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The role of tristetraprolin in cancer and inflammation

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1. ABSTRACT

Messenger RNA decay is a critical mechanism to control the expression of many inflammation- and cancer-associated genes. These transcripts are targeted for rapid degradation through AU-rich element (ARE) motifs present in the mRNA 3' untranslated region (3'UTR). Tristetraprolin (TTP) is an RNA-binding protein that plays a significant role in regulating the expression of ARE-containing mRNAs. Through its ability to bind AREs and target the bound mRNA for rapid degradation, TTP can limit the expression of a number of critical genes frequently overexpressed in inflammation and cancer. Regulation of TTP occurs on multiple levels through cellular signaling events to control transcription, mRNA turnover, phosphorylation status, cellular localization, association with other proteins, and proteosomal degradation, all of which impact TTP's ability to promote ARE-mediated mRNA decay along with decay-independent functions of TTP. This review summarizes the current understanding of post-transcriptional regulation of ARE-containing gene expression by TTP and discusses its role in maintaining homeostasis and the pathological consequences of losing TTP expression.

2. INTRODUCTION

Messenger RNA turnover is a tightly regulated process that plays a central role in the regulation of mammalian gene expression (1). The significance of this level of regulation is evident in disease pathogenesis, where loss of post-transcriptional regulation contributes to overexpression of many genes encoding growth factors, inflammatory mediators, and proto-oncogenes (2, 3). A conserved mRNA sequence element found in a majority of these inflammation- and cancer-associated genes is the adenylate and uridylate-rich element (ARE). This *cis*-acting regulatory element when present within the 3' untranslated region (3' UTR) of the mRNA mediates events controlling the fate of the transcript through interaction with *trans*-acting RNA-binding proteins. The significance of AREs as post-transcriptional regulatory elements is evident in that approximately 8-10% of the human transcriptome are ARE-containing transcripts (4).

One of the best-characterized ARE-binding proteins is tristetraprolin (TTP). TTP is the prototypic member of the TIS11 family of RNA-binding proteins and the human TIS11 family consists of three members: TTP

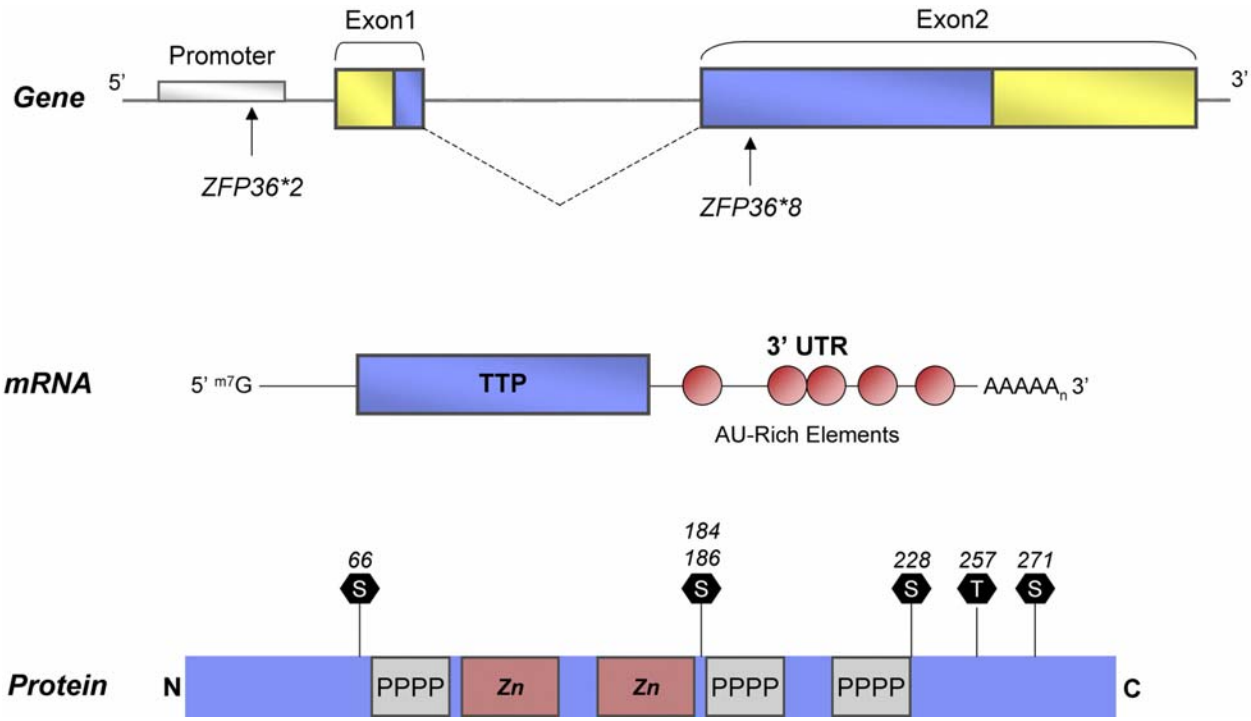

Figure 1. Schematic showing the organization of TTP gene, mRNA, and protein. Human TTP gene, ZFP36, consists of two exons and one intron. The arrows represent the genomic location of two SNPs that have been associated with rheumatoid arthritis. ZFP36*2 is located in the promoter, and ZFP36*8 lies in the protein coding region of the second exon. TTP mRNA contains ARE-motifs in the 3' UTR. TTP protein structure displays unique features including three tetra-proline (PPPP) repeats, two CCCH-type zinc (Zn) finger domains, and several serine/threonine phosphorylation sites.

