Posttranscriptional Gene Regulation: Novel Pathways for Glucocorticoids' Anti-inflammatory Action

Cristiana Stellato, M.D., Ph.D.

Department of Medicine, University of Salerno, Italy

(cstellato@unisa.it)

Abstract - - **Posttranscriptional gene regulation (PTR) is a fundamental biological process that integrates with the master transcriptional control of gene expression, in ways that only in the last decade have been increasingly understood [1, 2]. While epigenetic and transcriptional events shape cell response qualitatively, deciding the pattern of gene expression to 'switch on or off' in response to endogenous or environmental triggers, the key task of PTR is to act as a 'rheostat' and rapidly adapt the cellular response by providing the appropriate amplitude and timing to the protein expression patterns [3, 4]. The pivotal role of this mechanism comes to the forefront in inflammatory and immune response, where the changes in amplitude and duration in the expression of dangerous and protective genes are in delicate balance, and are critical in determining either the successful resolution of the immune response or its chronic overexpression [5]. This brief review introduces members of the main classes of molecules mediating the cytoplasmic arm of gene regulation, namely RNA-binding proteins and micro-RNA (miRNA), and summarizes experimental data that underscore the role of these molecules in the pathophysiology of chronic inflammation, as well as their promising value as mechanisms conveying the anti-inflammatory effect of synthetic glucocorticoids.**

Keywords - Inflammation, Glucocorticoids, RNA-binding Proteins, microRNA

I. INTRODUCTION

In the last decade, seminal studies have identified RNA-binding proteins (RBP) and microRNA (miRNA) as key mediators of PTR [6-8]. Both classes of molecules are regulated by the same signaling pathways – chiefly MAP kinases - that modulate transcriptional regulation, underscoring the highly integrative nature of the nuclear and cytoplasmic gene regulatory events[9, 10]. They are among the most abundant and well-conserved eukaryotic genes and share the property to coordinately regulate multiple genes through binding to conserved nucleic acid sequences. Often co-localized in cytoplasmic foci of mRNA metabolism (P bodies and stress granules), they can regulate each other's effects by competing for binding or complementing the binding of one another [11, 12]. As studies are increasingly revealing the complex interactions between these determinants of posttranscriptional gene regulation and their gene targets, the understanding of their potential involvement in the pathophysiology of many human chronic inflammatory diseases is a key task for the generation of novel therapeutic interventions.

1. Molecular Mediators of Posttranscriptional Gene Regulation in Inflammation: RNA-binding proteins and micro-RNAs.

Since their generation in the nucleus, mRNA molecules are kept as components of ribonucleoprotein (RNP) complexes, in association with a host of RNA-binding proteins (RBPs) that dynamically interact with the mRNAs and modulate their fate: from their correct splicing and nucleocytoplasmic transfer, to their turnover and translation rates [13]. Several conserved regions within the mRNA sequences, where RBPs associate with specific RNA Recognition Motifs (RRMs), have been identified and recently defined as USER (Untranslated Sequence Elements for Regulations), as they are preferentially –though not exclusively - located in the mRNA untranslated regions (UTR), particularly in the 3'UTR [1, 2, 14]. Among these, the Adenylate-Uridylate-Rich Elements (ARE) are the most conserved and well-characterized regulatory elements mediating changes in mRNA turnover and translation in immune and inflammatory genes [15]. ARE-mediated gene regulation has been described for inflammatory genes such as TNF-α, *IL2, IL3, IL-6, GM-CSF, COX-2, INOS, IL4,* and *IL13* and many more including numerous chemokines, although other USER have been found to be important, especially for T cell-derived genes [14]. Genome-wide studies examining the transcript pools selectively associated with distinct RBPs have established that functionally related mRNAs that share a USER, such as the ARE, can be coordinately regulated by one or more cognate RBPs [1, 16]. In order to regulate their targets' mRNA stability and/or translation, multiple ARE-binding proteins bind dynamically to the mRNA targets following the cues of signaling pathways triggered by proinflammatory stimuli [13] and acting either in a cooperative or exclusive fashion.

