이학석사 학위논문

암세포에서 TTP의 전사 후 조절을 통한 PFKFB3의 하향 조절에 대한 연구

Tristetraprolin posttranscriptionally down-regulates PFKFB3 in cancer cells

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ABSTRACT

The enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases 3(PFKFB3) catalyzes the first committed rate-limiting step of glycolysis and is upregulated in cancer cells. The mechanism of PFKFB3 expression upregulation in cancer cells has not been fully elucidated. The PFKFB3 3'-UTR is reported to contain AU-rich elements(AREs) that are important for regulating *PFKFB3* mRNA stability. However, the mechanisms by which *PFKFB3* mRNA stability is determined by its 3'-UTR are not well known. We demonstrated that tristetraprolin(TTP), an ARE-binding protein, has a critical function regulating *PFKFB3* mRNA stability. Our results showed that *PFKFB3* mRNA contains three AREs in the 3'-UTR. TTP bound to the 3rd ARE and enhanced the decay of *PFKFB3* mRNA. Overexpression of TTP decreased PFKFB3 expression and ATP levels but increased GSH level in cancer cells. Overexpression of PFKFB3 cDNA without the 3'-UTR rescued ATP level and GSH level in TTP overexpressing cells. Our results suggested that TTP post-transcriptionally downregulated PFKFB3 expression and that overexpression of TTP may contribute to suppression of glycolysis and energy production of cancer cells in part by downregulating PFKFB3 expression.

INTRODUCTION

The metabolic pathway of glycolysis converts glucose to pyruvate. Cancer cells prefer to metabolize glucose by glycolysis for energy production and provision of precursors for macromolecule biosynthesis even in the presence of oxygen. This phenomenon is known as the Warburg effect [1]. The altered metabolism of cancer cells is connected to enhanced glycolysis. The rate of glycolysis is controlled by different mechanisms. A critical modulator is the conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6P2) by 6-phosphofructo-1-kinase (PFK-1). This is the first committed step and the rate-limiting step of glycolysis [2].

PFK-1 is allosterically stimulated by fructose 2,6-bisphosphate (F2,6P2). F2,6P2 increases the affinity of PFK-1 for F6P, allowing enhanced F1,6P2 synthesis and glycolytic flux [3]. The steady-state concentrations of F26BP are controlled by the family of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) that interconvert fructose-6-phosphate (F6P)and F2,6P2. Humans have four isozymes of PFKFB: PFKFB1, PFKFB2, PFKFB3, and PFKFB4. These isozymes have tissue-specific expression patterns and distinct kinase-to-phosphatase activities [4,5]. Among the four isozymes, PFKFB3 is of particular interest because it is overexpressed in cancers [5-7]. In cancer cells, PFKFB3 expression is induced by stimuli such as oncogenic Ras signaling [8,9] and hypoxia [8]. Inhibition of PFKFB3 by small molecule antagonists [10], Ras inhibition [11], or knockdown of PFKFB3 [12] decreases glycolytic activity and suppresses tumor growth.

The mRNA of *PFKFB3* contains multiple copies of AU-rich elements (AREs), which are AUUUA-destabilizing motifs, in its 3'-UTR. The mRNA has a short half-life due to these motifs [13,14], indicating posttranscriptional regulation of PFKFB3 expression. Studies report 4that miR-206 and miR-26b interact with the 3′-UTR of *PFKFB3* mRNA, enhancing decay of *PFKFB3* mRNA and attenuation of glycolysis in cancer cells [15,16]. However, these miRNAs do not target AREs in the 3'-UTR of *PFKFB3* mRNA. The destabilizing function of AREs is believed to be through regulation by ARE-binding proteins [17]. One of the most well characterized ARE-binding proteins is tristetraprolin (TTP), which promotes degradation of ARE-containing transcripts [18,19]. TTP expression is reduced in many cancers [20-22]. This reduction may contribute to an increase in the levels of transcripts containing AREs in their 3'-UTRs.

We investigated the effect of TTP on the posttranscriptional regulation of PFKFB3 gene expression in human cancer cells. We report that overexpression of TTP decreased PFKFB3 expression levels in cancer cells. TTP bound to the 3rd ARE within the 3'-UTR of *PFKFB3* mRNA and promoted degradation of *PFKFB3* mRNA.

Mutations in the 3rd ARE prevented TTP binding to the motif and degradation of a luciferase gene containing the PFKFB3 3'-UTR. Overexpression of TTP in cancer cells decreased ATP synthesis and significantly inhibited the growth of cells, and transfection of *PFKFB3* cDNA without the 3'-UTR restored ATP synthesis and cell growth. Our findings suggested that TTP inhibited expression of PFKFB3 through interaction with the 3rd ARE in the *PFKFB3* 3'-UTR and that low levels of TTP observed in cancers [20] may be a key mechanism for upregulation of PFKFB3 observed inhuman cancers.

