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Tristetraprolin Regulates Prostate Cancer Cell Growth Through Suppression of E2F1

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The transcription factor E2F1 is active during G1 to S transition and is involved in the cell cycle and progression. A recent study reported that increased E2F1 is associated with DNA damage and tumor development in several tissues using transgenic models. Here, we show that E2F1 expression is regulated by tristetraprolin (TTP) in prostate cancer. Overexpression of TTP decreased the stability of E2F1 mRNA and the expression level of E2F1. In contrast, inhibition of TTP using siRNA increased the E2F1 expression. E2F1 mRNA contains three AREs within the 3′UTR, and TTP destabilized a luciferase mRNA that contained the E2F1 mRNA 3′UTR. Analyses of point mutants of the E2F1 mRNA 3′UTR demonstrated that ARE2 was mostly responsible for the TTP-mediated destabilization of E2F1 mRNA. RNA EMSA revealed that TTP binds directly to the E2F1 mRNA 3′UTR of ARE2. Moreover, treatment with siRNA against TTP increased the proliferation of PC3 human prostate cancer cells. Taken together, these results demonstrate that E2F1 mRNA is a physiological target of TTP and suggests that TTP controls proliferation as well as migration and invasion through the regulation of E2F1 mRNA stability.

Keywords: E2F1, ARE-binding protein, TTP, prostate cancer, gene regulation

Introduction

The transcription factor E2F family consists of eight members (E2F-1 to -8), which can be sorted into different functional groups. The E2F family forms heterodimers with different protein family members (DP1 and DP2), which bind to E2F sites and transactivate transcription [6]. Functionally, E2F1, E2F2, and E2F3 represent the group of growth-promoting transcription factors that induce quiescent cells to enter the G1/S phase of the cell cycle and pass through various growth-arrest signals [4]. It has been demonstrated that E2F1 is overexpressed in human cancers and is associated with advanced disease and poor prognosis in breast cancer and non-small-cell lung cancer patients [5, 21, 22]. Overexpressed E2F1 is related to lung and liver metastases of colorectal cancer and induces proliferation, hyperplasia, and p53-dependent apoptosis in E2F1 transgenic mice [7, 16]. Therefore, E2F1 plays a role as an oncogene in the cell and regulates multiple downstream targets relating to the cell cycle and proliferation.

The specific mRNA sequence element that regulates E2F1 decay is the AU-rich element (ARE) found in the 3′UTR of E2F1 mRNA. AREs are highly conserved sequences located in the 3′UTR of a variety of short-lived mRNAs – such as early response genes, cytokines, proto-oncogenes, and growth regulatory genes – that act as a post-transcriptional mechanism of regulation of expression [3]. The destabilizing mechanism of AREs is mediated by various ARE-binding proteins, such as HuR, NF90, BRF1, and tristetraprolin (TTP) [18, 19]. HuR and NF90 stabilize ARE-containing transcripts, whereas BRF1 and TTP decay their target genes. TTP mediates degradation of a number of ARE-containing transcripts and regulates cancer cell growth [1]. Low expression of TTP correlates with a high expression of proto-oncogenes in many cancers, altering tumorigenic phenotypes and patient prognosis. Thus, TTP is a critical regulator of the ARE-dependent mRNA decay mechanism, supporting the idea that E2F1 is a novel target of TTP and that regulation of E2F1 expression may affect cancer cell growth.

In the present study, we investigated the role of TTP in

the post-transcriptional regulation of E2F1 gene expression in human prostate cancer cell lines. Our data demonstrated that overexpression of TTP decreased E2F1 expression in prostate cancer cells. On the other hand, inhibition of TTP using siRNA increased the E2F1 expression. TTP was bound to the AUUUA motif in the 3′UTR and accelerated the AUUUA-motif-mediated decay of the E2F1 mRNA, and consequently suppressed the proliferation and tumor development of prostate cancer cells. Together, our results show that TTP inhibits the expression of E2F1 through binding to the ARE motif in the E2F1 3′UTR and that suppressing the cellular TTP level with siRNA increases the E2F1 level, which may contribute to the development and progression of prostate cancer.

Materials and Methods

Cells and Cell Preparation

Human prostate cancer cell lines (DU145, PC3, and LNCaP) were purchased from the American Type Culture Collection (Manassas, VA, USA) and were maintained in RPMI1640 media supplemented with 10% FBS (HyClone, Rogan, UT, USA) at 37°C in a humidified atmosphere of 5% CO_2.

For the MTT cell proliferation assay, cells were plated in triplicate at 1×10^4 cells/well in 96-well culture plates in RPMI1640. At the indicated times, CellTiter 96 Aqueous One Solution Reagent (Promega, USA) was added to each well according to the manufacturer's instructions and absorbance at 490 nm (OD_{490}) was determined for each well using a Wallac Vector 1420 Multilabel Counter (EG&G Wallac, Turku, Finland).