(TIS11, ZFP36), BRF-1 (TIS11b, ZFP36L1) and BRF-2 (TIS11d, ZFP36L2) (5). TTP was identified as an immediate-early response gene whose expression was induced in various cell types in response to phorbol ester, insulin, serum, and other mitogenic stimuli (6-9). The ZFP36 gene encodes a proline-rich, zinc finger protein of ~36 kDa with three repeats of the PPPP-motif giving rise to the more commonly used name of tristetraprolin or TTP (7). TTP's role in post-transcriptional gene regulation was identified in a seminal study from the Blackshear laboratory that established the role of TTP as a key player in promoting decay of ARE-containing mRNAs (10). Further advances have identified distinct signaling pathways that regulate the expression and function of TTP through protein phosphorylation, sub-cellular localization, and interaction with other cellular proteins. This review summarizes the current understanding of TTP's role in regulating gene expression, and how TTP is a necessary component in maintaining normal physiological processes. Additionally, we review how loss of TTP-mediated gene regulation can lead to severe pathological consequences, as evidenced in chronic inflammatory diseases and cancer.

3. TTP STRUCTURE, FUNCTION AND REGULATION

3.1. Gene

Tristetraprolin (TTP, TIS11, ZFP36, NUP475, GOS24) was initially identified as a TPA (12-*O*-tetradecanoylphorbol-13-acetate) inducible sequence in cultured murine fibroblasts (8, 9). The discovery of murine TTP facilitated the identification of human and rat homologs (11-13). Concurrent work demonstrated that expression of TTP mRNA displayed a pattern characteristic of immediate-early response genes, showing low-to-undetectable levels of TTP under quiescent growth conditions and rapid transient induction in TTP mRNA levels when cells were stimulated with insulin, serum or growth factors (6, 7, 9, 14).

In humans, ZFP36 gene is located on chromosome 19q13.1 (12), and consists of two exons and one intron (Figure 1).

Characterization of the 5'-proximal region of ZPF36 had identified various promoter elements implicated in regulating transcription in response to different stimuli (6, 15-17). To this extent, Lai *et al* found that a conserved 5'-proximal region of the TTP promoter that is essential for the serum-inducibility of TTP has consensus binding sites for several transcription factors, such as SP1, EGR-1, and AP2 (17). In addition, transcriptional regulatory elements identified in the single intron of murine *Zfp36* were shown to promote serum-stimulation of TTP expression, possibly through a concerted mechanism involving the proximal 5' promoter elements (16). Ogawa and colleagues found that transforming growth factor-beta (TGF-beta) treatment of human T cells induced expression of TTP mRNA, and this

induction was mediated through the binding of Smad3/4 transcription factors to the putative Smad-responsive binding elements present in the human TTP promoter (18). Consistent with this, other studies have shown that various growth-inhibitory cytokines (e.g. TGF-beta, interferons) as well as anti-inflammatory compounds and natural products such as glucocorticoids, green tea, and cinnamon can promote TTP transcription, which implies that the role of these factors in controlling immune responses and inflammation is in part through induction of TTP expression (18-23).

3.2. Messenger RNA

The human ZFP36 gene encodes for a mRNA transcript ~1.7 kb in length (Figure 1) with the protein coding region starting from the initial AUG, which is considered to be in the optimum context for translation initiation (7). Within the 3' UTR of TTP mRNA are contained AU-rich motifs that contribute to rapid mRNA decay and allows for TTP expression to return to low levels within 2-4 h after transcriptional induction (6, 7, 10, 13). The mitogen-activated protein kinase 38 (p38 MAPK) has been shown to promote TTP mRNA expression by altering ARE-mediated decay (24). This is consistent with findings showing increased stability of the ARE-mRNAs tumor necrosis factor-alpha (TNF-alpha) and cyclooxygenase-2 (COX-2) by p38 MAPK pathway activation (25). Moreover, TTP has been shown to bind to its own ARE and autoregulate its expression through a negative feedback loop (24).

3.3. Protein

TTP is the prototypic member of the CCCH-type zinc finger family of RNA-binding proteins. The amino acid sequence of TTP is abundant in proline (16%) and serine (17.5%) residues, and contains three proline-rich motifs, each represented as PPPP (Figure 1) (7). Another unique feature of TTP is the presence of two conserved tandem zinc-finger domains of the $CX_8CX_5CX_3H$ sequence (6, 13, 26). The integrity of TTP's zinc fingers was shown to be necessary for ARE binding and mRNA decay function (27). TTP protein levels are normally low and predominantly nuclear in quiescent cells, whereas mitogenic stimulation of cells induces TTP expression and promotes rapid TTP translocation from the nucleus into the cytoplasm (28). Phillips and colleagues found that the two zinc fingers of TTP are required for its nuclear import, however the ability of the zinc fingers to mediate nuclear import appears to be unrelated to its ARE-binding activity indicating that nuclear accumulation and RNA binding may involve different amino acid residues in the zinc finger structure (26). Furthermore, export of TTP from the nucleus requires a nuclear export sequence (NES) present at the amino terminal end of the protein and mutants with truncated NES are entirely nuclear (26).

3.4. Phosphorylation of TTP

Initial work characterizing the induction of TTP by serum-stimulation indicated it to be a phosphoprotein due to the apparent shift in its electrophoretic mobility (29). Mass spectrometric and site-directed mutagenesis studies have identified major sites of phosphorylation in human TTP (Figure 1) (30). These include S^{66}, S^{184}, S^{186}, S^{228}, T^{257}, and T^{271}, which correspond to S^{58}, S^{176}, S^{178}, S^{220}, T^{250}, and T^{264} in murine TTP. TTP is a downstream target of phosphorylation through a variety of signaling pathways, such as ERK/MAPK, p38 MAPK, JNK, and PKB/AKT (31-36). Various protein kinases, such as p38 MAPK, p38 MAPK-activated protein kinase 2 (MK2), and ERK2, have been reported that can directly phosphorylate TTP *in vitro* and/or *in vivo*, and TTP is a predicted kinase target based on phosphorylation motifs of PKA, glycogen synthase kinase 3 (GSK3), and ERK1 (31-37).