Exemplary of these complementary actions are the two ARE-binding proteins HuR and tristetraprolin (TTP). They both regulate mRNA transcripts binding to a group of heterogeneous and distinct, but partially overlapping ARE highly conserved in the 3'UTR of inflammatory and

immune genes' mRNAs, such as TNFα, IL-6, GM-CSF, COX-2, INOS, CCL2 and more [17-19].

While the ubiquitous RBP HuR is functionally characterized mainly as a positive regulator of mRNA stability and translation, TTP limits the inflammatory response by accelerating the mRNA decay of its targets [13].   In particular, cell activation induces HuR nucleocytoplasmic shuttling [20-24], an event reflecting its functional activation, which leads to increased protein output for several inflammatory genes, by making their mRNA more stable and/or by increasing their translation rates [22, 25-31] acting through multiple, often independent mechanisms yet to be fully characterized [32].

An important functional counterpart of HuR-mediated increase in inflammatory gene expression is TTP. Tristetraprolin is a member of a small family of CCCH zinc finger proteins that include TTP, Butyrate-response factor (BRF)-1 and BRF-2 [33]. Tristetraprolin promotes mRNA decay through binding of its zinc finger RRM domain to an ARE consisting in adjacent UUAU/UUAU half-sites [34-36].   Stress and inflammatory stimuli induce TTP as an immediate early response gene in numerous cell types, including T cells, macrophages, epithelial cells and fibroblasts, where it predominantly resides in the cytoplasm [37].   TTP is a potent endogenous negative regulator of TNFα-induced inflammation through a negative feedback loop on its activity: in fact, TNF-α induces TTP synthesis, which in turn leads to rapid destabilization and decay of TNF-α mRNA [38].   Besides TNF-α, TTP also increases the mRNA decay of many targets regulated in opposite fashion by HuR [17, 39-42].   Additional TTP-regulated transcripts have been identified in a genome-wide study of mouse embryonic fibroblasts (MEFs) isolated from TTP-knockout (TTP$^{-/}$) mice [18], and in mouse macrophages in which TTP expression was silenced [43]. The importance of TTP in limiting the inflammatory response has been clearly demonstrated also *in vivo* using TTP-deficient mice, which develop severe inflammatory arthritis, autoimmune dysfunction and myeloid hyperplasia through the deregulated expression of TNF-α and GM-CSF [44].

Micro-RNAs (miRNA) are small (approximately 20-25 nucleotides long), endogenous RNAs encoded by one of the largest and most conserved family of genes in eukariotes, which regulate gene expression at a post-transcriptional level mainly with a repressive outcome [45], though miRNA-mediated increase in gene translation has also been reported in cell-cycle processes [46]. It is estimated that up to one third of the human genome may be subject to regulation by miRNAs [47]. Such large control is exerted through the coordinate effect of a single miRNA on multiple transcripts, which are often functionally related, which harbor the seed regions and display other complex structural features dictating functional miRNA:mRNA interaction.   Furthermore, a single transcript is targeted by multiple miRNAs, such

that a highly combinatorial miRNA profile is what ultimately fine-tunes the protein levels of a high number of targets. MiRNAs act through multiple mecanisms, mediating either mRNA degradation or translation repression of their targets, though they also convey indirect effects on epigenetic and transcriptional regulatory factors [48, 49]. Research in the past decade has revealed their key role in fundamental biological processes such as cell development, proliferation and apoptosis [50], and consequently the immune system and the inflammatory response has resulted to be greatly influenced by miRNA functions[51-56].   Alteration of miRNA expression/function has been increasingly revealed in disease pathophysiology, including inflammatory and allergic diseases [55, 57-60]. As such, modulation of miRNA function, either with miRNA mimics or antagonists (denominated antagomiRs [53]), has increasingly pursued as a strategy to convey therapeutic effect [61, 62].   Importantly, recent studies show that miRNA and RBP can interact through common or overlapping site(s) of their target mRNAs, and their interaction can bring either cooperative or antagonistic outcomes [11, 63, 64].   Thus, it is critical to understand not only how these regulatory factors are modulated by immune activation, but also how their relative contribution is influenced by their relation with other components of the ribonucleoprotein complex.

