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Control of Cytokine mRNA Expression by RNA-binding Proteins and microRNAs

ABSTRACT

Cytokines are critical mediators of inflammation and host defenses. Regulation of cytokines can occur at various stages of gene expression, including transcription, mRNA export, and post-transcriptional and translational levels. Among these modes of regulation, post-transcriptional regulation has been shown to play a vital role in controlling the expression of cytokines by modulating mRNA stability. The stability of cytokine mRNAs, including TNFα, IL-6, and IL-8, has been reported to be altered by the presence of AU-rich elements (AREs) located in the 3′-untranslated regions (3′UTRs) of the mRNAs. Numerous RNA-binding proteins and microRNAs bind to these 3′UTRs to regulate the stability and/or translation of the mRNAs. Thus, this paper describes the cooperative function between RNA-binding proteins and miRNAs and how they regulate AU-rich elements containing cytokine mRNA stability/degradation and translation. These mRNA control mechanisms can potentially influence inflammation as it relates to oral biology, including periodontal diseases and oral pharyngeal cancer progression.

INTRODUCTION

The regulation of inflammatory cytokines is critical for innate cellular processes such as proliferation and angiogenesis, as well as responses to exogenous stimuli including radiation, stress, and infection (Khabar, 2005). The aberrant expression of cytokines has been correlated with inflammatory diseases, autoimmune disorders, and cancer (Audic and Hartley, 2004). Thus, the expression of cytokines and pro-inflammatory factors, including interleukin-1 (IL-1), IL-4, IL-6, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), prostaglandin E_2 (PGE_2), cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMP), and basic fibroblast growth factor (bFGF), is highly regulated at many levels, including gene transcription, messenger ribonucleic acid (mRNA) translation, and mRNA degradation (Stoecklin *et al.,* 2006). All of these regulatory pathways are controlled by multiple biological networks, in particular, post-transcriptional gene regulation, which determines the fate of mRNA in association with RNA-binding proteins (RBPs) and microRNAs (miRNAs). The order of cytokine gene expression and the relative duration of the various inflammatory events are among the hallmarks of the gene activation process. This route is probably the result of interplay among the elements that regulate transcriptional induction, transcriptional repression, and mRNA stability. It has been proposed that the differences in mRNA stability exert a strong influence on the temporal order of gene expression, in some cases overriding that of the transcriptional control elements (Hao and Baltimore, 2009). The Hao and Baltimore study illustrates that the transcripts of cytokines that are expressed early have abundant AU-rich elements in their 3′-untranslated regions (3′UTRs), whereas those expressed later have fewer. The authors conclude that two intrinsic characteristics of genes, mRNA stability and transcriptional control, manage the kinetics of gene expression induced by pro-inflammatory cytokines. The changes in transcriptional regulation that regulate cytokine expression have been reviewed elsewhere (Pries and Wollenberg, 2006; van Kempen *et al.*, 2006). Thus, the review presented here focuses on the relationship between and among RBPs, miRNAs, and cytokine mRNAs in the post-transcriptional regulation of cytokine expression. It also discusses the potential implications of these relationships in oral biology and pathobiology.

AU-RICH ELEMENTS MARK CYTOKINE mRNAs

The regulation of mRNA stability is an important step in the control of overall gene expression. The changes in mRNA stability in most cells are

mRNA TRANSCRIPT

Figure 1. Diagram of cytokine mRNA and sites of post-transcriptional regulation. Inflammatory cytokines are regulated post-transcriptionally through both the 5′- and 3′-untranslated regions (UTRs). The 5′UTR dictates mRNA translation initiation, whereas the 3′UTR dictates mRNA turnover. Cytokines are highly regulated *via* their AU-rich elements (AREs). TNF-α, IL-6, IL-8, and COX-2 AREs are described in the diagram.