Based on the observation that mitogen-stimulated phosphorylation of TTP coincided with its rapid cytoplasmic trafficking, Taylor and colleagues evaluated the effect of protein phosphorylation on TTP's subcellular localization (28, 29). Phosphorylation of murine TTP at S^{220} (S^{228} in human TTP (30)) did not appear to have an effect on its nucleo-cytoplasmic shuttling, however this observation did not address the role of other phosphorylated residues in nucleo-cytoplasmic transport of TTP. The role of p38 MAPK/MK2-mediated TTP phosphorylation at serine residues S^{52} and S^{178} in mouse TTP (S^{60} and S^{186} in human TTP (30)), allowed for enhanced interaction between TTP and 14-3-3 adapter proteins (35, 38-40). This interaction with 14-3-3 protected TTP from dephosphorylation by protein phosphatase 2A (PP2A), and promoted its stability and cytoplasmic localization (38, 40).

The effect of phosphorylation on controlling TTP's ability to bind and target ARE-containing mRNAs for degradation has also been investigated. Some studies have provided evidence showing that TTP phosphorylation does not impact its ability to bind ARE-RNA sequences or promote decay (30, 33, 35, 39). By contrast, *in vitro* studies have indicated that unphosphorylated TTP displays stronger affinity for ARE-RNA sequences than phosphorylated TTP, suggesting that phosphorylation inhibits RNA-binding, and the unphosphorylated form of TTP is active in targeting mRNA for rapid decay (31, 34, 41).

Since mRNA decay is a multi-step process involving many factors, it is plausible that phosphorylation of TTP can modulate its interaction with other proteins, including components of the RNA decay machinery. In support of this, a recent finding has implicated that phosphorylation of TTP through activation of the p38 MAPK pathway blocks recruitment of CAF1 deadenylase complex and inhibits TTP-directed deadenylation of ARE-containing mRNAs (42). Interestingly, phosphorylation of TTP by MK2 kinase had no effect on the ARE-binding activity of TTP suggesting that deadenylase interaction with TTP may be impacted by phosphorylation (42). Based on these observations, it can be reasoned that stimulus-dependent induction of TTP expression followed by its phosphorylation and subsequent accumulation in an inactive form controls its ability to promote ARE-mediated decay during the initial phase of an inflammatory response

