC or si TTF-1 at 0 and 72 h and incubated with or without TGF-β1 for 144 h.
TTF-1 inhibits TGF-β-mediated EMT

Discussion

In the present study, we showed that TTF-1 inhibits EMT in response to TGF-β and restores epithelial phenotypes in lung adenocarcinoma cells, leading to suppression of cell migration and invasion. TTF-1 abrogated TGF-β-mediated induction of Snail and Slug, which regulate the changes in gene expression patterns that underlie EMT (9). On the other hand, expression profiles of other factors that have been implicated in EMT, such as ZEB1 (21), SIP1 (15), HMGA2 (24), and Twist1 (25), suggested that they are not involved in either TGF-β-mediated EMT or the effect of TTF-1 in A549 cells (data not shown). The mechanism of how TTF-1 inhibits TGF-β-mediated EMT could be explained by multiple mechanisms. One is the suppression of Smad-mediated transcription of EMT-inducing molecules, such as Snail and Slug (Fig. 3), as suggested by the recent findings that Smad3 physically interacts with TTF-1 and regulates its transcriptional activity (19, 20). We have also shown the importance of another pathway; i.e., attenuation of autocrine TGF-β signaling by TGF-β2 down-regulation (Fig. 5).

Accumulating evidence of genomic analyses revealed that TTF-1 gene is amplified in 10% to 15% of lung adenocarcinomas, and in vitro studies further support the concept that TTF-1 acts as a lineage-specific oncogene (26–28). On the other hand, the functional significance of TTF-1 in other subsets of lung adenocarcinomas, wherein TTF-1 expression is reduced or lost, still remains to be elucidated.

It is reported that TTF-1 expression is high in well-differentiated carcinomas and relatively low in poorly differentiated carcinomas (13). According to the classification of lung adenocarcinomas into terminal respiratory unit (TRU) type and...
non-TRU type by Yatabe and colleagues, the majority of TTF-1–positive cases showed TRU morphology. Conversely, most of adenocarcinomas with TRU morphology were TTF-1 positive (29). These observations suggest that loss of TTF-1 expression is associated with poor differentiation of adenocarcinomas. Therefore, we believe that recent data showing the oncogenic role of TTF-1 do not exclude the possibility that TTF-1 might act as a tumor suppressor in another subset of lung adenocarcinomas, possibly combined with the mutation or amplification of other oncogenes.

We found that TGF-β suppresses the expression of TTF-1, and this effect was inhibited by LY364947. Expression of TTF-1 might be sustained by the feed-forward mechanism through binding of TTF-1 to its own promoter (30). We have also shown that TTF-1 can attenuate TGF-β signaling by down-regulation of TGF-β2. TGF-β signaling is often positively modulated through the induction of TGF-β ligands of different isoforms (31). Thus, enhancement of autocrine TGF-β signaling may accelerate the decrease of TTF-1 expression, and conversely, TTF-1 may attenuate autocrine TGF-β signaling. Because TTF-1 exerts a tumor suppressive effect through inhibition of EMT, these findings delineate a novel pathway that TGF-β accelerates lung cancer progression.

Three isoforms of TGF-β ligands show different expression profiles during lung branching morphogenesis. Whereas TGF-β1 expression is prominent throughout the mesenchyme, TGF-β2 is mainly localized to the epithelium of the developing distal airways. TGF-β2 may be critical for determining the epithelial cell behavior in a cell autonomous fashion. TTF-1 is expressed at the tip of the developing distal airway and may play a role in the maintenance of the epithelial polarity. Reciprocal regulations between TTF-1 and TGF-β signaling, involved in lung branching morphogenesis, may be recapitulated in lung adenocarcinoma cells.

Loss of TTF-1 expression may be associated with poor differentiation of adenocarcinomas, and our results showed that TTF-1 inhibits EMT and invasiveness of lung adenocarcinoma cells. Some clinical studies showed that TTF-1 positivity is a good prognostic indicator in patients with non–small cell lung cancer. Taken together, our present study sheds light on the new functional aspect of TTF-1, which can inhibit cancer progression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 9/8/08; revised 12/25/08; accepted 1/5/09; published OnlineFirst 3/17/09.
Grant support: KAKENHI grants-in-aid for scientific research and Global Center of Excellence Program for “Integrative Life Science Based on the Study of Biosignaling Mechanisms” from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We thank Y. Morishita, C. Iwata, A. Komuro, and T. Shirakihara for technical support and all the members of the Molecular Pathology Laboratory.
References


Thyroid Transcription Factor-1 Inhibits Transforming Growth Factor-β–Mediated Epithelial-to-Mesenchymal Transition in Lung Adenocarcinoma Cells

Roy-Akira Saito, Tetsuro Watabe, Kana Horiguchi, et al.


Updated version

Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-08-3490

Supplementary Material

Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2009/03/16/0008-5472.CAN-08-3490.DC1

Cited articles

This article cites 31 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/7/2783.full#ref-list-1

Citing articles

This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/69/7/2783.full#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.