Plasmids and siRNAs

The pcDNA6/V5-TTP and pcDNA6/V5-E2F1 constructs have been described previously [30, 47]. Three oligonucleotides containing ATTTA motifs of the E2F1 mRNA 3′UTR were PCR-amplified using EmeraldAmp GT PCR Master Mix (Takara, Ohtsu, Japan) and the following primer set: full length of E2F1 3′UTR 5′-CTC GAG CAG GGC TTG GAG GGA CCA G-3′ and 5′-GCG GCC GCA CAA CAA AAA CCT TTA CTG G-3′ (restriction enzyme sites underlined). The PCR products were inserted into the *Xho*I/*Not*I sites of the psiCHECK2 *Renilla*/firefly dual-luciferase expression vector (Promega). Point-mutated constructs in which the AUUUA was substituted with AGCGA were PCR amplified using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) and the following specific primer set: E2F1 ARE1 (ATT TAA TTT A to AGC GAA GCG A) 5′-CCT GTT TGG AAA CAG CGA AGC GAT ACC CCT CTC CTC-3′ and 5′-GAG GAG AGG GGT ATC GCT TCG CTG TTT CCA AAC AGG-3′; E2F1 ARE2 (ATT TAT TTA to AGC GAG CGA) 5′-CTT TAA TGG AGC GTT AGC GAG CGA TCG AGG CCT CTT TG-3′ and 5′-CAA AGA GGC CTC GAT CGC TCG CTA ACG CTC CAT TAA AG-3′; E2F1 ARE3 (ATT TAT TTA to AGC GAG CGA) 5′-GGC CTG GGT GAG CGA GCG ATT GGG AAA GTG AG-3′ and 5′-CTC ACT TTC CCA ATC GCT CGC TCA CCC AGG CC-3′ (point mutation site underlined).

siRNAs against human TTP (TTP-siRNA) (siTTP) and control siRNA (scRNA) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Transfections and Luciferase Assay

Cells (5×10^6) were transfected with various kinds of plasmid constructs using the jetPRIME (Polyplus, NY, USA) according to the manufacturer's instructions.

Lysates of the transfected cells were mixed with luciferase assay reagent (Promega) and the chemiluminescent signal was measured in a Wallac Victor 1420 Multilabel Counter (EG&G Wallac). *Renilla* luciferase activity of psiCHECK2/E2F1-ARE-WT was normalized to firefly luciferase in each sample.

Electrophoretic Mobility Shift Assay (EMSA)

The biotinylated RNA probes for wild-type (Oligo-ARE-WT, 5′-CUU UAA UGG AGC GUU AUU UAU UUA UCG AGG CCU CUU UGG-3′) and mutant (Oligo-ARE-MUT, 5′-CUU UAA UGG AGC GUU AGC GAG CGA UCG AGG CCU CUU UGG-3′) were synthesized by ST Pharm. Co., Ltd (Seoul, Korea). Cytoplasmic extracts were prepared from DU145, PC3, and LNCaP cells using NE-PER nuclear and cytoplasmic extraction reagent (Thermo Pierce Biotechnology Scientific, USA). RNA EMSA was performed using the Lightshift Chemoluminescent EMSA Kit (Thermo Pierce Biotechnology) according to the manufacturer's instructions. Briefly, 10 fmol of biotinylated RNA was combined with 4 μg of cytoplasmic protein of cells in a binding buffer. For the supershift EMSA, anti-TTP antibody (ab36558; Abcam, USA) and anti-V5 antibody (20-783-70389; GenWay) or control antibody (Sigma, St. Louis, MO, USA) was added to the reaction mixture. The reaction mixtures were resolved on 5% non-denaturing polyacrylamide gels in 0.5× Tris borate/EDTA buffer. Gels were transferred to nylon membrane (Hybond-N^+; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) in 0.5× Tris borate/EDTA at 100 V and 4°C for 1 h.

The RNAs were cross-linked to the membrane and detected using streptavidin-horseradish peroxidase binding and chemiluminescence.

SDS-PAGE Analysis and Immunoblotting

Proteins were resolved by SDS-PAGE, transferred to Hybond-P membranes (GE Healthcare Bio-Sciences Corp.), and probed with the appropriate dilution of anti-TTP antibody (Sigma), anti-E2F1 antibody (Bethyl Laboratories, USA), and anti-actin antibody (Cell Signaling, USA). Immunoreactivity was detected using the ECL detection system (GE Healthcare Bio-Sciences Corp.). Films were exposed at multiple time-points to ensure that images were not saturated.