Table 1. RBP Regulation of Specific Cytokine mRNAs

RBP[a]	Cytokine/ARE[b] mRNAs	Stability
TTP	IL-8	destabilized
	VEGF	destabilized
	IL-3	destabilized
	TNF-α	destabilized
	COX-2	destabilized
	IL-10	destabilized
AUF-1 and 2	IL-8	destabilized
	TNF-α	destabilized
	COX-2	destabilized
	GM-CSF	destabilized
HuR	IL-8	stabilized
	TNF-α	stabilized
	VEGF	stabilized
	COX-2	stabilized
BRF-1 and 2	COX-2	destabilized
TIAR and TIA-1	TNF-α	destabilized
	c-myc	destabilized
CUGBP1	TNF-α	destabilized
CUGBP2	COX-2	stabilized

[a]RBP, RNA-binding protein.
[b]ARE, AU-rich element.

ultimately reflected at the protein level. Thus, mRNA decay of the gene is crucial for homeostasis and normal cell survival. The stability of mRNAs is determined by *cis*-acting sequences in the 3′UTRs that promote the degradation of mRNA. A well-studied *cis*-acting element consisting of adenine- and uridine-rich sites dictates the fate of mRNA. The clusters of adenine- and uridine-rich elements (AREs) found in mRNAs encoding cytokines were first identified over 25 years ago (Caput *et al.*, 1986) and were subsequently confirmed by various studies showing that cytokines, chemokines, lymphocytes, proto-oncogenes, and pro-inflammatory genes are subject to ARE-mediated decay (Shaw and Kamen, 1986; Hamilton *et al.*, 2007). AREs provide binding sites for trans-acting factors, such as RBPs, that can subsequently regulate the stability and/or translation of mRNA. The basic motif of which the ARE are comprised are pentamers of AUUUA, nonamers of UUAUUUAUU, and AU-rich clusters composed of linked pentamers and/or nonamers (Wilusz *et al.*, 2001). On the basis of sequences and their decay kinetics, AREs have been grouped into various classes. Typically, Class I AREs contain several AUUUA pentamers scattered throughout the 3′UTR within or near a U-rich region (*e.g.*, c-myc and c-fos). Class II AREs contain overlapping copies of the nonamer UUAUUUAUU within a U-rich region [*e.g.,* cytokine mRNAs such as tumor necrosis factor (TNF) and GM-CSF]. Class III AREs contain mostly U-rich regions but no significant AUUUA pentamers (*e.g.*, c-jun). Bakheet *et al.* (2006) created a database of human ARE mRNAs (ARED; http://brp.kfshrc.edu.sa/ARED/) based on the patterns of AUUUA motifs. Based on their analysis, the percentage of mRNAs within the human genome that contain AREs is about 8%. Fig. 1 illustrates some of the best examples of AREs located at the distal 3′UTR of the mRNA transcripts of cytokines, such as GM-CSF, TNF, IL-2, IL-3, IL-6, and IL-8, as well as pro-inflammatory factors like COX-2. Thus, AREs are critical elements in controlling the expression of genes at the post-transcriptional level.

INTERACTION OF CYTOKINE mRNAs WITH RNA-BINDING PROTEINS

Cytokine mRNA expression is restricted in resting cells through continuously active mRNA decay mechanisms. Induction of

mRNA decay pathways allows for attenuation of the cellular production of cytokines through interactions with RBPs (Anderson, 2009). AREs facilitate the binding of RBPs that degrade or stabilize the mRNA transcripts, often in association with other proteins. Several proteins that can bind to ARE segments have been identified, including tristetraprolin (TTP), human antigen-related protein (HuR), butyrate response factor-1 and butyrate response factor-2 (BRF-1 and BRF-2), ARE/poly(U)-binding/degradation factor (AUF-1), T-cell-restricted intracellular antigen-1 (TIA-1), and TIA-1-related protein (TIAR). However, only subsets of RBPs have been shown to influence the stability or translational efficiency of their target mRNAs. Cytokine mRNAs and their functional interactions with important RBPs are summarized in Table 1. A detailed description of each RBP and their association with cytokine mRNAs are discussed in the Appendix.