MATERIALS AND METHODS

1. Cell culture

Human cancer cell lines A549, H1299, H1975, MCF-7, and MDA-MB-231were from the Korean Cell Line Bank (KCLB-Seoul, Korea). MDA-MB-231 cells were cultured in Dulbecco's modified Eagle medium. A549, H1299, H1975, and MCF-7 cells were cultured in RPMI 1640 media. All cell lines were supplemented with 10% heat-inactivated fetal bovine serum (Welgene, Korea) and maintained at 37℃ in a humidified atmosphere of 5% CO2.

2. Plasmids, small interfering RNAs, transfections, and dual-luciferase assays

The pcDNA6/V5-TTP construct was described previously [23]. The pCMV3-PFKFB3-GFPSpark construct was from Sinobiological (HG13230-ACG). MDA-MB-231 and H1299 cells were transfected with pcDNA6/V5-TTP or pCMV3-PFKFB3-GFPSpark using the TurboFect™ *in vitro* transfection reagent (R0531, Thermo Scientific). Small interfering RNAs (siRNAs) against human TTP (TTP-siRNA, sc-36760) and control siRNA (scRNA, sc-37007) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). MCF-7 and A549 cells were transfected with TTP-siRNA or scRNA using Lipofectamine™ RNAiMAX (13778-150, Invitrogen) and harvested after 24 h incubation. Expression of TTP or *PFKFB3* mRNA and protein was analyzed by RT-PCR and western blots. Full-length *PFKFB3* 3'-UTR containing all three AREs was amplified from MDA-MB-231 cDNA using PCR primers *PFKFB3*-3′-UTR Full-U, 5'- CCGCTCGAGGGCAGACGTGTCGGTTCCAT-3'; *PFKFB3*-3′-UTR-Full-D, 5'-ATAAGAA TGCGGCCGCCCAATTAACAATGAAACCCA-3'. Underlined sequences are restriction enzyme sites. PCR products were inserted into the *XhoI/NotI* sites of the psiCHECK2 Renilla/Firefly dual-luciferase expression vector (Promega) to generate psiCHECK2-*PFKFB3* 3'-UTR-Full. Oligonucleotides containing the third AUUUA motifs (Oligo-ARE3 W) of the *PFKFB3* mRNA 3'-UTR were synthesized by ST Pharm. Co., Ltd. (Korea). Oligo-ARE-F, 5'-TCGAGATTTTGATAGCAGATGTGCTATTTA TTTATTTAATATGTATAAGGAGCCTAAAGC-3'; Oligo-ARE-R, 5'-GGCCGCTTTAGGCTCC TTATACATATTAAATAAATAAATAGCACATCTGCTATCAAAATC-3'. Oligonucleotides were inserted into the XhoI/NotI sites of the psiCHECK2 expression vector. A mutant oligonucleotide with AGCA substituting for the AUUUA motif (Oligo-ARE3M) was also synthesized and ligated into the *XhoI/NotI* site of psiCHECK2. Oligo-ARE-M-F, 5'-TCGAGATTTTGATAGCAGATGTGCTAGCAATATGTATAAGGAGCCTAAAGC-3'; Oligo-ARE-M-R,5'-GGCCGCTTTAGGCTCCTTATACATATTGCTAGCACATCTGCTATCAAA ATC-3'. For luciferase assays, cells were co-transfected with indicated psiCHECK2-*PFKFB3* 3′-UTR constructs and pcDNA6/V5-TTP using in vitro transfection reagent (TurboFectTM, R0531,Thermo Scientific). Transfected cells were

lysed with lysis buffer and mixed with luciferase assay reagent (017757, Promega). Chemiluminescent signal was measured using a microplatereader (SpectraMax L, Molecular Devices). Firefly luciferase was normalized to Renilla luciferase for each sample. Luciferase assays represent at least three independent experiments, from three wells per transfection.

3. SDS-PAGE analysis and immunoblotting

Proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes (10600001, GE Healthcare) and probed with appropriate dilutions of anti-TTP (SAB4200505, Sigma), anti-PFKFB3 (#13123, Cell signaling) and anti-β-actin (A5441, Sigma). Immunoreactivity was detected using an ECL detection system (GE Healthcare). Films were exposed at multiple time points to ensure images were not saturated. If required, band densities were analyzed with NIH image software and normalized by comparison to densities of internal control β-actin bands.