Quantitative Real-Time PCR

For RNA kinetic analysis, we used actinomycin D and assessed E2F1 mRNA expression by quantitative real-time PCR. Quantitative real-time PCR was performed using the BioRad (CFX96 Optics Module, Biorad, CA, USA) by monitoring in real-time the increase in fluorescence of the SYBR Green dye (iQ SYBR Green Supermix, Biorad). The specificity of each primer pair was confirmed by melting curve analysis and agarose gel electrophoresis. PCR primer pairs were as follows: TTP: 5′-CGC TAC AAG ACT GAG CTA T-3′ and 5′-GAG GTA GAA CTT GTG ACA GA-3′; E2F1: 5′-TGC CCT GAG GAG ACC GTA G-3′ and 5′-GGT GAC ACT ATG GTG CAG AG-3′.

Migration/Invasion Assay

The *in vitro* migration and invasive properties of cell lines DU145, PC3, and LNCaP were studied using Boyden chambers (NeuroProbe, USA) pre-coated with collagen (Sigma) and matrigel (BD Biosciences, USA) and incubated for 1 h at 37°C. The bottom wells were filled with 27 µl of media with 2% serum medium. A total of 1×10^5 cells/56 µl were seeded into the upper compartment and incubated for 24 h at 37°C and 5% CO_2. After incubation for 24 h, cells in the upper surface of the filter were removed by using a cotton swab, and those attached to the lower surface of the filters were stained using Diff-Quik reagents (Sysmex Co., Japan) and counted (five fields/well). The migration/invasion percentage was expressed as the percentage of invading cells through the collagen and matrigel. A representative graph of six independent experiments is reported.

Statistics

For statistical comparisons, p values were determined using the Student's t-test.

Results

E2F1 Expression is Inversely Correlated with TTP Expression in Human Prostate Cancer Cell Lines

Analysis of the human E2F1 mRNA 3′UTR revealed the presence of one AUU UAA UUU A motif and two doubles of AUU UAU UUA, which are sufficient for TTP binding and for regulation of stability. Therefore, it is possible to speculate that E2F1 is a novel target of TTP through post-transcriptional regulation.

To confirm this hypothesis, we first examined TTP and E2F1 expression levels by western blotting and quantitative real-time PCR in three human prostate cancer cell lines. The expression level of TTP was high in DU145 and PC3 cells but extremely low in LNCaP cells. In contrast, the level of E2F1 expression in PC3 and LNCaP cells was high compared with that in DU145 (Fig. 1A). We next examined whether the down-regulation of TTP effects E2F1 expression by using a siRNA against TTP to reduce the expression level of TTP in DU145 and PC3 cells. Down-regulation of TTP by treatment with siTTP significantly increased the expression level of E2F1 (Figs. 1B and 1C). However, treatment with a nonspecific siRNA (scRNA) did not decrease the expression level of endogenous TTP, nor did it induce a change in E2F1 expression.

We also tested whether TTP affects the expression of E2F1 by transiently transfecting LNCaP cells with a TTP expression vector (pcDNA/V5-TTP) or the pcDNA/V5 empty vector as a negative control. Overexpression of TTP in LNCaP was confirmed by western blotting and quantitative real-time PCR (Fig. 1D). Collectively, these results indicate that the expression of E2F1 is regulated by changes in TTP expression in human prostate cancer cells.

TTP Decreases the Expression Level of Luciferase mRNA Contacting the E2F1 3′UTR

TTP protein regulates mRNA stability through binding to the AREs within the mRNA 3′UTR. Analysis of the 1,246 bp human E2F1 3′UTR revealed the presence of one AUU UAA UUU A motif and two doubles of the AUU UAU UUA motifs (Fig. 2A). To determine whether the 3′UTR of E2F1 mRNA is required for TTP-mediated destabilization of E2F1 mRNA, we transfected a luciferase reporter gene linked to the E2F1 3′UTR fragment containing all three AREs in the plasmid psiCHCK into DU145 and PC3 cells. When DU145 and PC3 cells with a high expression level of TTP were treated with siRNA against TTP to down-regulate TTP expression (Figs. 1A and 1B), the luciferase activity was increased (Figs. 2B and 2C) compared with that of DU145 and PC3 cells with the scRNA. In contrast, when LNCaP cells with a low expression level of E2F1 were transfected with the pcDNA6/TTP vector and overexpressed TTP, the luciferase activity was dramatically inhibited compared with LNCaP cells transfected with the empty vector pcDNA6/V5 (Fig. 2D). These results demonstrate that the 3′UTR of E2F1 mRNA is important for TTP-mediated down-regulation of E2F1 expression.