2. Does deregulation of PTR impair the resolution of the inflammatory response?

Despite great advancements in the understanding of the basic mechanisms conveying PTR in inflammation [3], evidence of the pathophysiological impact of PTR in human allergic, infectious and inflammatory diseases is lagging behind - although data pointing at its importance are accumulating.   To begin with, pro-inflammatory signaling pathways known to be activated in allergic and inflammatory diseases, such as the stress-activated MAP kinases, are now known to be key regulators of PTR and in particular, of the activation and function of RBPs [13, 65]. For example, in asthmatic patients, the ERK1/2 and p38 pathways, which regulate key cell secretory function and proliferation, are strongly upregulated in airway epithelium and BAL macrophages, and their increase correlates with asthma severity and response to GC therapy [66, 67]. These two pathways are established, potent regulators of TTP and HuR, by inhibiting the first and activating the latter [68, 69]. These evidences strongly suggest that alteration of the components within the ribonucleoprotein complex that regulates mRNA decay and translation may play a pathogenetic role in chronic inflammatory disorders such as asthma or rheumatoid arthritis.   It can be hypothesized that inflammatory and/or microbial triggers may produce an aberrant shift of the PTR balance towards increased mRNA stability and translation of the gene pool coordinately regulated through these mechanisms, determining an increase in the amplitude and prolongation in time of the gene expression profile defined

transcriptionally.

3. *Posttranscriptional mechanisms in glucocorticoid's anti-inflammatory action: a novel focus for anti-inflammatory strategies.*

It is firmly established that the mechanism of the antiinflammatory action of synthetic glucocorticoids (GC) critically relies on their ability to influence gene expression through transcriptional regulation [70]. More recent data, however, indicate that control of posttranscriptional pathways could constitute a critical component of an efficient anti-inflammatory action [71, 72]. Understanding the pathophysiological role of PTR mediators, such as RBPs and miRNAs, and to what extent they may convey an anti-inflammatory function may lead to novel anti-inflammatory strategies.

GC have been known to accelerate the mRNA decay of a growing number of cytokines, chemokines and other pro-inflammatory molecules, but the molecular mechanisms by which GCs act on post-transcriptional events are still very poorly understood [71]. Glucocorticoids can impact PTR in different ways. An important indirect effect occurs through the induction by GC of MAP Kinase Phosphatase-1 (MKP-1), a phosphatase that inhibits p38 MAP kinase activity, which induces stabilization of many mRNA species relevant to inflammation (cytokines, chemokines, cyclooxygenase-2) [73]. Mouse lacking MKP-1 demonstrate a diminished response to GC [74].