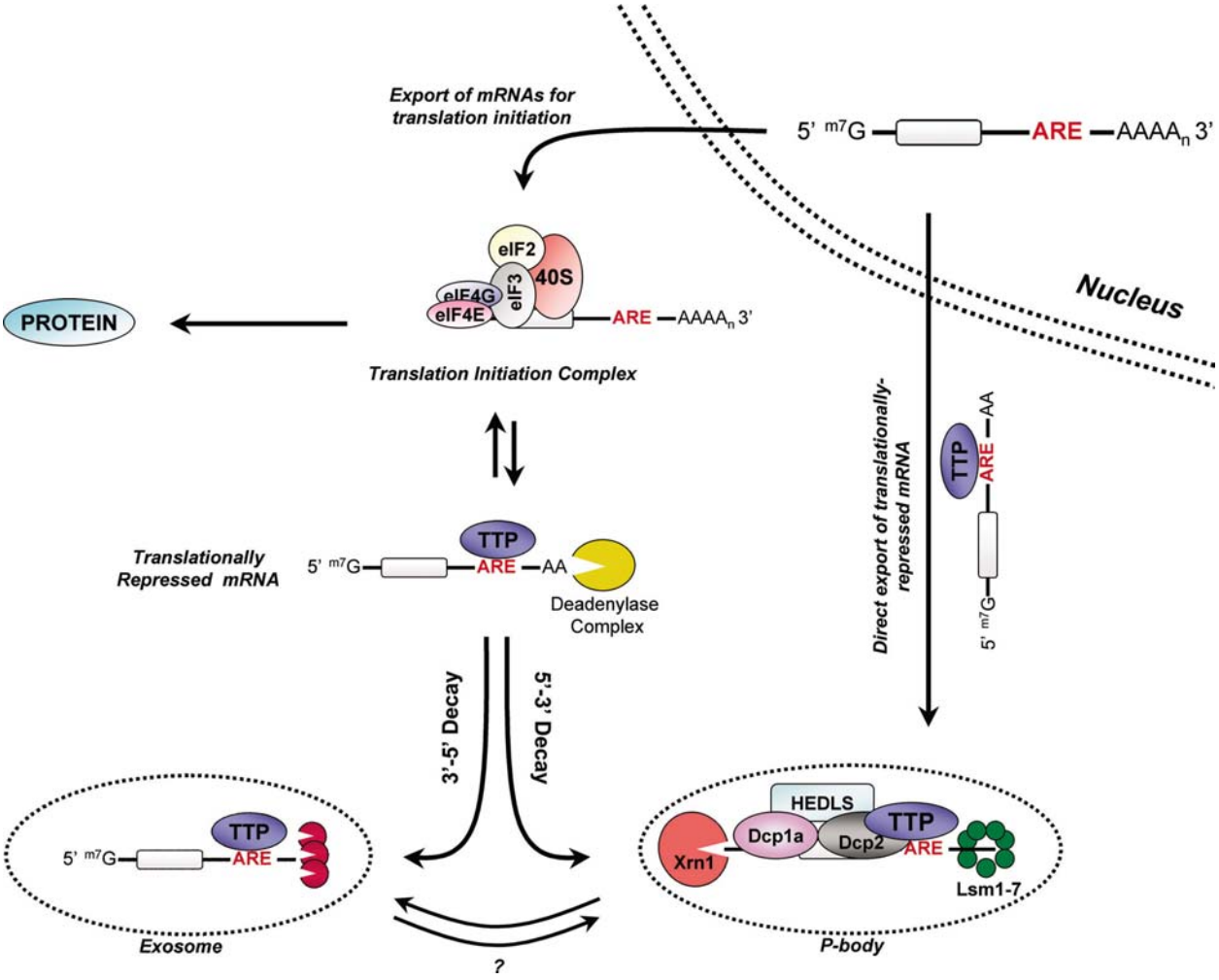

Figure 2. TTP's role in ARE-mediated mRNA decay. Processed mRNA transcripts are exported from the nucleus into the cytoplasm wherein the translation initiation complex is assembled. Cytoplasmic TTP associates with these transcripts *via* ARE binding and further recruits the deadenylase complex. Following deadenylation, the mRNA transcript can undergo 5'- 3' decay in P-bodies, where the transcript is decapped and the mRNA is stored or decayed by the exonuclease Xrn1. Alternatively, TTP can facilitate 3'- 5' exosome-mediated decay of target mRNAs. Under conditions of stress, TTP can be recruited to stress granules and may facilitate delivery of translationally-repressed ARE-containing mRNAs from stress granules to P-bodies for degradation.

(41). As the stimulus dissipates, phosphorylation-dependent repression of TTP is relieved, leading to targeted decay of ARE-containing mRNAs expressed during inflammation.

3.5. ARE-binding and mRNA decay

Based on its immediate-early gene expression pattern, nuclear localization, and zinc-finger structure, TTP was initially hypothesized to be a transcription factor acting downstream of growth factor signaling (29). Insight into its role as a mRNA decay factor came from the characterization of TTP knockout mice, which display a systemic inflammatory syndrome due to chronic TNF-alpha excess (43). In an attempt to elucidate the mechanism underlying this apparent TNF-alpha elevation, Carballo and colleagues found that TTP could directly bind to the ARE within the 3'UTR of TNF-alpha mRNA and target it for rapid degradation, thereby attributing excessive TNF-alpha levels to loss of TTP-mediated mRNA decay (10). Further efforts determined that TTP preferentially binds the nonameric sequence motif UUAUUUAUU, the core destabilizing element of many ARE-containing mRNAs (44-49). The ARE-binding activity of TTP was shown to be dependent on the CCCH residues of TTP's two zinc-finger domains and a single cysteine to arginine mutation in either of the zinc-finger domains attenuated ARE binding (27, 50). Interestingly, this non-binding mutant of TTP exert a dominant-negative effect by inhibiting TTP-mediated decay of TNF-alpha mRNA (50). Since its discovery, TTP has been identified to bind the AREs present in various inflammatory mediators and tumor promoting factors and limit their expression *via* rapid ARE-mediated mRNA decay (Table 1).

A primary mechanism underlying TTP-mediated ARE-mRNA decay lies in its capacity to promote mRNA deadenylation (Figure 2). TTP's ability to promote poly(A)

Table 1. TTP mRNA Targets

Cellular Process	mRNA Target
Inflammation and Cancer	Ccl2 (22) Ccl3 (22) CD86 (104) c-fos (105) cIAP2 (106) c-myc (107) COX-2 (54, 100) DUSP1 (104) E6-AP (93) E47 (53) GM-CSF (52) IDO (104) Interleukins (IL-2, IL-3 , IL-6, IL-10, IL-12) (108-112) IFN-gamma (113) iNOS (114) LATS2 (115) MHC (Class 1B and F) (104) MMP-1 (89) PAI-2 (116) SOD2 (104) TNF-alpha (10) TTP (117) uPA (89) uPAR (89) VEGF (86)
Cell Cycle	Cyclin D1 (107) MIP-2 (112) p21 (105) Plk3 (118)
Other	1,4-galactosyltransferase (119) Ier3 (120) PITX2 (121)

tail shortening in target mRNAs required the two zinc-finger domains of TTP necessary for the ARE binding (27, 51). Mammalian mRNA decay utilizes three major deadenylation complexes, the Ccr4/Caf1/Not (Caf1) complex, the poly A-specific ribonuclease (PARN) complex, and the Pan2/Pan3 complex (52-54). Among these, the Caf1 deadenylase complex has been implicated in ARE-mediated decay and co-immunoprecipitation experiments have identified a direct association between TTP and Caf1 (47, 54). *In vitro* studies have shown that TTP can promote PARN-dependent deadenylation of ARE-containing mRNAs, however TTP-mediated stimulation of PARN activity was independent of direct physical association, indicating a possible indirect interaction through other proteins to exist (47, 55). The Pan2/3 deadenylase complex has been suggested to catalyze initial poly(A) shortening, however no interaction was detected between TTP and Pan2/3 , nor did Pan2/3 depletion have any effect on TTP-directed deadenylation (42, 56)