4. RNA kinetics, quantitative real-time PCR, and semiquantitative RT-PCR

For RNA kinetic analysis, we used actinomycin D (A9415, Sigma) and assessed *PFKFB3* mRNA expression using quantitative real-time PCR (qRT-PCR). Two micrograms DNase Itreated total RNA was reverse transcribed using oligo-dT (79237, Qiagen) and MMLV reverse transcriptase (3201, Beamsbio) according to the manufacturer's instructions. QRT-PCR was performed by monitoring increased fluorescence in real-time with SYBR Green dye (MasterMix-R, Abm) using a real-time PCR system (StepOnePlusTM, Applied Biosystems). Semiquantitative RT-PCR (semi-qRT-PCR) used Taq polymerase 2X premix (Solgent) and appropriate primers. PCR primer pairs were: TTP, 5'-CGCTACAAGACTGAGCTAT-3' and 5'-GAGGTAGAACTTGTGACAGA-3'; PFKFB3, 5'- ATCTACCTGAACGTGGAGTCCGTCTG-3' and 5'- TCAGTGTTTCCTGGAGGAGTCAGC-3'; GAPDH, 5'-ACATCAAGAAGGTGGTGAAG-3' and 5'-CTGTTGCTGTAGCCAAATTC-3'. The mRNA half-life was calculated by nonlinear regression of mRNA at 15-, 30-, 45-, 60- and 75-min timepoints following addition of actinomycin D using GraphPad Prism 5.00 software based on a one-phase exponential decay model.

5. Ribonucleoprotein immunoprecipitation assays

To determine binding of TTP to PFKFB3 ARE, ribonucleoprotein immunoprecipitation (RNP) assays were conducted as described previously [23]: 1 x 10^7 MDA-MB-231 cells were cotransfected with 10 μg pcDNA6/V5-TTP and psiCHECK2-PFKFB3-Oligo-ARE3 W or psiCHECK2- PFKFB3-Oligo-ARE3 M. At 24 h after transfection, cell suspensions were incubated in 1% formaldehyde for 20 min at room temperature. Reactions were stopped with 0.25 M glycine (pH 7.0) and

cells were sonicated in modified radioimmune precipitation assay buffer containing protease inhibitors (Roche Applied Science). RNP complexes were immunoprecipitated using protein G-agarose beads preincubated with 1 μg anti-V5 Tag antibody (GWB-7DC53A, Genway Biotech) or 1 μg isotype control (Sigma). After crosslink reversion at 70℃ for 45 min, RNA was isolated from immunoprecipitates and treated with DNase I (Qiagen). From the RNA, cDNA was synthesized and the Renilla luciferase gene amplified by PCR using Taq polymerase and Renilla luciferase-specific primers (Up, 5'-ACGTGCTGGACTCCTTCATC-3'; and Down, 5'-GACACTCTCAGCATGGACGA-3'). TTP proteins in immunoprecipitated samples were detected by western blot using anti-V5 Tag.

6. ATP assays

Cellular ATP levels were measured using CellTiter-Glo luminescent cell viability assay kits (G7571; Promega) according to the manufacturer's instructions. MDA-MB-231cells were plated on 96-well white-walled plates with clear bottoms in 100 μl culture medium, and 100 μl CellTiter-Glo reagent was added to each well. Contents were mixed for 2 min on an orbital shaker to induce cellular lysis, followed by incubation at room temperature for 10 min to stabilize the signal. Luminescence was recorded immediately.

7. GSH assay

GSH level was measured using glutathione colorimetric assay kits (K261-100; BioVision) according to the manufacturer's instructions: Cells (1×10^6) were collected by centrifugation at 700 x g for 5 min and glutathione buffer was added to lysis the cells. Lysate (80 ul) was added to 96-well plates with 160 ul reaction buffer and 20 ul of substrate solution to generate GSH. Absorbance at 415 nm was determined for each well a plate reader/spectrophotometer (EPOCH2, BioTek, America).

8. Statistical analysis

For statistical comparisons, p values were determined using Student's t-test or one-way analysis of variance (ANOVA) and $p < 0.05$ was consider significant.