Studies conducted by our laboratory and others indicate that GC induce TTP [75] [19]. In our study, genome-wide analysis using mouse embryonic fibroblasts (MEF) from TTP$^{-/-}$ and wild type (WT) littermates revealed that GC-mediated changes in gene expression were strikingly dependent from TTP, with a loss of GC sensitivity for more than 80% of genes that were GC-responsive in WT MEFs [19]. We confirmed the loss of GC-mediated action for several TTP targets in cultured human primary bronchial epithelial cells, using siRNA-mediated TTP ablation. Conversely, in a mouse model of LPS-induced peridontal inflammation, TTP overexpression displayed an antiinflammatory effect through accelerated turnover of TNF-α mRNA [76]. These studies indicate that the impact of posttranscriptional regulation in the mechanism of anti-inflammatory action of GCs is larger than ever appreciated and it involves molecules, like TTP, that directly modulate mRNA stability. However, As TTP is regulated by TNFα and other inflammatory stimuli both at the level of expression *and* functional state by MAP kinases, which functionally inactivate TTP [69, 77-79], in order to exploit TTP-mediated action GC would need to increase TTP levels <u>and</u> regulate its functional state, by controlling the inflammatory signaling that suppress it or by supporting the cellular pathways that counteract the inflammation. Therefore, we are currently testing the hypothesis that if TTP would be increased in level <u>and</u> functionally active under GC control, its physiologic role in the termination of the inflammatory response would contribute significantly to the GC-mediated shutdown of inflammatory cytokines, by allowing increased mRNA decay for the many ARE-bearing inflammatory transcripts under TTP control. More recently, we and others [80, 81] have characterized a novel cytoplasmic role of the glucocorticoid receptor (GR) in posttranscriptional gene control, describing that in airway epithelial cells, GR may also function as an RNA-binding protein. Proteins functionally characterized as transcription factors, such as the GR, and RBPs have several features in common, such as stimulus- or ligand-induced nucleocytoplasmic shuttling function and the ability to coordinately regulate multiple genes through binding to conserved nucleic acid sequences. Some regulatory factors can bind to both DNA and RNA, thus influencing both transcription and mRNA fate. For instance, the zinc finger protein NF-90 can regulate transcription of IL-2, but can also bind to and stabilize the IL-2 mRNA [82]. Initial studies done in rat smooth muscle cells reported that the GR interact with the mRNA of the chemokine CCL2, and that the presence of the GR in the RNP complex was necessary for its degradation to occur [80]. This work identified a novel role of GR in mRNA turnover, and raised questions about the scope and mechanisms underlying this process. Given the importance of GC action in modulating inflammatory gene expression in human airway epithelium, and given the relevance of other RBPs, such as TTP, in GC action [19], we sought to investigate whether the GR would act as an RBP in human airway epithelial cells, reasoning that, as RBPs can coordinately regulate subsets of functionally related transcripts that share a common recognition motif, such action may regulate not only few targets but a substantial subset of genes[1]. We first identified the association of GR with the chemokines' CCL2 and CCL7 mRNAs by immunoprecipitation of ribonucleoprotein complex (RNP-IP) and biotin pull-down in the human airway epithelial cell line BEAS-2B. In the case of CCL2, the binding site was mapped to the 5'UTR of the transcript. Furthermore, we aimed at identifying the full complement of transcripts that could bind to GR using a ribonomic approach, and computationally searched for a common GR recognition motif within the identified GR target transcripts. We demonstrate that the GR in human lung epithelial cells is capable of associating with almost 500 transcripts, and we validated such association for a subset of them. Finally, we identified by computational analysis a novel, GC-rich motif, for which we confirmed GR association, present in the 5' UTRs of 7889 predicted mRNA targets, or in the entire sequences of 25,672 predicted mRNA targets (21% of the UniGene transcript pool) [81].

Overall, these data indicate that the GR can mediate GC action beyond its nuclear functions in transcriptional control, and that it may directly participate, via association with mRNA, in GC-mediated control of cytoplasmic mechanisms of gene expression. Though these data indicate an intriguing novel regulatory mechanism for the GR, much remains to be tested to

understand its relevance in the global anti-inflammatory action of GC: for example, whether the functional role of the RNA motif is indeed to convey acceleration of mRNA decay, as documented for CCL2 and CCL7, and whether other GR motifs can be found in genes expressed upon inflammatory cell activation. To this end, multiple motifs have been documented for the RBP TIAR, which binds to U-rich motifs within the 3'UTR of its targets with high affinity when conveying translational repression, while its low-affinity binding to a C-rich element decreases upon cellular stress [83]. Likewise, it can be postulated that GC treatment, as well as a host of intra- and extracellular triggers, could modulate the GR binding to the RNA motifs in many ways. For example, this process could be modified by stimulus-induced changes in the RBP and microRNA potentially associated with GR in the RNP complex, and/or by action upon the association of GR-bound RNA with cytoplasmic foci of regulated mRNA decay and translation, such as P bodies and stress granules, where these functions are critically regulated during inflammation. On the structural level, much remains to be discovered on the cytoplasmic GR:mRNA association, starting from the identification of the GR domain interfacing with the GC-rich motif and the stoichiometry of this binding: what we know so far is that the GR lacks a conserved RNA-binding domain (RBD), present in other transcription factors that also bind to mRNA, like NF-90 and in nucleolin [84, 85]. In addition to a specific RBD, GR could also associate to other RBP components that bind directly to RNA.