Deadenylated mRNAs can take the route to decay either through a 5'- 3' or 3'- 5' decay pathway (Figure 2) (1, 57). At the 3' end, mRNA can be degraded by a multi-protein exosome complex (1). Initial studies had indicated that ARE-mRNAs are degraded primarily in the 3'- 5' direction by the exosome, and that TTP plays a key role in this pathway through its ability to interact with the exosome and recruit it to the AU-rich mRNAs (58). More recently, TTP's involvement in mediating decay occurring at the 5' end is becoming better understood. Decay is initiated with removal of 7-methyl guanosine cap by the decapping complex Dcp1/Dcp2, leaving the mRNA body susceptible to degradation by the 5'- 3' exonuclease Xrn1 (59, 60) (Figure 2). These components of the 5'- 3' decay complex have been found to co-localize at small cytoplasmic foci known as processing (P)-bodies (PBs), which also contain factors involved in translational silencing, and miRNA- and siRNA-mediated mRNA silencing (61-65). Through its ability to associate with P-body components such as Dcp1a, Dcp2, Hedls, hEdc3, and Xrn1, TTP can promote localized decay of ARE-containing mRNAs at P-bodies (66-68). Further studies to understand the role of TTP in P-body formation identified that when the cellular mRNA decay machinery is limiting or TTP is overexpressed, TTP could nucleate P-body formation and deliver ARE-mRNAs to P-bodies as a means to sequester them away from polysomes (69). Moreover, various studies have provided evidence that TTP can also localize to discrete sub-cellular structures in the cytoplasm called stress granules (SGs), which assemble in response to cellular stress, and comprise subsets of translationally arrested RNAs, stalled translational machinery such as 40S ribosomal subunit, eIF4E and eIF4G, and other ARE-binding proteins such as TIA-1 and TIAR (39, 70, 71). This ability of TTP to interact with components of P-bodies and stress granules exemplifies a potential mechanism by which TTP can mediate dynamic trafficking of cellular mRNAs between these two centers of RNA metabolism (72, 73). Although it remains to be determined, it is possible that depending upon the cellular requirements TTP mediates shuttling of translationally repressed mRNAs from stress granules to P-bodies, thereby establishing it as a versatile and essential link with regard to mRNA turnover. Recent work has implicated TTP as a novel mediator of microRNA-dependent post-transcriptional regulation (74). TTP was shown to be required for the ability of the ARE-targeting microRNA miR-16 to regulate ARE-mRNA turnover. Although TTP does not directly bind to miR-16, it associates with the argonaute (Ago) protein family members and promotes miR-16-mediated decay of ARE-containing mRNAs (74).

The ability of TTP to regulate mRNA turnover requires ARE-binding and recruitment of components of the cellular mRNA decay machinery to ARE-containing transcripts. This suggests that the ARE-mRNA pool, which comprises upto 5 - 8% of the entire transcriptome, can potentially be regulated by TTP (4). However, the parameters that influence substrate selection or decay kinetics are still not clear. Hau and colleagues have demonstrated that TTP binds to AU-rich sequences from TNF-alpha, IL-2, GM-CSF, IL-3, and c-fos transcripts with high affinity, however poor binding was seen for a U-rich sequence in c-myc mRNA (67). Consistent with this, TTP-dependent deadenylation and decay rates were shown to correlate with the binding affinities of these sequences, implicating that the sequence and context of AU-rich elements can influence degradation of target mRNAs. Novel mRNA targets for TTP were identified by global analysis of stabilized transcripts in embryonic fibroblasts from wild-type and TTP-deficient mice (120). Of 250 mRNAs stabilized in the absence of TTP, only 23 were found to contain potential AU-rich binding sites for TTP. A similar genome-wide assessment using TTP-deficient

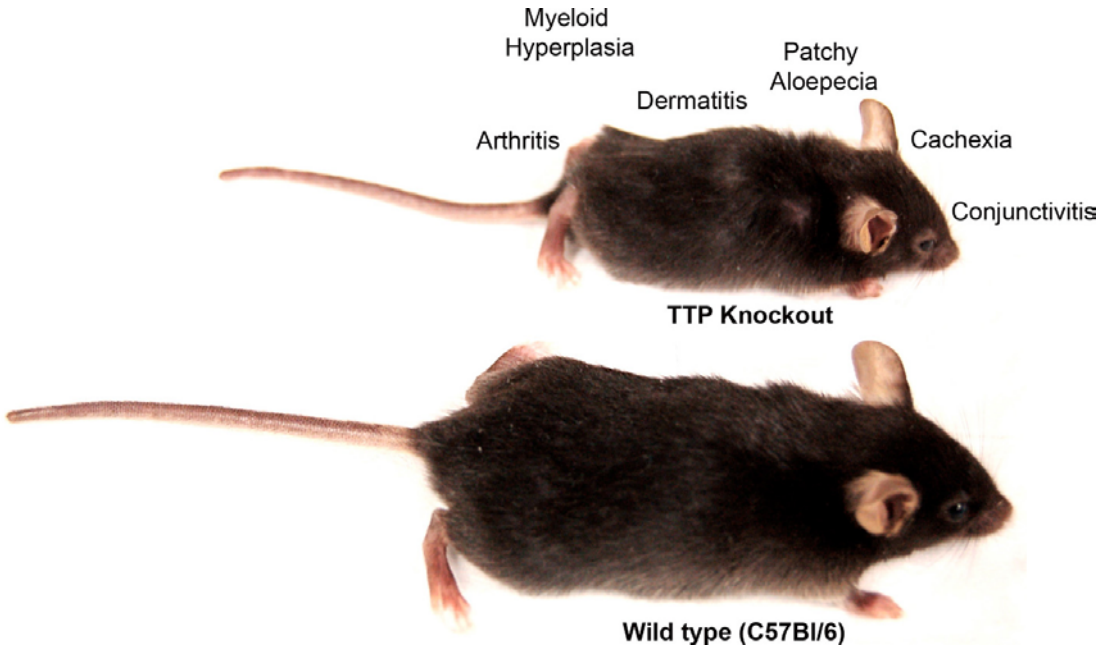

Figure 3. TTP knockout mouse. Eight-week old TTP knockout mice and wild-type C57Bl/6 are shown. Reduction in weight gain is apparent in the TTP knockout mouse. Symptoms of alopecia and dermatitis can be visualized as patches on skin showing hair loss and mild discoloration, respectively.

macrophages revealed that of all the ARE-containing transcripts associated with TTP, only a subset showed enrichment in TTP-deficient cells, implying that the ones remaining unchanged are either inherently resistant to TTP-mediated mRNA degradation (in that cell type) or are being rescued from TTP-mediated decay pathway *via* their interactions with other regulatory proteins (111). Furthermore, the roles of other RNA-binding proteins that may compete for ARE-binding, including TTP family members BRF-1 and BRF-2 and the stability factor HuR, can be major determinants influencing TTP-mediated regulation of target mRNAs (5). The differences seen in TTP's target profile therefore may not only be indicative of substrate abundance, but may also reflect cell-type dependent variations in the expression and function of other ARE-binding proteins. Together, these findings support that the association of an ARE-mRNA with TTP and the fate of the transcript depends on the integration of signals from multiple regulatory factors in a cell-specific manner.

