Recent advances in the role of RNA-binding protein, tristetraprolin, in arthritis

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ABSTRACT
The expression levels of cytokines and chemokines are strictly regulated at transcriptional and post-transcriptional levels. These small proteins are closely related to inflammatory diseases such as rheumatoid arthritis (RA). The purpose of this review is to highlight the potential utilization of tristetraprolin (TTP) as a therapeutic target in treating RA. TTP is the most notable and well-characterized RNA-binding protein that destabilizes mRNA of pro-inflammatory cytokines. TTP is thought to play an important role in RA because its target mRNA includes a lot of inflammatory cytokines such as TNFα. Post-translational modifications, especially phosphorylation, seem to be critical for the anti-inflammatory effects of TTP. Importantly, various mouse models, many of which are consistent with in vitro studies, are now available to elicit a more detailed understanding of the pathogenic role of TTP. The results of these multidisciplinary studies indicate that it is possible to improve inflammation by controlling TTP activity. Through this review, I propose that the use of recently developed mouse models and establishment of clever designs to target TTP will greatly contribute to future drug development to treat RA.

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1. Introduction
Changes in the expression of cytokines and chemokines are closely related to inflammatory diseases. It is important to understand the underlying mechanisms that regulate the gene expression of these proteins in diseases, because these molecules are potent therapeutic targets. For example, patients with rheumatoid arthritis (RA), one of the most common and representative inflammatory diseases characterized by high levels of cytokines and chemokines, have benefited from recent advances in biological drugs targeting these proteins [1,2].

The half-lives of mRNAs encoding cytokines and chemokines tend to be short. Previous reports have demonstrated that mRNA stability has a substantial influence on gene expression, which is comparable to transcriptional regulation [3]. One can imagine that a longer mRNA half-life of cytokine or chemokine can lead to an aberrant inflammatory process observed in diseases. Therefore, it is important to determine detailed mechanisms that regulate the mRNAs involved in cytokine or chemokine metabolism, so as to be able to identify novel targets for the treatment of cytokine/chemokine-related inflammatory diseases.

The half-life of mRNA is regulated through the interaction between cis elements at the 3′-untranslated region (3′-UTR) of the target mRNA and RNA-binding protein (RNA-BP) [4]. Historically, many RNA-BP have been reported and their pathological significance has been studied. In recent years, novel RNA-BPs such as Roquin [5] and Regnase-1 [6,7] have been reported, and their target mRNA has been identified as an important cytokine for inflammation. The discovery of Roquin and Regnase-1 has attracted attention since a new mechanism was proposed for regulation of gene expression by RNA-BP and because of the importance of these molecules in the immune system and inflammation [8,9]. In this review, tristetraprolin (TTP), one of the most classical and most elucidated RNA-BP and the possibility of targeting TTP as a treatment strategy for RA will be discussed.

2. Regulation of mRNA metabolism by RNA-binding proteins

2.1. Regulatory elements in mRNA
Metabolism of target mRNA is carried out by recognition of the cis-element in 3′-UTR of the mRNA by RNA-BP. One of the well-known 3′-UTR elements is the adenyate-uridyate-rich element (ARE) that is characterized by repeats of AUUUA [10]. Importantly, the ARE is one of the target motifs for
rapid degradation of cytokine and chemokine mRNAs [11]. Representative target mRNAs for RNA-BPs are listed in Table 1. Although many cytokines and chemokines contain ARE elements, other cis-elements are also present in cytokine and chemokine mRNAs that are recognized by RNA-BPs. These include GAIT (IFNγ-activated inhibitor of translation), endo (endoribonuclease cleavage site), CRD (coding region determinant of instability), CDE (constitutive decay element), and GRE (GU-rich element). However, it is obvious through past reports that ARE is most frequently found in important pathogenic cytokines and chemokines.

2.2. RNA-binding proteins

RNA-BPs, such as T-cell intracellular antigen-1 (TIA1), TIA1-related protein (TIAR), TTP, and human antigen R (HuR), are trans-acting factors that regulate the stability and translation efficacy of target mRNA that harbor ARE in their 3'-UTR. TIA1 and TIAR inhibit the translation of target mRNAs. TTP accelerates target mRNA decay, while HuR is known to stabilize the target mRNA [11]. These RNA-BPs bind to target mRNAs through unique sequences found in the 3'-UTR that forms secondary conformations, such as stem loops or internal bulges.