SIGNALING PATHWAYS LINKED TO CYTOKINE mRNA STABILITY

The ability of RBPs to interact with cytokine mRNAs and regulate their expression is also controlled by post-translational modifications. Such modifications are mediated by kinases and phosphatases that may change the binding efficiency of RBPs to the ARE sites within mRNAs and thus alter their gene expression. The p38 MAP kinase pathway is a well-studied system that illustrates how signaling mechanisms direct mRNA stability. As shown in Fig. 2 and highlighted in the Appendix, p38/MK2 signaling is required for TTP phosphorylation, which in turn promotes the sequestration of TTP, an event that is partially dependent upon binding to 14-3-3 proteins. These proteins

inhibit the activity of TTP by preventing TTP association with the stress granules in which the mRNA is stored and triaged. Down-regulation of TTP levels then leads to increased cytokine production, because TTP can no longer bind to the AREs and destabilize mRNA. (For additional details, please see the Appendix).

CYTOKINES AND mRNA STABILITY

TNF-α

The pro-inflammatory cytokine TNF has been extensively studied for its role in inflammatory diseases. Analysis of TNF reporter gene expression demonstrated that its ARE strictly inhibited translation of the mRNA (Han *et al.*, 1990). Further evidence for the role of the ARE was also observed in a mouse model, where deletion of the ARE from the TNF gene affected mechanisms responsible for TNF mRNA destabilization and translational repression in hematopoietic and stromal cells. In stimulated conditions, TNF ARE was required for both the relief and reinforcement of message destabilization and translational silencing. Notably, deletion of ARE caused chronic inflammatory arthritis and Crohn's-like inflammatory bowel disease (Kontoyiannis *et al.,* 1999), emphasizing the importance of limiting the expression of critical pro-inflammatory genes through ARE-mediated decay. Recently, we have shown that MAPK phosphatase-1 (MKP-1) is a negative regulator of the host inflammatory response that controls the half-lives of IL-6, IL-10, and TNF-α mRNAs through association with AUF1 (Yu *et al.*, 2011). The results from this study suggested that the half-lives of IL-6, IL-10, and TNF-α mRNAs were significantly increased in bone marrow macrophages derived from MKP-1 knock-out (KO) mice compared with macrophages derived from MKP-1 wild-type (WT) mice after infection with LPS. Thus, our work provides new mechanistic insights into MKP-1 signaling and regulation of cytokine mRNA stability through AUF1 in response to inflammatory stimuli. The interaction between TTP and TNF-α mRNA has been well-studied; specifically, loss of TTP function during the activation of the p38 pathway lengthens the TNF-α mRNA half-life from 37 minutes in unstimulated cells to 90 minutes in stimulated cells (Deleault *et al.*, 2008), dramatically increasing TNF-α cytokine production. Thus, TNF is an excellent target for the prevention of cancer progression. Interestingly, the nuclear factor kappa B (NFκB) has been found to be associated with oral cancer development and plays an essential role in the suppression of TNF-mediated apoptosis (Chen *et al.*, 2002). Hence, targeting signaling kinases associated with TNF
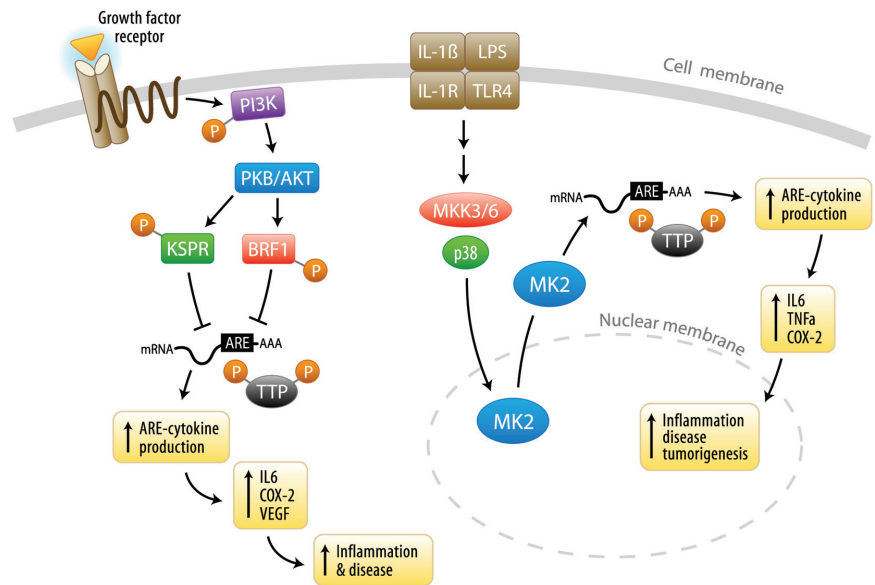
Figure 2. Signaling pathways that regulate cytokine AREs post-transcriptionally. The p38 pathway is the best-described signaling pathway that regulates AU-rich element (ARE)-mediated cytokine degradation. Activation of IL-1β receptors and the Toll-like receptor (TLR) family stimulates p38 MAPK signaling *via* signaling intermediates, including MKK3/6. Active p38 dictates mRNA stability through activation of MK2, which subsequently phosphorylates and inactivates TTP. Once inactivated, phosphorylated TTP dissociates from the ARE region of cytokine transcripts to enhance mRNA stability and translation, thereby causing an increase in cytokine secretion that can promote chronic inflammation and inflammatory disease progression if not properly regulated. In addition, the phosphatidylinositol-3 kinase (PI3K) pathway can also post-transcriptionally regulate mRNAs through AKT phosphorylation of RBPs. This phosphorylation alleviates the mRNAs of RBPs that normally destabilize the mRNA and results in increased mRNA stability and thus increased cytokine protein production and secretion, which, if not properly controlled, can induce chronic inflammation.