RESULTS

1. TTP decreases PFKFB3 expression

TTP is an ARE-binding protein that downregulates the expression of genes containing ARE in their mRNA 3'-UTR [18,19]. PFKFB3 mRNA has a short half-life due to AREs in its 3'-UTR [13,14]. Therefore, expression of PFKFB3 might be posttranscriptionally regulated by TTP. To confirm this hypothesis, we examined TTP expression by RT-PCR in five human cancer cell lines: MCF-7, MDA-MB-231, A549, H1299 and H1975. Expression of TTP was high in MCF-7 and A549 cells but low in MDA-MB-231, H1299 and H1975 cells (Fig. 1A). We next determined if overexpression of TTP downregulated PFKFB3 expression in MDA-MB-231 and H1299 cells. Cells were transfected with a TTP expression vector (pcDNA6/V5-TTP) or the empty pcDNA6/V5 vector as a negative control. Overexpression of TTP in MDA-MB-231 and H1299 cells was confirmed by RT-PCR and western blots (Fig. 1B, C). Overexpression of TTP significantly inhibited the level of PFKFB3 mRNA and protein (Fig. 1B, C).

We tested if inhibition of TTP affected PFKFB3 expression using MCF-7 and A549 cells expressing high levels of TTP. These cells were transfected with siRNA against TTP to reduce TTP expression. Although treatment with nonspecific siRNA (scrambled siRNA) did not induce a change in PFKFB3 expression, inhibition of TTP by siRNA significantly increased PFKFB3 expression (Fig. 1D, E). These results indicated that TTP downregulated PFKFB3 expression in human cancer cells.

2. TTP decreases expression of luciferase mRNA containing the PFKFB3 3′-UTR

Consistent with previous reports [13,14], we detected three AUUUA ARE motifs in the 2603-base pair human PFKFB3 3'-UTR (Fig. 2A). To determine if downregulation of PFKFB3 expression by TTP was mediated through the PFKFB3 mRNA 3′-UTR, we used a luciferase reporter gene linked to the full PFKFB3 3′-UTR containing all three AREs in the plasmid psiCHECK2. When MDA-MB-231 cells were transfected with a plasmid overexpressing TTP, luciferase activity from the full PFKFB3 3′-UTR was significantly inhibited (Fig. 2B). The third ARE has overlapping AUUUA motifs (AUUUAUUUAUUUA). To determine if the third ARE in PFKFB3 responded to TTP, we generated a luciferase reporter gene linked to oligonucleotides (Oligo-ARE3 W) containing the 3rd ARE in the plasmid psiCHECK2. To determine the importance of the third ARE, we prepared a luciferase reporter gene with mutant oligonucleotides (oligo-ARE3 M, with AGCA substituting for the AUUUA sequences). OligoARE3 W responded to TTP but oligo-ARE3 M did not (Fig. 2B). Taken together, these results demonstrated that the 3rd ARE motif in the PFKFB3 3′-UTR was responsible for TTP inhibition of PFKFB3 expression in

MDA-MB-231 cells.

3. TTP binds to the 3rd ARE, ARE3, in the PFKFB3 mRNA 3′-UTR

TTP downregulates the expression of ARE-containing genes by binding to ARE motifs within mRNA 3'-UTRs [18,19]. To determine if TTP interacted with the 3rd ARE of the PFKFB3 3′-UTR, MDA-MB-231 cells were cotransfected with pcDNA6/V5-TTP and psiCHECK2-oligoARE3 W (oligo-ARE3 W) or psiCHECK2-oligo-ARE3 M (oligo-ARE3 M). After immunoprecipitation with anti-V5 or control antibody (immunoglobulin G), the presence of TTP was determined by Western blots using anti-V5 (Fig. 3A, bottom). Total RNA was extracted from immunoprecipitates and luciferase mRNA was analyzed by RT-PCR using PCR primers specific to the luciferase gene. Amplified PCR products were observed in immunoprecipitates from cells cotransfected with oligo-ARE3 W and pcDNA6/V5-TTP (Fig. 3A, top). No PCR products were detected in samples from cells cotransfected with pcDNA6/V5-TTP and oligo-ARE3 M (Fig. 3A, top). PCR products were also not detected in immunoprecipitates obtained using control antibody. To determine if TTP-induced inhibition of PFKFB3 expression resulted from changes in PFKFB3 mRNA stability, the half-life of this mRNA was measured by qRT-PCR. MDA-MB-231 cells were transfected with pcDNA6/V5-TTP (MDA-MB-231/TTP) or the pcDNA6/V5 control vector (MDA-MB-231/pcDNA). In control MDA-MB-231/pcDNA cells, the half-life of PFKFB3 mRNA was 5.275 h after actinomycin D treatment. In TTP-overexpressing MDA-MB-231/TTP cells, the half-life was 1.45 h (Fig. 3B). These results demonstrated that TTP interacted with the 3rd ARE of PFKFB3 and enhanced degradation of PFKFB3 mRNA.