Next, we confirmed whether these three AREs are responsible for TTP activity by comparing WT-ARE and ARE-MUT. We first used luciferase genes linked to various point mutants of the E2F1 3′UTR. TTP decreased the luciferase activity of the luciferase reporter gene with the full-length 3′UTR of E2F1 (FL-ARE 1w/2w/3w: WT) by 66.6%. Three parts of single point-mutated constructs (E2F1 FL-ARE M1, M2, and M3) responded to TTP similarly, although slightly less than the full-length E2F1 3′UTR (62.7%, 63.1%, and 59.2%), and double point-mutated constructs of E2F1 FL-ARE M1/2 (1m/2m/3w) abrogated

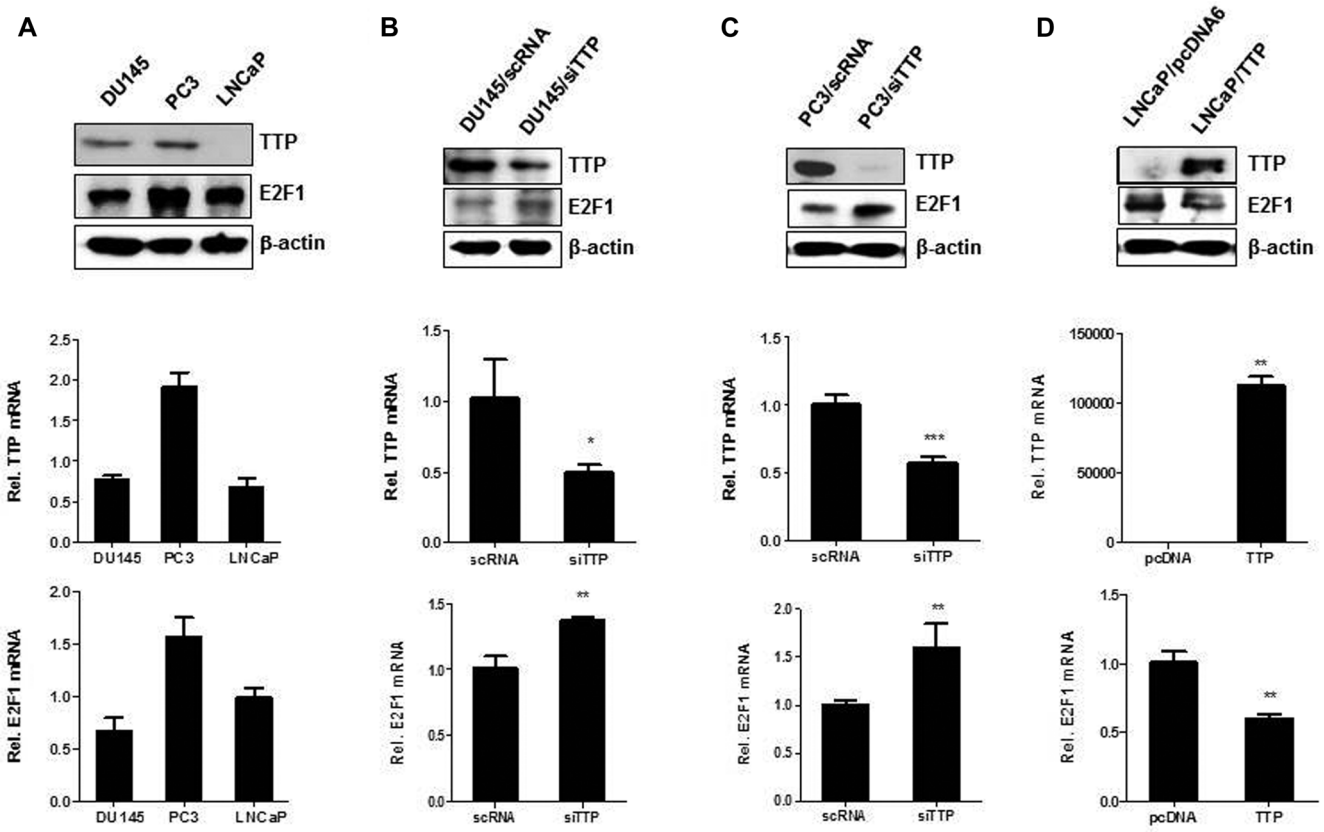

Fig. 1. TTP inhibits E2F1 expression in human prostate cell lines.

(**A**) The levels of TTP and E2F1 expression in three human prostate cancer cell lines were determined by real-time PCR and western blot analysis. (**B**, **C**) DU145 and PC3 cells were transfected with TTP-siRNA (siTTP) or control-siRNA (scRNA). (**D**) LNCaP cells were transfected with pcDNA6/V5-TTP or pcDNA6/V5. β-actin was detected as a loading control for real-time PCR and western blot analysis. Results represent means ± SD of three independent experiments (*$P < 0.01$).

the inhibitory effect of TTP on the reporter gene activity (41.7%). However, E2F1 FL-ARE M1/3 and M2/3 reacted to TTP similarly to the full-length E2F1 3′UTR (62.4% and 57.7%). These results suggest that ARE1 and ARE2 within the E2F1 3′UTR are responsible for the inhibitory effect of TTP.