provides an additional measure of controlling inflammatory gene expression.

IL-6

Interleukin-6 (IL-6) has many functions in homeostatic regulation, including a role in the immune system, in induction of inflammation, in bone resorption and production, and in various other cellular processes (Keller *et al.*, 1996). Transcriptional and post-translational regulation of IL-6 is critical for maintaining non-pathologic levels of the cytokine and to control both the magnitude and duration of the IL-6 response. Also, loss of IL-6 regulation contributes to the abundance of inflammatory infiltrate in inflammatory diseases and can contribute to the etiology of some cancers (Trikha *et al.*, 2003). In addition, constitutive overexpression of IL-6 is associated with the pathogenesis of rheumatoid arthritis, systemic juvenile arthritis, and Crohn's disease (Hirth *et al.*, 2002; Souza *et al.*, 2008). TTP and TTP-related proteins, BRF-1 and BRF-2, have a major regulatory role for IL-6. Overexpression studies show that TTP, BRF-1, and BRF-2 induce the degradation of mRNAs containing cytokine AREs (Stoecklin *et al.,* 2003; Sully *et al.,* 2004). IL-6 levels are up-regulated in TTP⁻ᐟ⁻ mice, but not to the same extent as TNF-α, suggesting that redundant pathways exist to regulate IL-6 mRNA stability (Taylor *et al.*, 1996). We have identified that TTP expression in HNSCC

was found to be inversely correlated with the secretion of IL-6, and, interestingly, knockdown of TTP increased IL-6 mRNA stability. Conversely, overexpression of TTP in HNSCC cells led to decreased secretion of IL-6 (Van Tubergen *et al.*, 2011). Analysis of these data, together, suggests that TTP plays a critical role in cytokine mRNA stability in HNSCC.

COX-2

Cyclooxygenase-2 (COX-2), expressed at low levels in the stomach, kidney, and intestines (Kujubu *et al.,* 1991), catalyzes the transformation of arachidonic acid to prostaglandin E_2 (PGE_2), the most important COX-2-produced mediator of inflammation. Because COX-2 is an upstream mediator of several inflammatory cytokines, it is a potential target for inflammatory inhibitors that will treat arthritis, Crohn's disease, and other inflammatory diseases. COX-2 expression is itself induced by multiple pro-inflammatory mediators such as IL-1, TNF-α, and LPS, which induce COX-2 mRNA transcription and translation. Activation of p38 stabilizes the mRNA transcripts of COX-2 *via* their effects on TTP, HuR, and AUF-1 (Lasa *et al.*, 2000; Sengupta *et al.*, 2003). The role of HuR in associating with and increasing the stability of COX-2 mRNA has been better described than other RBPs (Subramaniam *et al.*, 2008). Increased cytoplasmic HuR expression has been noted in several cancer types, and in HNSCC it contributed to the increased COX-2 expression observed during tumorigenesis and metastasis (Cha *et al.,* 2011). In particular, the study showed that cytoplasmic HuR expression was significantly associated with COX-2 expression and lymph node metastasis and distant metastasis. Thus, the cytoplasmic expression of HuR appears to be associated with COX-2 expression in OSCCs, and HuR can regulate COX-2 expression in oral cancer. HuR also correlated with COX-2 expression in salivary mucoepidermoid carcinomas (Cho *et al.,* 2007). Thus, HuR plays a major role in controlling COX-2 expression in HNSCC.