Research on the role of miRNA in GC anti-inflammatory action is still in its infancy. The effect of GC in a mouse model of LPS-induced inflammation was minimal on global miRNA profiling in the lungs [86], as it was in a study of miRNA profiling in bronchial biopsies of mild asthmatics before and after therapy with inhaled glucocorticoids [87]. Given that miRNA expression is cell-specific, discrete changes may have been masked by the evaluation of tissue samples rather than purified cells. In line with this consideration, in fact, recent data indicate that GC potently inhibits in a macrophage cell line the expression of miR101, a suppressor of MKP-1 [88] and strongly repress miRNA expression during glucocorticoid-induced apoptosis of primary rat thymocytes [89].

II. CONCLUSION

In conclusion, growing data indicate the existence of relevant, yet understudied molecular mechanisms involved in the physiologic control of the inflammatory response that could provide a different paradigm of anti-inflammatory approach, shifting from the control of master transcriptional mechanisms to that of posttranscriptional regulatory events. RNA-binding proteins and miRNA are increasingly seen as critical and targetable effector molecules of inflammation.

Considering that both molecular species coordinatley regulate functionally related pools of transcripts through common regulatory elements, intervening at the structural and functional interface between RBPs, miRNAs and their targets may convey a relatively specific anti-inflammatory outcome.

REFERENCES

1. Keene, J.D., *RNA regulons: coordination of post-transcriptional events.* Nat Rev Genet, 2007. **8**(7): p. 533-43.

2. Wilusz, C.J. and J. Wilusz, *Bringing the role of mRNA decay in the control of gene expression into focus.* Trends in Genetics, 2004. **20**(10): p. 491-497.

3. Anderson, P., *Post-transcriptional regulons coordinate the initiation and resolution of inflammation.* Nat Rev Immunol, 2010. **10**(1): p. 24-35.

4. Hao, S. and D. Baltimore, *The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules.* Nat Immunol, 2009. **10**(3): p. 281-8.

5. Anderson, P., *Intrinsic mRNA stability helps compose the inflammatory symphony.* Nat Immunol, 2009. **10**(3): p. 233-4.

6. Shyu, A.B. and M.F. Wilkinson, *The double lives of shuttling mRNA binding proteins.* Cell, 2000. **102**(2): p. 135-8.

7. Anderson, P., et al., *Post-transcriptional regulation of proinflammatory proteins 10.1189/jlb.1103536.* J Leukoc Biol, 2004. **76**(1): p. 42-47.

8. Baltimore, D., et al., *MicroRNAs: new regulators of immune cell development and function.* Nat Immunol, 2008. **9**(8): p. 839-845.

9. Saj, A. and E.C. Lai, *Control of microRNA biogenesis and transcription by cell signaling pathways.* Current Opinion in Genetics & Development, 2011. **21**(4): p. 504-510.

10. Dean, J.L., et al., *The involvement of AU-rich element-binding proteins in p38 mitogen-activated protein kinase pathway-mediated mRNA stabilisation.* Cell Signal, 2004. **16**(10): p. 1113-21.

11. Bhattacharyya, S.N., et al., *Relief of microRNA-mediated translational repression in human cells subjected to stress.* Cell, 2006. **125**(6): p. 1111-24.