4. ROLE OF TTP IN INFLAMMATION

The physiological role of TTP was first investigated by Taylor and colleagues by targeted disruption of the Zfp36 gene in mice (43). TTP-deficient mice appeared normal at birth, but within 1-8 weeks after birth developed a systemic autoimmune inflammatory syndrome characterized by cachexia, conjunctivitis, dermatitis, erosive polyarticular arthritis, and patchy alopecia (Figure 3). This inflammatory phenotype impacted the rate of weight gain where $TTP^{-/-}$ mice displayed 2- to 5-fold lower weights than the $TTP^{+/+}$ littermates and subcutaneous fat was essentially absent in these animals (43). Interestingly, this phenotype was dependent upon complete loss of TTP, since TTP haploinsufficiency did not attribute to this inflammatory phenotype.

Occurring with the systemic inflammatory syndrome exhibited in $TTP^{-/-}$ mice were several hematopoietic abnormalities including myeloid hyperplasia that resulted in enlarged spleen and lymph nodes, and thymic atrophy (43). Cellular studies revealed that the myeloid progenitor cells isolated from spleen and bone marrow of $TTP^{-/-}$ mice do not display hyperproliferative behavior in culture, nor did they respond differently to growth factor treatment, suggesting that the observed hyperplasia might be a consequence of increased *in vivo* hematopoietic signaling (43). Inflammatory abscesses were also observed in the liver and heart of $TTP^{-/-}$ mice along with high titers of antinuclear and anti-DNA antibodies in the serum.

On account of its resemblance to other chronic disorders where excess TNF-alpha expression is a contributing factor, the hyper-inflammatory phenotype of $TTP^{-/-}$ mice was attributed to overabundance of circulating TNF-alpha. Injecting TTP-deficient mice with antibodies to TNF-alpha at an early age prevented all aspects of the inflammatory phenotype, suggesting a potential role of TTP in regulation of TNF-alpha production *in vivo* (75, 76). To this extent, bone marrow-derived macrophages showed a marked decrease in the TNF-alpha mRNA decay in $TTP^{-/-}$ cells stimulated with endotoxin, resulting in a 5-fold increase in TNF-alpha expression (10, 77). Studies aimed at elucidating the mechanism of TNF-alpha elevation in $TTP^{-/-}$ mice demonstrated a functional interaction between TTP and TNF-alpha mRNA through the ARE present in the

3'UTR of TNF-alpha (10, 27). In support of this, mice deficient in TIA-1, an ARE-binding protein implicated in translational regulation, show increased levels of TNF-alpha and develop mild symptoms of arthritis, whereas, mice lacking both TIA-1 and TTP have more severe arthritis than either TIA-$1^{-/-}$ or $TTP^{-/-}$ mice, suggesting a crucial coordination between post-transcriptional and translational control of proinflammatory gene expression (78). Further studies using bone marrow stromal cells derived from $TTP^{-/-}$ mice revealed that the ARE-containing granulocyte monocyte-colony stimulating factor (GM-CSF) mRNA was another physiological target of TTP (52). Loss of TTP-mediated ARE-mRNA turnover therefore resulted in overexpression of GM-CSF in $TTP^{-/-}$ mice and contributed to myeloid hyperplasia in these mice (52).

As evidenced from the knockout mouse model, TTP is a critical physiological regulator of various pro-inflammatory cytokines, and altered TTP expression can be speculated to influence the onset and severity of inflammatory syndromes in humans, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and ulcerative colitis (UC). Previous studies have reported an inverse relationship between TTP expression in synovial tissue and the degree of inflammation with regard to the serum levels of C-reactive protein (CRP) in RA patients (79). To this extent, Carrick *et al* investigated the role of TTP in the development of autoimmune disorders by examining polymorphisms in the human ZFP36 gene across a large cohort of healthy individuals and patients with autoimmune diseases (80). In this study, 28 polymorphisms were identified out of which the single nucleotide polymorphism (SNP) 36*8, a C to T transition in exon 2 was associated with a higher incidence of RA in African-American individuals. Since the presence of this SNP is not predicted to alter the amino acid sequence of TTP, its functional consequence has been argued to negatively impact transcription, translation, or the stability of TTP mRNA. In a similar study of Japanese individuals, Suzuki *et al* identified a single nucleotide polymorphism, SNP359 A/G (reported as ZFP36*2 by Carrick *et al* (80)), within the ZFP36 promoter region that impacted promoter activity (81). Although no differences were observed in the allele frequencies of SNP359 A/G between healthy individuals and RA patients, the presence of the minor G allele inhibited TTP promoter activity approximately two-fold compared to the A allele. Moreover, patients with the GG genotype were observed to have an early age of disease onset when compared to the AA/AG genotypes suggesting that this SNP could possibly modulate disease activity by negatively impacting TTP expression on a transcriptional level. These findings indicate that genetic polymorphisms in the ZFP36 gene can alter physiological expression of TTP, and may serve as useful markers in determining disease activity and treatment options in inflammatory disorders.

TNF-alpha is a prominent activator of the NF-kappaB signaling cascade (82), and TTP is involved in controlling NF-kappaB signaling through its ability to mediate degradation of TNF-alpha mRNA (27). In this regard, recent studies have revealed a novel function of TTP in regulating NF-kappaB-dependent transcription that is independent of its ability to degrade TNF-alpha mRNA (83, 84). Schichl and colleagues demonstrated that attenuation of NF-kappaB activity by TTP expression was in part due to TTP's ability to interfere with the nuclear translocation of the p65 subunit of NF-kappaB (84). In another study, Liang *et al* found that TTP could physically interact with histone deacetylases (HDACs), HDAC-1, HDAC-3, and HDAC-7, and function as a co-repressor for NF-kappaB-dependent transcription (83). Together, these findings have identified novel cellular interactions of TTP that not only synergize with its mRNA-decay function to contribute to efficient regulation of pro-inflammatory gene expression, but also implicate a potential role in regulating inflammatory signal transduction.