IL-8

Interleukin-8 (IL-8) modulates inflammatory response and is a potent angiogenesis stimulator. Tight regulation of IL-8 production is critical for cell function during infection, tissue damage, and cellular homeostasis (Li *et al.*, 2003). Like other cytokines, IL-8 is highly regulated at many stages of expression in the cell, including at the post-transcriptional level (Villarete and Remick, 1996). RBPs target IL-8 mRNA through the AREs in its 3′UTR to increase or decrease cell stability. Multiple proteins have been identified that alter IL-8 mRNA half-life. IL-8 is targeted by HuR, which stabilizes the transcript when activated by inflammatory factors (Choi *et al.,* 2009). Interestingly, HuR and AUF-1 are both capable of stabilizing IL-8 mRNA in human saliva (Palanisamy *et al.,* 2008), and IL-8 mRNA present in human saliva has been shown to be a prognostic marker for oral cancer (St John *et al.,* 2004).

VEGF

Vascular endothelial growth factor (VEGF) facilitates wound healing and tumor progression by promoting angiogenesis. It is regulated post-transcriptionally by AREs in the 3′UTR. VEGF mRNA half-life is stabilized under hypoxic conditions (106 ± 9 min), as compared with normal levels of oxygen (43 ± 6 min) (Levy *et al.*, 1997), through increased expression of HuR (Levy *et al.*, 1998). HuR inhibition in hypoxic cells decreases VEGF expression, and overexpression of HuR stabilizes VEGF only under hypoxic conditions, suggesting that other protein factors and regulatory molecules, such as miRNAs, influence VEGF expression.

Therapeutic Interventions Modulate Cytokine mRNA Stability *via* ARE-binding Proteins

Several RBPs have been identified since the cloning of the first ARE-binding protein (ARE-BP), AUF1. Studies have begun to elucidate the roles of specific ARE-BPs and their target mRNAs in pathological inflammation. For example, TTP was the first ARE-BP recognized to have an effect on inflammation in intact animals, due to its deleterious effect on TNF mRNA stability (Carballo *et al.*, 1998). More recently, several ARE-BPs have been shown to be involved in the inflammatory response *in vivo* (Phillips *et al.,* 2004; Katsanou *et al.,* 2005; Sadri and Schneider, 2009). However, because the complete knockout (KO) of these proteins, which are known to be involved in embryonic development and inflammation, is deleterious, there is an urgent need for tissue-specific KO approaches. Conversely, we have shown that overexpression of TTP in an experimental model of inflammatory bone loss results in significant reductions of IL-6, TNF-α, and prostaglandin-E_2 (Patil *et al.*, 2008). In fact, our *in vivo* analyses indicated a significant protective effect from inflammation-induced bone loss and inflammatory infiltrate in animals over-expressing TTP compared with reporter controls. These findings provide experimental evidence that mRNA stability is a valid therapeutic target in inflammatory bone loss. At the same time, small-molecule inhibitors are starting to emerge to inhibit the functions of ARE-BPs. For example, HuR RNA-binding activity is inhibited by small-molecule inhibitors isolated from microbial origins (Meisner *et al.*, 2007). It would thus be fascinating to identify several other inhibitors for different ARE-BPs and test how these proteins interact with ARE containing cytokine messages for the potential prevention of inflammation in head and neck cancers. Based on clinical importance, ARE-mediated mRNA turnover is of high interest for the design and development of novel therapeutics. However, since not many drugs have been identified or developed that directly target ARE-BPs, the modulation of signaling pathways involved in either their synthesis or their intracellular trafficking provides a powerful strategy for chemical interference with ARE-driven mRNA stability. How chemical modulation of ARE-mediated cytokines would affect mRNA stability has already been reviewed in detail elsewhere (Eberhardt *et al.*, 2007; Cheneval *et al.*, 2010). Also, we have previously reviewed the significance of the p38 MAPK pathway in periodontal disease progression and the potential therapeutic consequences of pharmacological antagonism in the treatment of periodontal diseases (Kirkwood and Rossa, 2009). As a result, we will not discuss these points further. However, it is important to note that these studies illustrate that the current most promising targets to modulate