12. Jing, Q., et al., *Involvement of MicroRNA in AU-Rich Element-Mediated mRNA Instability.* Cell, 2005. **120**(5): p. 623-634.

13. Anderson, P., *Post-transcriptional control of cytokine production.* Nat Immunol, 2008. **9**(4): p. 353-9.

14. Vavassori, S. and L.R. Covey, *Post-transcriptional regulation in lymphocytes: the case of CD154.* RNA Biol, 2009. **6**(3): p. 259-65.

15. Khabar, K.S., *The AU-rich transcriptome: more than interferons and cytokines, and its role in disease.* J Interferon Cytokine Res, 2005. **25**(1): p. 1-10.

16. Keene, J.D. and S.A. Tenenbaum, *Eukaryotic mRNPs may represent posttranscriptional operons.* Mol Cell, 2002. **9**(6): p. 1161-7.

17. Carballo, E., W.S. Lai, and P.J. Blackshear, *Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability.* Blood, 2000. **95**(6): p. 1891-9.

18. Lai, W.S., et al., *Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts.* Mol Cell Biol, 2006. **26**(24): p. 9196-208.

19. Ishmael, F.T., et al., *Role of the RNA-binding protein tristetraprolin in glucocorticoid-mediated gene regulation.* J Immunol, 2008. **180**(12): p. 8342-53.

20. Wang, J.G., et al., *LFA-1-dependent HuR nuclear export and cytokine mRNA stabilization in T cell activation.* J Immunol, 2006. **176**(4): p. 2105-13.

21. Yarovinsky, T.O., et al., *Early Exposure to IL-4 Stabilizes IL-4 mRNA in CD4+ T Cells via RNA-Binding Protein HuR.* J Immunol, 2006. **177**(7): p. 4426-4435.

22. Seko, Y., et al., *Selective Cytoplasmic Translocation of HuR and Site-specific Binding to the Interleukin-2 mRNA Are Not Sufficient for CD28-mediated Stabilization of the mRNA.* J. Biol. Chem., 2004. **279**(32): p. 33359-33367.

23. Atasoy, U., et al., *ELAV protein HuA (HuR) can redistribute between nucleus and cytoplasm and is upregulated during serum stimulation and T cell activation.* J Cell Sci, 1998. **111 (Pt 21)**: p. 3145-56.

24. Xu, Y.Z., et al., *RNA-binding protein HuR is required for stabilization of SLC11A1 mRNA and SLC11A1 protein expression.* Mol Cell Biol, 2005. **25**(18): p. 8139-49.

25. Ma, W.J., et al., *Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein.* J Biol Chem, 1996. **271**(14): p. 8144-51.

26. Dean, J.L., et al., *The 3' untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR.* Mol Cell Biol, 2001. **21**(3): p. 721-30.

27. Ming, X.F., et al., *Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase.* Mol Cell Biol, 2001. **21**(17): p. 5778-89.

28. Dixon, D.A., et al., *Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells.* J Clin Invest, 2001. **108**(11): p. 1657-65.

29. Goldberg-Cohen, I., H. Furneauxb, and A.P. Levy, *A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR.* J Biol Chem, 2002. **277**(16): p. 13635-40.

30. Rodriguez-Pascual, F., et al., *Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene. Involvement of the RNA-binding protein HuR.* J Biol Chem, 2000. **275**(34): p. 26040-9.

31. Atasoy, U., et al., *Regulation of Eotaxin Gene Expression by TNF and IL-4 Through Messenger RNA Stabilization: Involvement of the RNA-binding protein HuR.* J Immunol, 2003. **171**(8): p. 4369-4378.

32. Hinman, M.N. and H. Lou, *Diverse molecular functions of Hu proteins.* Cell Mol Life Sci, 2008.

33. Carrick, D.M., W.S. Lai, and P.J. Blackshear, *The tandem CCCH zinc finger protein tristetraprolin and its relevance to cytokine mRNA turnover and arthritis.* Arthritis Res Ther, 2004. **6**(6): p. 248-64.