Table 1 shows that TTP targets important cytokines and chemokines such as TNFα [12,13]. With recent advances, this list seems to be growing; a more detailed list of TTP-targeted mRNAs can be found in the review written by Blackshear et al. [14]. These facts suggest that TTP is extremely important in inflammatory diseases and define TTP as an anti-inflammatory molecule.

3. The regulation of TTP function

TTP belongs to a family of CCCH-type zinc finger proteins that promote the decay of pro-inflammatory cytokine or chemokine mRNAs that harbor ARE in their 3'-UTR. TTP binds AREs through their zinc-finger domains. The CCR4 (carbon catabolite repressor protein 4)–NOT (negative on TATA-less) deadenylase complex is recruited to TTP-bound mRNA, resulting in deadenylation followed by translational arrest and a rapid degradation of the mRNA [15]. The binding of TTP to its target mRNA depends on the phosphorylation of the molecule.

3.1. Phosphorylation of TTP

Phosphorylation of TTP is induced by growth factors or cytokines and is important for its function [16]. The activity of TTP to destabilize mRNA is abrogated by the phosphorylation of Ser52 and Ser178 in mouse TTP, by the mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2), a downstream kinase of MAPK p38 signaling [17]. The phosphorylation of the serine residues in TTP allows binding of the 14-3-3 protein to TTP, which in turn prevents TTP from binding to its target mRNA [18,19]. Therefore, phosphorylation of TTP by p38/MK2 signal prevents TTP from reducing inflammatory cytokines and chemokines. The phosphorylated TTP escapes destruction by proteasome leading to an increase in its expression level in the cell [20]. Thus, p38/MK2 signal promotes accumulation of inactive form of TTP and stabilization and translation of mRNA of various pro-inflammatory cytokines and chemokines for progression of inflammation [21].

3.2. De-phosphorylation of TTP

Sun et al. [22] determined that protein phosphatase-2A (PP2A) de-phosphorylates TTP. The ablation of a catalytic subunit of PP2A by siRNA increases TTP phosphorylation and facilitates its complex formation with 14-3-3 protein. As a consequence, the expression of TNFα is up-regulated in a cell with less PP2A activity [22]. Therefore, it can be said that PP2A is an activator for TTP. Another phosphatase that is responsible for TTP activity is mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1, DUSP1), a corticosteroid-inducible gene [23]. MKP-1 keeps TTP inactive via de-phosphorylation of MAPK p38. These data explain, at least in

| RNA element | RNA-BPs | Target genes |
|-------------|---------|--------------|
| ARE         | TTP     | CCL2, CCL3, CXCL1, GM-CSF, IFNγ, IL-1β, IL-2, IL-3, IL-6, IL-10, IL-17, TNFα |
| HuR         |         | CCL11, IFNγ, IL-12, TNFα |
| KSRP        |         | IL-8         |
| AUF1        |         | IL-1β       |
| YB-1, AUF-1, PIN1 |     | GM-CSF     |
| TIA1, TIAR, FAR1 |   | TNFα      |
| GAIT        | GAIT complex | CCR5, CCR4, CCR6, CCL22 |
| endo        | MChiP1  | IL-1β, IL-6, IL-12b |
| CRD         | HuR     | IL-4        |
|             | KSRP    | IFNβ       |
| CDE         | SRC3    | TNFα       |
| GRE         | CUGBP1  | TNFR2      |

Table 1. Examples of cytokine and chemokine mRNAs subject to regulation by RNA-BPs.
part, how corticosteroid represses TNFα mRNA through its 3′-UTR [24].

4. TTP in mouse model

Many studies have used mice models to analyze the function of TTP and most were found to be consistent with the results of in vitro studies. Besides, mice models are important as they provide the opportunity to try treatment strategies before clinical use.