Table 2. Mechanisms Demonstrating Cooperative Function between miRNAs and RBPs[a] in the Repression/Activation of Shared Target Cytokine mRNAs

Mechanism	Cytokine	miRNA(s)	RBP(s)	Reference(s)
1[b]	TNFα	miR-16	TTP	Jing *et al.*, 2005
1	TNFα	miR-125bmiR-221miR-579	TTP and TIAR	El Gazzar and McCall, 2010
2[c]	IL10	miR-4661	TTP	Ma *et al.*, 2010
3[d,e]	TNFα	miR-369-3	FXR1 and Ago2	Vasudevan and Steitz, 2007; Vasudevan *et al.*, 2007
3[f]	VEGFA	miR-297miR-299	hnRNP L	Jafarifar *et al.*, 2011

[a]RBPs, RNA-binding protein(s).
[b]mRNA decay and translational inhibition due to miRNA and RNA-BP co-dependence.
[c]mRNA stabilization due to competition between RNA-BPs and miRNAs.
[d]Environmental effects on mRNA stability mediated through miRNAs and RNA-BPs.
[e]Dependent on stage of cell cycle.
[f]Dependent on normoxic/hypoxic conditions.

ARE-regulated mRNA stability are the various signaling pathways involved in post-transcriptional gene regulation. Thus, interfering with the signaling pathways should alter the pattern of ARE-BPs and regulate the stability of cytokine mRNAs in oral biology and medicine.

MICRORNA-MEDIATED REGULATION OF CYTOKINE EXPRESSION LEVELS

miRNAs are a specific class of evolutionarily conserved small (19-25 nucleotides) endogenous non-coding RNAs that mediate gene expression at the post-transcriptional level (Rana, 2007). By base-pairing to partially or perfectly complementary sites in the 3′UTR of mRNAs, miRNAs can induce either translational repression or mRNA degradation of the target gene (Rana, 2007). However, new evidence suggests that miRNAs act predominantly to decrease target mRNA levels rather than to inhibit translation (Guo *et al.*, 2010). Human miRNAs regulate diverse cellular and molecular processes, including cellular proliferation, differentiation, and apoptosis, and are predicted to regulate > 60% of all protein-encoding genes within the human genome (Rana, 2007; Friedman *et al.*, 2009). Thus, it is not surprising that miRNAs would also be important regulators of the innate immune system and the expression of cytokines during inflammation. Indeed, the dysregulation of miRNAs could potentially contribute to inflammatory diseases and cancer pathogenesis.

miRNAs are believed to regulate cytokine expression through several mechanisms. These include direct miRNA targeting of the cytokine mRNAs, miRNA regulation of the network of cytokine signaling (including receptors and transcription factors), and miRNA-mediated regulation of cytokine mRNAs *via* their association with RBPs (O'Neill *et al.*, 2011). The evidence for the direct targeting of cytokine mRNAs by miRNAs is limited, with very few targets experimentally verified. The miRNA regulation of cytokine signaling pathways has been extensively examined in several recent review articles (Bak and Mikkelsen, 2010; O'Neill *et al.*, 2011). Therefore, we chose to focus on the areas relating to miRNA-assisted RBP regulation of cytokine mRNAs. More specifically, we examine several mechanisms that demonstrate the cooperative function of miRNAs with RBPs in the repression/activation of shared target cytokine

mRNAs (see Table 2). The mechanisms include: (1) mRNA decay and translational inhibition due to miRNA and RBP cooperation; (2) mRNA stabilization due to competition between RBPs and miRNAs; and (3) the environmental effects on mRNA stability, mediated through miRNAs and RBPs.