TTP Destabilizes the AHRR mRNA

We next determined whether the decreased expression of E2F1 resulted from changes in the stability of E2F1 mRNA by measuring the half-life of this mRNA by quantitative real-time PCR in LNCaP cells transfected with pcDNA/V5-TTP (LNCaP/TTP) or empty vector (LNCaP/pcDNA). Overexpression of TTP in LNCaP cells was confirmed by western blot analysis (Fig. 1D). While E2F1 mRNA was stable until 30 min after actinomycin D treatment in empty-vector-transfected cells (LNCaP/pcDNA), the half-life was significantly reduced at 30 min after treatment in TTP-transfected cells (LNCaP/TTP) (Fig. 3A). Our data indicate that the elevated TTP expression contributes to a decrease in E2F1 expression levels through the destabilization of E2F1 mRNA.

TTP Binds to AREs Within the AHRR mRNA 3′UTR

To determine whether TTP interacts with ARE2 of E2F1 3′UTR, RNA EMSA was conducted using a biotinylated RNA probe containing the wild-type or mutant ARE2 of E2F1. The RNA probe for RNA EMSA is the same region of the ARE2 fragment for the luciferase assay (Fig. 2E). Cytoplasmic extracts were prepared from DU145, PC3, and LNCaP cells transfected with pcDNA/TTP to overexpress TTP and were incubated with the biotinylated RNA probe containing wild-type or mutant ARE2 of E2F1. This RNA-protein complex was reduced according to the concentration of TTP-antibody and V5-antibody with pre-incubation in the reaction mixture. There was no change when the mixture was pre-incubated with anti-IgG as a negative

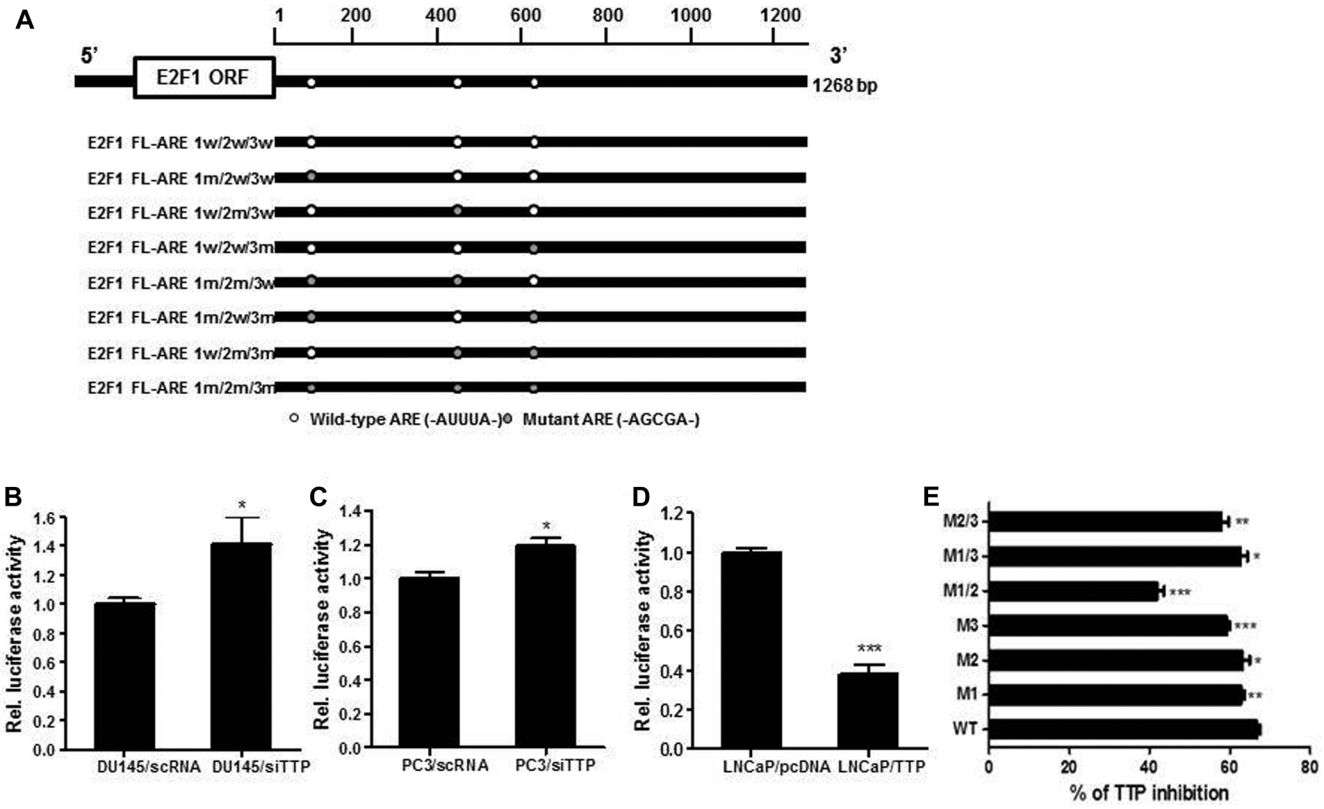

Fig. 2. ARE2 within the E2F1 mRNA 3′UTR is important for the inhibitory effect of TTP.