4. TTP reduces ATP levels but increases GSH levels of cancer cells

PFKFB3 is a key enzyme required for glycolytic flux. Inhibition of PFKFB3 reroutes glucose metabolism from glycolytic flux to the pentose phosphate pathway (PPP) [24,25]. We determined the effects of TTP expression on cellular levels of ATP, a product of glycolysis, and GSH, a product of the PPP. Significant decreases in ATP content were observed in TTP-overexpressing MDA-MB-231 cells. TTP overexpression increased GSH levels in MDA-MB-231 cells (Fig. 4A-C). Ectopic expression of PFKFB3 rescued the ATP content and GSH levels in TTP-overexpressing MDA-MB-231 cells (Fig. 4A-C). These results demonstrated that overexpression of TTP reduced expression of PFKFB3, followed by decreased glycolysis and increased PPP in cancer cells.

FIGURES

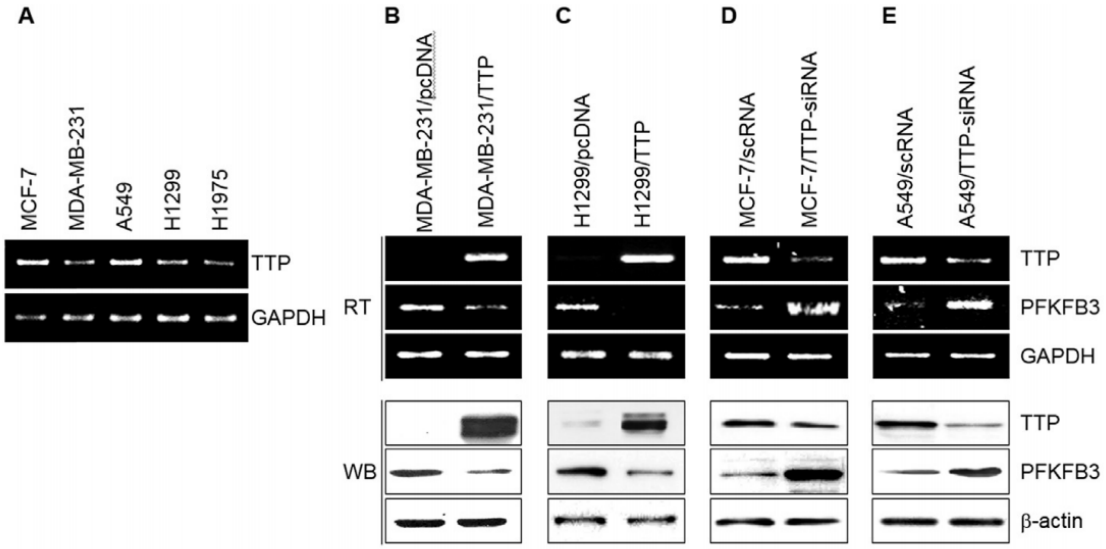

Figure 1. PFKFB3 expression is downregulated by TTP in cancer cells. (A) TTP expression in MCF-7, MDA-MB-231, A549, H1299, and H1975 cells was determined by RT-PCR. (B & C) Overexpression of TTP decreases PFKFB3 expression in cancer cells. (B) MDA-MB-231 and (C) H1299 cells were transiently transfected with pcDNA6/V5-TTP or pcDNA6/V5 for 24 h. Cells were analyzed by RT-PCR (top) and Western blot (bottom) for TTP and PFKFB3. (D & E) Inhibition of TTP by siRNA increased PFKFB3 expression in cancer cells. (D) MCF-7 and (E) A549 cells were transiently transfected with TTP-specific siRNA or scRNA for 24 h. Cells were analyzed by RT-PCR (top) and Western blot (bottom) for TTP and PFKFB3.