mRNA Decay and Translational Inhibition Due to miRNA and RBP Co-dependence

One of the first examples of miRNA-assisted RBP regulation of a cytokine mRNA was observed for TNF-α. Jing *et al.* (2005) reported that a miRNA, miR-16, had a partial sequence match with the TNF-α ARE and that the ARE-mediated decay of TNF-α was dependent on both TTP and miR-16. Based on their data, it was hypothesized that miR-16-bound RNA-induced silencing complex (RISC) assisted TTP binding to the ARE, which subsequently induced mRNA degradation by recruiting deadenylation and/or exosomal proteins. Additionally, a more recent study found that three miRNAs, miR-125b, miR-221, and miR-579, were up-regulated during LPS-induced tolerance (a state that causes TNF-α mRNA to be degraded), and that these miRNAs were capable of either associating with TTP to accelerate TNF-α mRNA decay or blocking TNF-α translation, possibly through recruitment of the translational inhibitor TIAR (El Gazzar and McCall, 2010). Alternatively, another study found that HuR binding to the c-Myc 3′UTR repressed c-Myc expression by recruiting let-7/RISC to an adjacent site on the c-Myc 3′UTR (Kim *et al.*, 2011). However, in this case HuR was not found to interact with RISC. Thus, it was proposed that HuR binding to mRNA possibly changes the local conformation of the mRNA, unmasking the let-7 recognition site that triggers a reduction in both mRNA levels and translation.

mRNA Stabilization Due to Competition between RBPs and miRNAs

The above examples illustrate how miRNAs and RBPs can function together to promote cytokine mRNA destabilization and/or inhibit translation. Conversely, RBPs and miRNAs can also compete with one another to promote mRNA stability. Bhattacharyya *et al.* (2006) found that HuR reverses miR-122-mediated repression of CAT-1 translation by binding to a
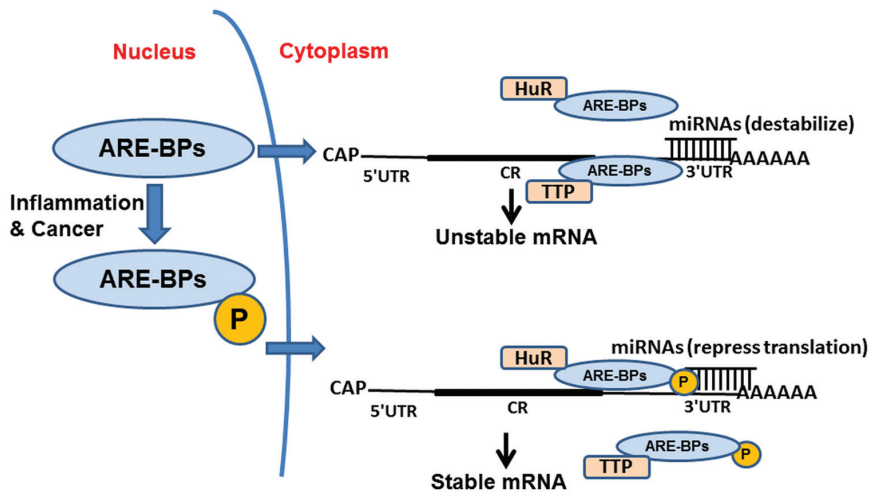
Figure 3. Model of the cooperative function between RNA-binding proteins (RBPs) and miRNAs in the regulation of cytokine mRNAs. Deregulation of miRNAs or RBPs in cancer can be due to the altered expression, localization, activity, or stability of these regulators. The mRNA is presented linearly for simplicity. CR represents a coding region. Under normal conditions, both the AU-rich element-binding protein TTP and miRNAs are involved in the destabilization of cytokine mRNAs, and the non-phosphorylated form of HuR disassociates from mRNAs. However, in response to inflammation and cancer progression, HuR becomes phosphorylated and translocates to the cytoplasm, where it stabilizes mRNAs along with miRNAs that are known to repress translation. This action releases the mRNAs from TTP- and miRNA-mediated destability and repression, respectively. Moreover, inflammation induces TTP phosphorylation and disassociates it from mRNAs along with destabilizing miRNAs.