(**A**) Schematic representation of the luciferase reporter constructs used in this study. Fragments derived from E2F1 mRNA 3′UTR were cloned downstream of the luciferase reporter gene in the psiCHECK2 luciferase expression vector. The white circles represent the wild-type (w) motif AUUUA and the grey circles represent the mutated (m) motif AGCGA. (**B**–**D**) Inhibition of the luciferase reporter containing E2F1 3′UTR by TTP suppression or overexpression. DU145 and PC3 cells were co-transfected with luciferase reporter plasmid containing three AREs of E2F1 mRNA 3′UTR and TTP-siRNA or control-siRNA (**B** and **C**). LNCaP cells were co-transfected with luciferase reporter and pcDNA/V5-TTP or empty pcDNA/V5 control vector (**D**). Cells were harvested and *Renilla* luciferase activity was normalized to firefly activity. The luciferase values obtained from cells co-transfected with luciferase construct and control-siRNA or pcDNA6/V5 were set to 1. Results represent means ± SD of three independent experiments (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (**E**) Mapping of the sequence in the E2F1 mRNA 3′UTR required for TTP inhibition of luciferase activity. LNCaP cells were co-transfected with luciferase reporter construct as described for (**A**) and pcDNA6/V5-TTP or empty pcDNA6/V5. TTP-induced inhibition of luciferase activity observed with each construct was compared with that obtained with pcDNA6/V5-TTP. The results shown represent means ± SD of three independent experiments (*$P < 0.01$).

control (Fig. 3B). These results indicate that TTP directly interacts with the ARE2 of E2F1 mRNA 3′UTR.

Change of TTP Expression Level Affects the Tumor Progression of PC3 Cells

We also examined whether the changes of TTP expression level affect the proliferation of cancer cells. We compared the growth rates of PC3 cells transfected with pcDNA6/V5-TTP or siRNA-TTP. Overexpressed TTP significantly suppressed the growth of PC3 cells compared with that of PC3 transfected with control empty vector. To determine whether down-regulation of E2F1 restores the growth of pcDNA6/V5-TTP-transfected PC3 cells, cells were co-transfected with pcDNA/V5-TTP and pcDNA/V5-E2F1. Transfection with pcDNA6/V5-E2F1 increased the growth of PC3 cells by 90% compared with that of PC3 cells transfected with pcDNA6 control vector (Fig. 4A, upper). In contrast, treatment with siRNA against TTP increased the growth of PC3 cells compared with that of PC3 cells treated with nonspecific siRNA. We also confirmed that co-treatment with siRNA-TTP and siRNA-E2F1 recovered the growth of PC3 cells (Fig. 4A, bottom). These results show that changes of TTP expression level affect the growth of cancer cells through the regulation of E2F1 expression.

An invasion and migration assay was carried out to assess whether TTP plays a role in E2F1-mediated migration