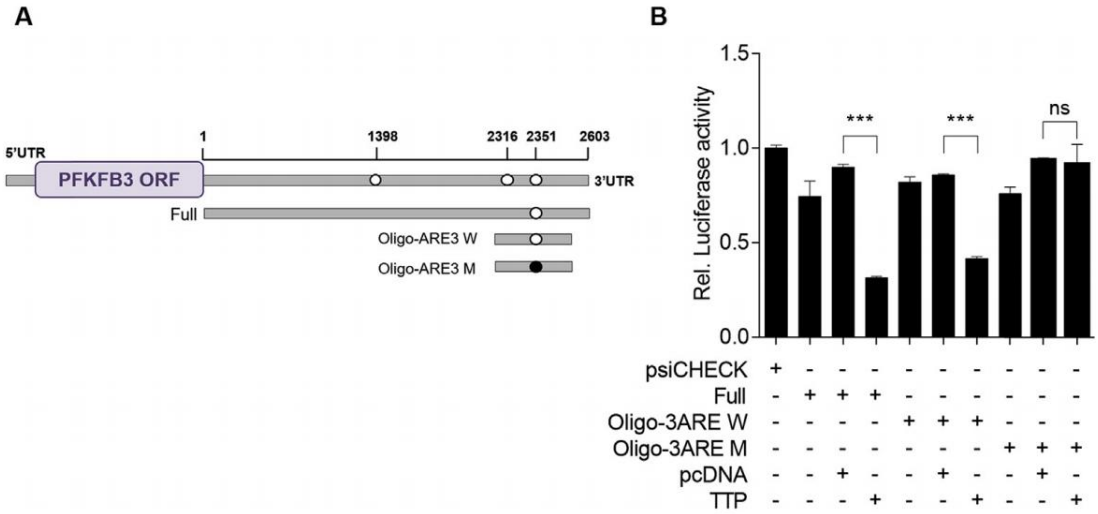

Figure 2. AU-rich element within the PFKFB3 mRNA 3'UTR is essential for TTP-mediated inhibition of PFKFB3 expression. (A) Schematic representation of luciferase reporter constructs used in this study. (B) The 3rd ARE within the PFKFB3 3′-UTR was necessary for TTP-mediated inhibition of PFKFB3. Full-length (Full) and oligonucleotides (Oligo) from the 2603-base pair PFKFB3 3′-UTR were cloned downstream of a luciferase reporter gene in the psiCHECK2 luciferase expression vector. White circles, wild-type (W) AREs; black circle, mutated (M) ARE. MDA-MB-231 cells were cotransfected with pcDNA6/V5-TTP and psiCHECK2 luciferase reporter constructs containing full-length, oligo-ARE3 W or oligoARE3 M. After normalization, luciferase activity from MDA-MB-231 cells transfected with psiCHECK2 vector alone was set to 1.0. Data are mean ± SD (n = 3; ***p < 0.001; ns, not significant).