This implied that FXR1 was associated only with the activating miRNP complex (Steitz and Vasudevan, 2009). In addition to cell cycling, other environmental factors, such as UV exposure and hypoxic stress, can also regulate the crosstalk between miRNAs and RBPs in the repression/activation of shared target mRNAs (Glorian *et al.*, 2011; Jafarifar *et al.*, 2011). In the case of hypoxia, a common feature of neoplastic microenvironments, tumor-associated macrophages induce the expression of the cytokine VEGFA, a critical process for tumor progression and metastasis. Jafarifar *et al.* (2011) recently reported that two miRNAs, miR-297 and miR-299, normally endogenous negative regulators of VEGFA expression in human monocytic cells, could be negatively modulated by heterogeneous nuclear RNP L (hnRNP L) (Jafarifar *et al.*, 2011). More specifically, it was observed that during normoxia, miR-297 and miR-299 target the CA-rich element (CARE) in the VEGFA 3′UTR and negatively regulate VEGFA expression. However, during hypoxia, miRNA-mediated repression was reversed due to the translocation of hnRNP L from the nucleus to the cytoplasm and its increased binding to the CARE region in the VEGFA 3′UTR.

shared site on the ARE-rich CAT-1 3′UTR (Bhattacharyya *et al.*, 2006). Interestingly, a similar mechanism was reported in the regulation of cytokine IL-10 expression levels. miR-4661, which contains a seed region that is complementary to ARE sequences, was demonstrated to up-regulate both IL-10 mRNA and protein levels upon transfection into LPS-stimulated RAW264.7 macrophages by competing with TTP for binding to the ARE sequence in IL-10 mRNA, thus protecting the mRNA from TTP-mediated degradation (Ma *et al.*, 2010). Fig. 3 illustrates the model of competition between ARE-BPs and miRNAs in the regulation of mRNA stability.

Environmental Effects on mRNA Stability Mediated through miRNAs and RBPs

In addition to the intricate interplay between miRNAs and RBPs in the regulation of cytokine expression, there is another layer of complexity that needs to be considered—the environment. Vasudevan and colleagues (Vasudevan and Steitz, 2007; Vasudevan *et al.*, 2007) reported that miR-369-3 can bind directly within a region of the TNF-α ARE and activate translation in quiescent cells through the recruitment of two RBPs, FXR1 and AGO2, two factors usually considered negative regulators. In contrast, in proliferating cells, miR-369-3 was found to repress TNF-α expression (Vasudevan *et al.*, 2007). Interestingly, in reporter pull-out experiments, only AGO2 was detected in the repressing ribonucleoprotein (RNP) complex, not FXR1 (Vasudevan *et al.*, 2007; Steitz and Vasudevan, 2009).

CONCLUSIONS

Overall, mRNA stability is critical for cytokine production. Moreover, it plays an important role in inflammatory disease progression. For example, cytokines, which activate multiple signaling cascades in inflammation, including ERK, JNK, NF-κB, and p38 MAPK, are highly regulated *via* mRNA stability and translation mechanisms. Moreover, loss of post-transcriptional regulation of cytokine mRNAs can dramatically increase cytokine production, leading to tissue destruction and increased mortality. Given the onset of cytokine production, there are several features of post-transcriptional control that play a critical role in their maintenance (Fig. 2). In summary, the above mechanisms illustrate how the crosstalk between miRNAs and RBPs can regulate the repression/activation of shared target cytokine mRNAs. Moreover, these examples illustrate how specific environmental stressors, such as hypoxia, can promote cancer by modulating the interaction between miRNAs and RBPs with the shared target cytokine mRNA, affecting its expression levels. A better understanding of how miRNAs function together with RBPs, in regulating the expression of shared cytokine mRNAs, could potentially lead to improved therapies for cancer and inflammatory diseases.

ACKNOWLEDGMENTS

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