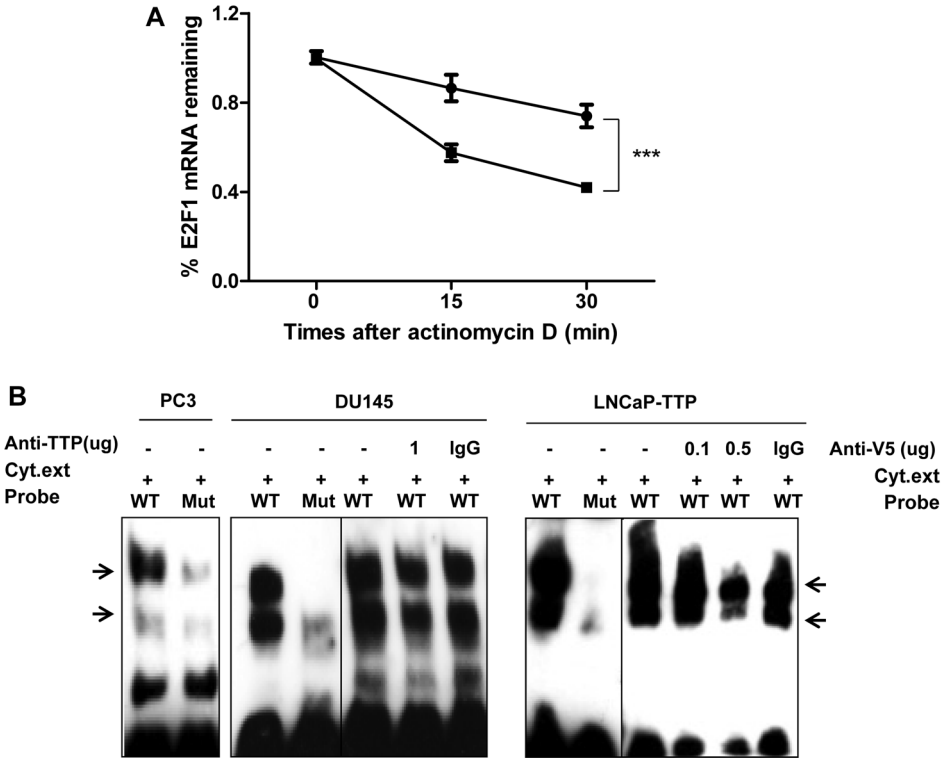

Fig. 3. TTP directly binds to AREs within the E2F1 mRNA 3′UTR.
(**A**) LNCaP cells were transfected with pcDNA6/V5-TTP or pcDNA6/V5. Expression of E2F1 mRNA in LNCaP cells was determined by quantitative real-time PCR at the indicated times after the addition of 5 μg/ml actinomycin D. Results represent means ± SD of three independent experiments (***$P < 0.0001$). (**B**) An RNA EMSA was performed by mixing cytoplasmic extracts containing 4 μg of total protein from PC3, DU145, and LNCaP cells transfected with pcDNA6/V5-TTP cells with 10 fmol of biotinylated wild-type and mutant probe. Anti-TTP or anti-V5 or control antibody was added to the reaction mixtures. The binding reactions were then separated by electrophoresis on a 5% polyacrylamide gel under non-denaturing conditions. Arrows indicate the position of the TTP-containing band.

and invasion by PC3 and DU145 cells. The PC3 cells were transfected with pcDNA/V5-TTP and pcDNA/V5-E2F1, and co-transfected with these two expression vectors, incubated for 24 h and then were plated in Boyden chambers (Fig. 4B). Whereas migration and invasion of PC3 cells were increased by overexpression of E2F1, migration and invasion were dramatically inhibited when PC3 cells were transfected with pcDNA/V5-TTP or co-transfection with pcDNA/V5-TTP and pcDNA/V5-E2F1. We confirmed that treatment with siRNA against E2F1 decreased the migration and invasion of PC3 cells; however, co-treatment with siRNA against TTP and E2F1 restored the migration and invasion properties of PC3 cells (Fig. 4B). Taken together, these data show that TTP is an important regulator of E2F1 expression and may be involved in promoting proliferation as well as migration and invasion by prostate cancer cells.

Discussion

E2F1 is known to exert various effects on cell growth and tumor development in a cell-type- and gene-specific manner. In this study, we showed that TTP post-transcriptionally regulates the expression of E2F1 through binding to its ARE within the E2F1 3′UTR. Overexpression of TTP decreased E2F1 expression, whereas suppression of TTP expression increased it through stabilization of E2F1 mRNA. RNA EMSA assays showed that mutation of the AUUUA motif in the E2F1 3′UTR abrogates the binding of TTP to the E2F1 3′UTR. This suggests that TTP is one of the key molecules involved in down-regulation of E2F1 expression.

TTP is an ARE-binding protein that promotes degradation of ARE-containing mRNAs [2, 8–14]. Brennan *et al.* [1] reported that TTP expression is significantly decreased in many cancers, and it is possible to suppose that the reason for the increased level of E2F1 in tumor cells, which exert various effects on cell growth and tumor development, may result from the low expression of TTP. Supporting this