5. ROLE OF TTP IN CANCER

Initiation and progression of tumorigenesis is characterized by aberrant overexpression of ARE-containing genes that encode factors promoting cell growth, inflammation, angiogenesis, and invasion (2, 3). This observed overexpression of ARE-containing genes is an outcome of defects in ARE-mediated mRNA decay, primarily due to alterations in *trans*-acting regulatory factors. Consistent with this, several lines of evidence have indicated that loss of TTP expression is a consistent feature in a variety of human malignancies, and re-expression of TTP induces cell type-specific growth inhibitory effects, implicating TTP as a potential tumor suppressor. In addition, a substantial body of evidence supports the idea that chronic inflammation can predispose an individual to cancer. Thus, as a physiological regulator of various pro-inflammatory mediators, TTP can exert anti-cancer effects through its ability to control anti-inflammatory gene expression.

5.1. Brain

Malignant gliomas are highly aggressive tumors originating from glial cells in the central nervous system. Previous work has demonstrated overexpression of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) in these tumors through selective ARE-mRNA stabilization by HuR, thereby promoting proliferation and angiogenesis (85). Along the same lines, Suswan and colleagues (55) examined the role of TTP in malignant glioma cells, and found that ectopic expression of TTP induced growth inhibitory effects through destabilization of VEGF and IL-8 mRNAs. Interestingly, TTP was expressed in both normal and brain tumor tissue, but the hyperphosphorylated forms of TTP predominated in gliomas, suggesting that phosphorylation of TTP may attenuate its ability to promote ARE-mediated decay in these tumors.

5.2. Breast

Suppression of TTP expression has been identified as a negative prognostic indicator in breast cancer that is associated with higher tumor grade and poor clinical outcome (86). In a recent study, Gebeshuber *et al* have demonstrated that this apparent loss of TTP expression in breast cancer could potentially be mediated by miR-29a, in cooperation with the oncogenic Ras

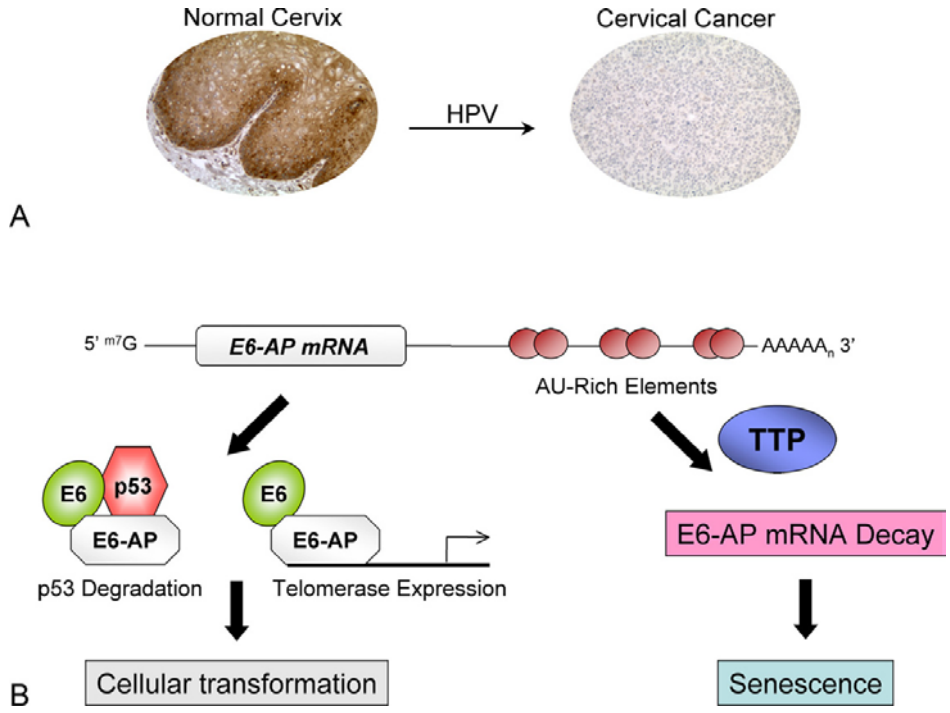

Figure 4. TTP-mediated regulation of E6-AP in cervical cancer. A) Immunohistochemistry of TTP expression in normal cervical tissue (left) and HPV-mediated cervical cancer (right). TTP expression (brown staining) is apparent in normal tissue and lost in tumor tissue. B) Binding of TTP to the ARE-containing mRNA of the ubiquitin ligase E6-AP targets it for rapid decay and subsequently counteracts HPV-mediated degradation of p53 tumor suppressor and hTERT transcription leading to cellular senescence.

signaling during breast cancer progression (87). MicroRNA profiling of human breast cancer cell lines and tissue specimens revealed a negative correlation between miR-29a and TTP levels, and overexpression of this microRNA in breast epithelial cells suppressed TTP expression. Restoring TTP expression to physiological levels subsequently led to reduced cell proliferation and invasion of metastatic breast cancer cells, which is consistent with previously reported anti-oncogenic effects of TTP in Ras-dependent mast cell tumor model (88). More recently, studies investigating the role of TTP in global regulation of ARE-gene expression in breast cancer have identified some novel ARE-mRNA targets namely, MMP1, uPA, and uPAR, that code for key proteins involved in invasion and metastasis (89).