4.1. TTP loss of function model

TTP knockout in mouse exhibits systemic inflammation [12]. TTP-deficient mice appear healthy at birth, but soon develop cachexia, erosive arthritis, dermatitis, conjunctivitis, glomerular mesangial thickening, and high titers of anti-DNA antibodies [12]. Since this striking phenotype resembles TNFα transgenic mice [25], the authors of the study injected monoclonal antibody against mouse TNFα and succeeded in preventing the development of the phenotype [12]. Consistently, mice deficient in both TTP and TNF receptors show absence of inflammatory phenotypes that are observed in TTP loss-of-function alone [26]. These in vivo experiments indicate that TTP is required to maintain normal levels of TNFα and to prevent aberrant inflammatory progression in mouse.

4.2. TTP gain of function model

Transgenic mice with high TTP expression driven by strong promoter was found to be embryonic lethal [27]. In order to solve this problem, Patial et al. [28] generated mice in which the ARE in 3′-UTR of TTP was deleted. The protein expression of TTP in cultured cells obtained from these mice was high, owing to the stabilization of the TTP mRNA in absence of ARE [28]. One of the most important findings of this study was that the mice were resistant to anti-collagen antibody induced arthritis. The authors proved that it is possible to treat inflammatory diseases by increasing the expression level of TTP [28]. Studies have also reported that changing the degree of phosphorylation, rather than changing the expression level of TTP can be used to treat inflammatory diseases. Ross et al. [29] generated transgenic mouse carrying a mutant form of TTP, wherein serine residues at 52 and 178 were replaced by alanine. Since these unphosphorylatable TTP mutants are prone to degradation by proteasome, their expression levels are low. However, their mRNA degradation function remains intact, because the mutated mice exhibited strongly attenuated inflammatory response to LPS. Thus, the equilibrium between non-phosphorylated and phosphorylated forms of TTP is a crucial factor determining the anti-inflammatory action of TTP [29].

The unphosphorylatable TTP mutant mice showed dramatic resistance to serum transfer-induced arthritis (STIA) [29], a mouse arthritis model developed through injection of arthritogenic K/BxN serum [30], suggesting that TTP also plays an important role in STIA. More importantly, the treatment of STIA with PP2A agonist showed reduced arthritis severity [31]. The two compounds that were used in these experiments, COG1410 (an apolipoprotein E peptide mimetic) and AAL(s) [a lipid derivative of the immunosuppressant FTY720 (fingolimod)], disrupt interaction between PP2A and its inhibitory protein complexes to activate PP2A. The free PP2A de-phosphorylates and activates TTP for degradation of cytokine mRNAs [31]. The principal of TTP regulation by phosphorylation and de-phosphorylation equilibrium are illustrated in Figure 1 [32].

5. Limitations for therapeutic strategy for RA by targeting TTP

Both mRNA and protein expression of TTP is up-regulated in the inflamed joints of RA [33]. Since the protein expression of TTP co-localizes with MK2, it is possible that activated MK2 facilitates the phosphorylation of TTP and prevents TTP from degradation by proteasome in RA synovia. Therefore, it has been thought that one of the most promising strategies to activate TTP for the purpose of inhibiting cytokine expression was to inhibit

![Equilibrium between TTP phosphorylated and de-phosphorylated forms is critical for TTP activity. Inflammatory stimuli activate MK2 via augmentation of MAPK p38 to phosphorylate TTP into its inactive form. PP2A agonist de-phosphorylates TTP into the active form and accelerates degradation of target mRNA. P indicates Ser-52 and Ser-178 phosphorylations. Modified from Ref. [32].](image-url)
upstream kinase MAPK p38 to inhibit MK2. In fact, several p38 inhibitors were effective in several autoimmune animal models. However, the results of clinical trials of small molecules to inhibit p38 have been disappointing. P38 inhibitors can bring about a transient improvement only in blood test parameters, which unfortunately does not sustain [34].

Another approach to activate TTP is to facilitate de-phosphorylation by phosphatase activation, which is very effective in ameliorating mouse arthritis model [30]. This is an important experiment that clearly recapitulated the principal that phosphorylation and de-phosphorylation equilibrium of TTP is closely related with inflammation (Figure 1). However, PP2A plays multiple roles in maintaining cell homeostasis, and activation of PP2A can exert unexpected adverse effects [32]. A more detailed understanding of the physiological functions of both TTP and PP2A will be critical to be able to device therapeutic strategies to target TTP.

Disclosure statement
No potential conflict of interest was reported by the author.

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