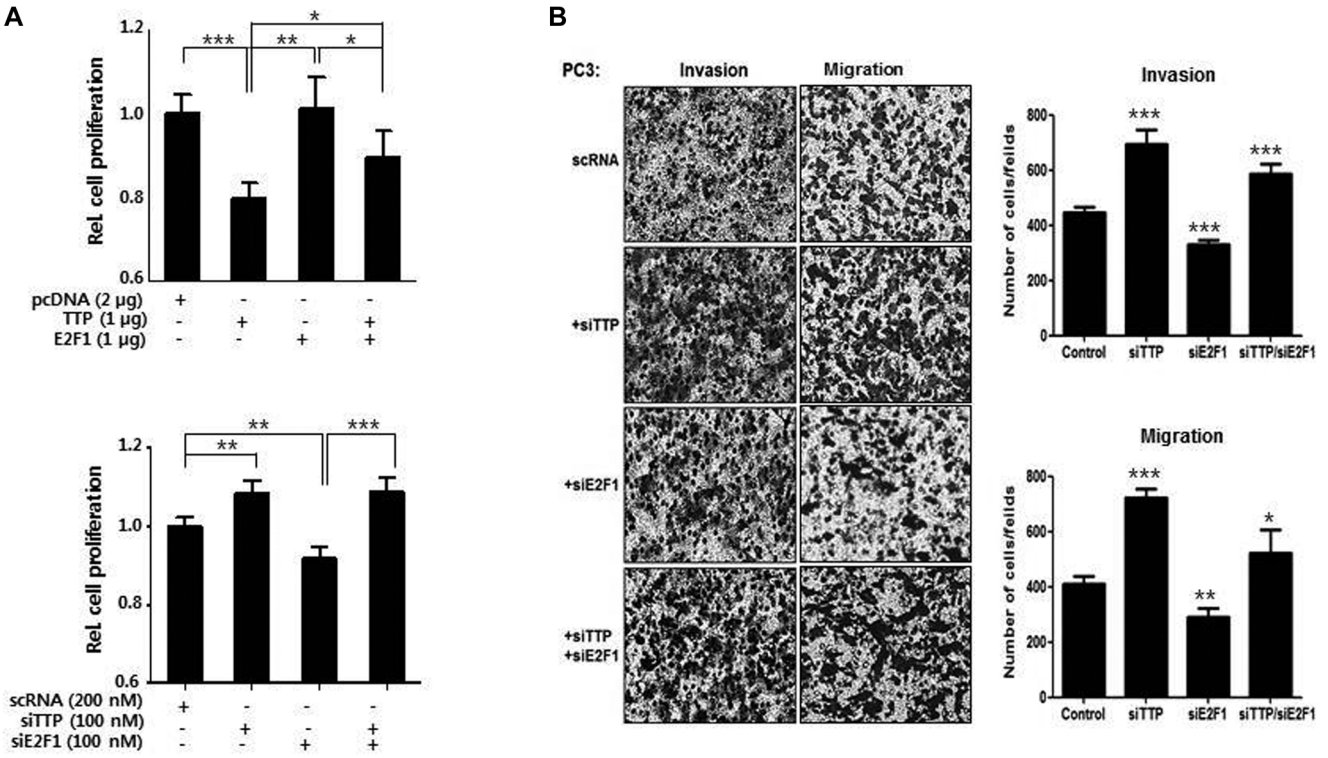

Fig. 4. Change of TTP expression level affects the tumor progression of PC3 cells.
(**A**) *In vitro* proliferation of PC3 cells transfected with control vector pcDNA6 and pcDNA6/V5-TTP or control-siRNA and TTP-siRNA. Cells were seeded at 1×10^4 cells per well in 96-well plates. Cell viability was performed at the indicated times using a MTT cell proliferation assay. The data represent the mean ± SD of three different experiments (*$P < 0.01$). (**B**) E2F1 was able to potently promote migration and invasion by PC3 cells after transfection of TTP as seen in a Boyden-chamber assay. Migrating and invading cells could be visualized using Diff Quick solution staining of the membranes and were counted (five fields/well). Graphical representation of the results, PC3 cells (*$P < 0.05$).

idea, we found that the inhibition of TTP using siRNA increased the E2F1 expression levels in prostate cancer cells in the absence of added growth factors. Moreover, overexpression of TTP suppressed the expression of E2F1 as well as cell proliferation and mobility. The expression of E2F1 regulated the growth of cells overexpressing or silencing TTP, indicating that the inhibitory effect of TTP on cell growth is mediated by the regulation of E2F1.

It has been reported that the oncogenic and pro-survival potential of E2F1 contributes to tumor development and promotes the cell cycle through regulating the transcription of genes that are essential for DNA synthesis and cell-cycle progression. These finding are important because they provide evidence that the development of effective cancer drugs may be possible by regulating E2F1 expression. The function of E2F1 is not only to induce the cells to enter the cell cycle but also to break the balance of growth and arrest signals [4]. Previous studies reported that abnormal expression of E2F1 was related to various human cancers, including erythroleukemia, breast, head and neck carcinomas, and non-small-cell lung carcinoma, where high levels of E2F1 were related to advanced disease and poor prognosis [5, 21, 22]. Moreover, overexpression of E2F1 elevated the development of skin carcinoma and protected cells from UV-induced apoptosis in a $p53^{-/-}$ background [17, 20]. As Lee *et al*. [15] reported, p53 stimulated by the genotoxic agent doxorubicin induces the expression of TTP in human cancer cells, supporting the idea that induction of TTP expression by p53 regulates E2F1 expression.

In this study, we found that TTP expression is important for post-transcriptional regulation of E2F1 expression and the growth of cancer cells. We determined that E2F1 mRNA contains AREs in the 3′UTR, and TTP destabilized its mRNA by binding to the E2F1 mRNA ARE. As a result, TTP-mediated down-regulation of E2F1 leads to an inhibition in cancer cell growth as well as migration and invasion abilities of prostate cancer cell lines. In addition, these findings, coupled with the fact demonstrating the

benefits of E21 inhibition in many cancer models, suggest that pharmacologic activation of TTP or induction of TTP expression could be a candidate for a chemotherapeutic drug.

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