5.3. Cervix

Cervical cancer is a high-risk human papillomavirus virus (HPV)-induced malignancy that develops as a consequence of the oncogenic functions of the viral oncoproteins E6 and E7 to inactivate the p53 and pRB tumor suppressor pathways, respectively (90, 91). Based on the findings of our laboratory and others, normal human cervix displays abundant TTP mRNA and protein expression (86, 92, 93). However, cervical cancer-derived tissues and cell lines showed loss of TTP expression suggesting that TTP may be an important player in the pathology of cervical cancer. Consistent with this, TTP re-expression in HPV18-transformed HeLa cervical cancer cells led to inhibition of cellular proliferation as a result of induction of cellular senescence (86, 93). Our studies have shown that TTP counteracted HPV-mediated cellular transformation by targeting the ARE-containing mRNA of the cellular ubiquitin ligase E6-associated protein (E6-AP) for rapid decay (Figure 4). This targeting of E6-AP mRNA for decay resulted in p53 protein stabilization and inhibition of telomerase (hTERT) gene expression ultimately leading to cellular senescence. Furthermore, previous studies have indicated that TTP overexpression can sensitize HeLa cells to certain proapoptotic stimuli such as TNF-alpha and staurosporine (86, 94, 95).

5.4. Colon

Normal physiology of colonic epithelium is an outcome of tightly regulated gene expression that controls constant epithelial cell turnover and self-renewal, and dysregulated ARE-mediated decay is a feature observed in colorectal cancer cells and tumors (96-98). This is evident in gene expression profiling of human colorectal cancers where selective enrichment of ARE-containing transcripts, including those involved in tumor progression and angiogenesis, is observed (99). Our recent work examining TTP expression in various stages of colorectal cancer has shown that loss of TTP expression occurs at early stages of tumorigenesis, and is concomitant with elevated HuR expression (100). Based on the opposing effects these RNA-binding proteins have on ARE-containing gene expression, it was found that loss-of-TTP and gain-of-HuR functions in colon cancer are required to promote expression of COX-2. Furthermore, ectopic expression of

TTP in colon cancer cells attenuates cell proliferation, and inhibits COX-2 expression, indicating that TTP can antagonize HuR-mediated overexpression of COX-2 in these cells. Other studies have identified tumor-suppressive effects of TTP in colon cancer cells through its ability to target VEGF and inhibit tumor vascularization (101).

5.5. Liver

Compared to normal liver, TTP expression in hepatocellular carcinoma (HCC) cell lines and tumors is significantly downregulated (102). Interestingly, this loss of TTP expression in HCC has been attributed to hypermethylation of a single CpG site in the Smad-binding region of the ZFP36 promoter. Inhibition of DNA methylation by 5-aza-deoxycytidine restored transcriptional induction of TTP in HCC cell lines in response to TGF-beta signaling, and subsequently led to TTP-mediated downregulation of c-myc mRNA and growth inhibition of HCC cells.

5.6. Other cancers

Tumors of lung, ovary, prostrate, and thyroid also show decreased TTP expression compared to the corresponding normal tissues, indicating that loss of TTP expression is a central event to the process of tumorigenesis. (86, 92). Recently, a study by Masuda *et al* has brought into light tissue-specific and age-dependent variations in the expression pattern of ARE-binding proteins (103). These findings show that TTP expression declined in tissues of advancing age with the exception of gastrointestinal tract and reproductive tissue, where TTP expression remained elevated throughout. This finding is in contrast to a cellular model of aging where human diploid fibroblasts undergoing replicative senescence displayed increased TTP levels. Since cellular senescence constitutes a natural tumor suppressive mechanism, understanding of the age and tissue distribution of TTP will assist in understanding how the expression and function of this post-transcriptional regulator is compromised during age-related cancers.

6. CONCLUSIONS

Over the last two decades, the role of TTP as a key player in post-transcriptional gene regulation has been established, particularly with regard to its function in promoting ARE-mediated decay of genes involved in cellular growth, inflammation, angiogenesis, and invasion. Based on our current knowledge, many distinct signaling events and pathways have been identified that appear to control TTP at the transcription, post-transcriptional, and post-translational level. For instance, the functional significance of TTP phosphorylation at various serine/threonine residues is evident in how this post-translational modification controls various aspects of protein behavior, including protein stability, sub-cellular localization, ARE-binding, and RNA decay. Further understanding of the cross-talk between the different signaling pathways that control TTP expression and mRNA decay function will aid in determining the varied impact these pathways have on gene expression. TTP has been shown to interact with components of RNA decay machinery within the 5'-3' and 3'-5' pathways. Since the two distinct mRNA decay mechanisms yield similar outcomes, an important question is to understand what role TTP has in specifying the selection between the two decay pathways. More recently, novel interactions of TTP with various cellular factors have been identified. Though independent of TTP's RNA-binding activity, these interactions synergistically act to control expression of ARE-containing transcripts, implicating a versatile role of TTP in regulating cellular RNA physiology.

As a physiological regulator of various inflammation- and cancer-associated factors, TTP has a fundamental ability to exert anti-inflammatory and tumor suppressor effects. It is evident from current research that loss of TTP expression allows for selective enrichment of an important subset of disease-associated factors through aberrant mRNA stabilization. This implies that loss of TTP-directed mRNA decay provides a significant growth advantage to cells and directly contributes to the respective disease etiology. Recent work has brought into light genetic and epigenetic factors that contribute to loss of TTP in cancer and inflammatory diseases, however our understanding of these mechanisms is still incomplete. Further efforts to understand the regulation of TTP expression and its function in normal versus diseased tissue is becoming exceedingly exciting for its wide-range of clinical applications in chronic inflammation and tumorigenesis.

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Abbreviations: TTP: tristetraprolin; ARE: AU-rich element; 3'UTR: 3' untranslated region; TNF-alpha: tumor necrosis factor-alpha; COX-2: cyclooxygenase-2; TGF-beta: transforming growth factor-beta; HPV: human papillomavirus virus

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