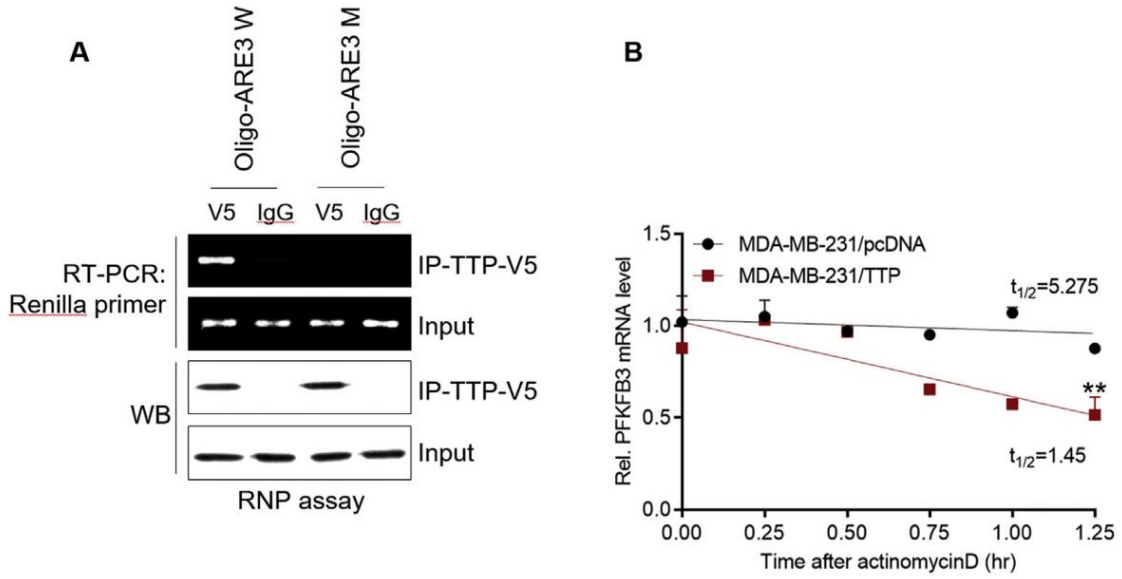

Figure 3. TTP enhances PFKFB3 degradation by binding to the ARE of the PFKFB3 3'-UTR. (A) Ribonucleoprotein immunoprecipitation assays. MDA-MB-231 cells were cotransfected with pcDNA6/V5-TTP and psiCHECK2 luciferase reporter constructs containing PFKFB3 oligo-3ARE W. The psiCHECK2 luciferase reporter construct containing mutant ARE-3, oligo-3ARE M was the negative control. At 24h after transfection, ribonucleoprotein complexes containing TTP were immunoprecipitated with protein G-agarose and anti-V5 or control antibody. Luciferase mRNA in immunoprecipitates was amplified by semi-qRT-PCR. TTP in immunoprecipitates was detected by Western blot with anti-V5. (B) TTP destabilized PFKFB3 mRNAs in cancer cells. MDA-MB-231 cells were transiently transfected with pcDNA6/V5-TTP or pcDNA6/V5 for 24 h. Expression of PFKFB3 in cells was determined by qRT-PCR at indicated times after addition of 5 μg/ml actinomycin D. mRNA half-life was calculated by nonlinear regression of mRNA levels at indicated times after addition of actinomycin D. Data are mean ± SD (n = 3; **p < 0.01).

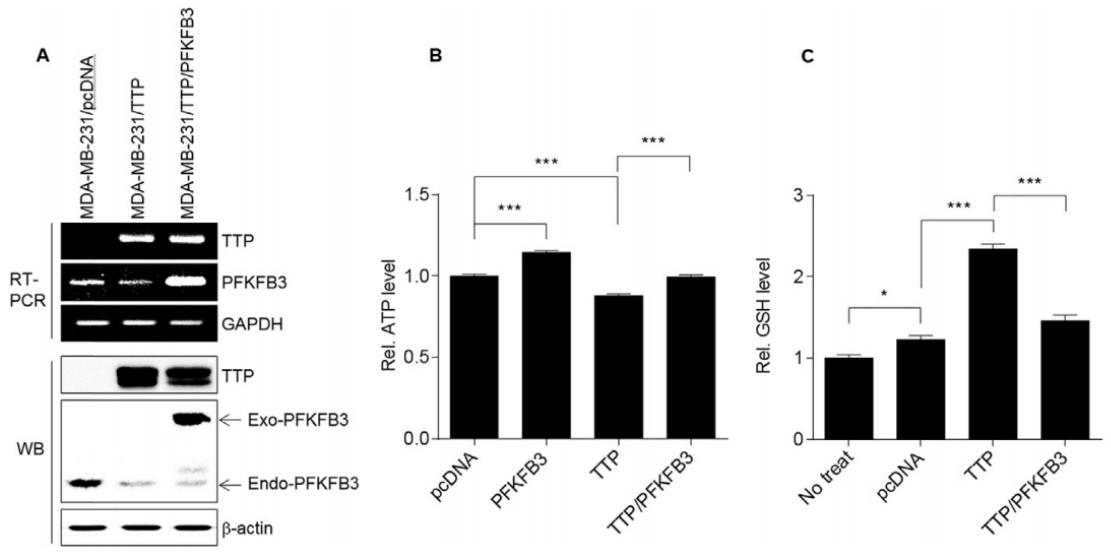

Figure 4. TTP-mediated downregulation of PFKFB3 decreases TTP level but increases GSH level in cancer cells. MDA-MB-231 cells were transiently transfected with empty vector pcDNA6/V5, pcDNA6/V5-TTP, or pcDNA6/V5-TTP and pCMV3-PFKFB3-GFPSpark for 48h. (A) TTP and PFKFB3 levels were determined by RT-PCR (top) and Western blot (bottom). Cells were analyzed for (B) ATP level and (C) GSH. Data are mean ± SD (n = 3; *p < 0.05; ***p < 0.001).

DISCUSSION

Cancer cells generally exhibit enhanced glycolysis. PFKFB3 is upregulated in a variety of tumor cells, and downregulation of PFKFB3 suppresses the growth of tumor cells by downregulating glycolytic flux [26]. PFKFB3 mRNA is reported to contain the AUUUA motif within its 3'-UTR and be important for destabilization of PFKFB3 mRNA [13,14]. In this study, we demonstrated that TTP, an ARE-binding protein, bound to the ARE motif of PFKFB3 mRNA and enhanced the decay of PFKFB3 transcripts. Mutation of the AUUUA motif in the PFKFB3 3'-UTR abrogated the binding of TTP to the PFKFB3 3'-UTR and TTP-induced inhibition of a PFKFB3 3'-UTR-containing luciferase gene. Our results suggest that TTP is a key negative regulator of PFKFB3 expression and inhibition of TTP led to increased PFKFB3 levels in cancer cells.

MicroRNAs such as miR-206 [15] and miR-26b [16] are reported to target PFKFB3 mRNA and downregulate PFKFB3 expression. Although these findings help explain the posttranscriptional regulation of PFKFB3 in cancer cells, they are not sufficient to explain the enhanced expression of PFKFB3 in cancer cells since the regulation may occur in other cells. In this study, we demonstrated that TTP inhibited PFKFB3 expression at the posttranscriptional level in cancer cells. Given previous reports that TTP expression is significantly decreased in many cancers [20], we hypothesized that the reason for the enhanced PFKFB3 level and enhanced glycolysis in cancer cells may be a low level of TTP. In support of this hypothesis, we found that inhibition of TTP by siRNA significantly increased PFKFB3 expression in cancer cells. In addition, restoring TTP expression suppressed the expression of PFKFB3 and ATP generation. Expression of PFKFB3 cDNA without the 3'-UTR restored the ATP generation in cells overexpressing TTP, indicating that the inhibitory effect of TTP on ATP generation was mediated by downregulation of PFKFB3.

Cancer cells preferentially use glycolysis for energy metabolism [1] and PFKFB3 is essential for glycolytic flux [7]. Inhibition of PFKFB3 is reported to suppress glycolytic flux while stimulating the PPP [24,25]. Thus, we predicted that inhibition of PFKFB3 would decrease intracellular levels of ATP, a glycolytic product, and increase GSH, a PPP product.

Consistent with this prediction, we found that downregulation of PFKFB3 by TTP decreased ATP and increased GSH levels in cancer cells. We previously reported that TTP overexpression decreases intracellular TTP levels through downregulation of α-synuclein [27] and hexokinase 2 (HK2) [28]. Thus, we hypothesized that even though we did not determine the effect of TTP overexpression on HK2 and α-synuclein levels, TTP overexpression might decrease intracellular ATP levels through downregulation of α-synuclein, HK2, and PFKFB3. We found that ectopic

expression of PFKFB3 rescued levels of ATP and GSH in TTP-overexpressed cancer cells, indicating that modulation of ATP and GSH levels by TTP was mediated by PFKFB3 downregulation. Collectively, our data suggested that TTP regulated balance between glycolysis and PPP through downregulation of PFKFB3 in cancer cells.

In conclusion, we demonstrated that TTP is important for the posttranscriptional regulation of PFKFB3 gene expression. We determined that PFKFB3 contains AREs in its mRNA 3'-UTR and TTP destabilized its mRNA by binding to the 3rd ARE of PFKFB3 mRNA. As a result, TTP-induced downregulation of PFKFB3 led to decreased glycolysis and intracellular ATP but increased GSH levels. This study therefore provided a molecular mechanism for the posttranscriptional regulation of PFKFB3 gene expression. Since TTP expression is inhibited in a variety of human cancer cells [20], PFKFB3 upregulation in cancer cells could be a consequence of low TTP levels in cancer. This study provided a molecular mechanism for the enhanced levels of PFKFB3 in cancer cells. TTP-mediated enhancement of PFKFB3 mRNA degradation expands our understanding of the regulation of PFKFB3 expression in cancer cells.

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국 문 요 약

6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases 3(PFKFB3) 효소는 당 분해의 첫 속도 제한 단계를 촉매하고 암 세포에서 상향조절 되어있다. 암세포에서 PFKFB3 발현 상향조절의 기작은 아직 완전히 설명되진 않았다. PFKFB3 3‘-UTR은 PFKFB3의 mRNA 안정성을 조절하는데 중요한 역할을 하는 AU-rich elements(AREs)를 포함하고 있다고 알려져 있다. 하지만 PFKFB3 mRNA 안정성이 어떻게 스스로의 3'-UTR에 조절되는지에 대한 기작은 잘 알려져 있지 않다. 이 논문에서 우리는 ARE에 결합하는 단백질인 tristetraprolin(TTP)가 PFKFB3 mRNA 안정성을 조절하는데 중요한 역할을 하는 것을 밝혔다. 또한 우리는 PFKFB3 mRNArk 3'-UTR에 AREs 3개를 지니고 있는 것을 확인했다. TTP는 3번째 ARE에 달라붙고 PFKFB3 mRNA의 분해를 촉진한다. 암세포에서 TTP 과발현은 PFKFB3 발현과 ATP 수준을 낮추었지만 GSH 수준은 증가시켰다. 3’-UTR이 없는 PFKFB3 cDNA의 과발현은 TTP를 과발현 하는 세포에서 ATP와 GSH의 수준을 낮췄다. 종합하자면 TTP는 PFKFB3의 수준을 전사 후 조절을 통해 하향조절하고 이에 따라 암세포에서 PFKFB3 발현의 하향조절을 통해 해당과정과 에너지 생산을 억제할 수도 있다는 것을 밝혔다.