34. Worthington, M.T., et al., *RNA binding properties of the AU-rich element-binding recombinant Nup475/TIS11/tristetraprolin protein.* J Biol Chem, 2002. **277**(50): p. 48558-64.

35. Blackshear, P.J., et al., *Characteristics of the interaction of a synthetic human tristetraprolin tandem zinc finger peptide with AU-rich element-containing RNA substrates.* J Biol Chem, 2003. **278**(22): p. 19947-55.

36. Brewer, B.Y., et al., *RNA Sequence Elements Required for High Affinity Binding by the Zinc Finger Domain of Tristetraprolin: CONFORMATIONAL CHANGES COUPLED TO THE BIPARTITE NATURE OF AU-RICH mRNA-DESTABILIZING MOTIFS.* J. Biol. Chem., 2004. **279**(27): p. 27870-27877.

37. Blackshear, P.J., *Tristetraprolin and other CCCH tandem zinc-finger proteins in the regulation of mRNA turnover.* Biochem Soc Trans, 2002. **30**(Pt 6): p. 945-52.

38. Carballo, E., W.S. Lai, and P.J. Blackshear, *Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin.* Science, 1998. **281**(5379): p. 1001-5.

39. Sawaoka, H., et al., *Tristetraprolin binds to the 3'-untranslated region of cyclooxygenase-2 mRNA. A polyadenylation variant in a cancer cell line lacks the binding site.* J Biol Chem, 2003. **278**(16): p. 13928-35.

40. Stoecklin, G., et al., *A novel mechanism of tumor suppression by destabilizing AU-rich growth factor mRNA.* Oncogene, 2003. **22**(23): p. 3554-61.

41. Ogilvie, R.L., et al., *Tristetraprolin down-regulates IL-2 gene expression through AU-rich element-mediated mRNA decay.* J Immunol, 2005. **174**(2): p. 953-61.

42. Fechir, M., et al., *Tristetraprolin Regulates the Expression of the Human Inducible Nitric-Oxide Synthase Gene.* Mol Pharmacol, 2005. **67**(6): p. 2148-2161.

43. Jalonen, U., et al., *Down-regulation of tristetraprolin expression results in enhanced IL-12 and MIP-2 production and reduced MIP-3alpha synthesis in activated macrophages.* Mediators Inflamm, 2006. **2006**(6): p. 40691.

44. Taylor, G.A., et al., *A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency.* Immunity, 1996. **4**(5): p. 445-54.

45. Bartel, D.P., *MicroRNAs: genomics, biogenesis, mechanism, and function.* Cell, 2004. **116**(2): p. 281-97.

46. Vasudevan, S., Y. Tong, and J.A. Steitz, *Switching from repression to activation: microRNAs can up-regulate translation.* Science, 2007. **318**(5858): p. 1931-4.

47. Lewis, B.P., C.B. Burge, and D.P. Bartel, *Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets.* Cell, 2005. **120**(1): p. 15-20.

48. Fabbri, M., et al., *MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B.* Proc Natl Acad Sci U S A, 2007. **104**(40): p. 15805-10.

49. Mertens-Talcott, S.U., et al., *The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells.* Cancer Res, 2007. **67**(22): p. 11001-11.

50. Williams, A.E., *Functional aspects of animal microRNAs.* Cell Mol Life Sci, 2008. **65**(4): p. 545-62.

51. O'Connell, R.M., et al., *Physiological and pathological roles for microRNAs in the immune system.* Nat Rev Immunol, 2010. **10**(2): p. 111-22.

52. Asirvatham, A.J., W.J. Magner, and T.B. Tomasi, *miRNA regulation of cytokine genes.* Cytokine, 2009. **45**(2): p. 58-69.

53. Krutzfeldt, J., et al., *Silencing of microRNAs in vivo with 'antagomirs'.* Nature, 2005. **438**(7068): p. 685-9.

54. Lindsay, M.A., *microRNAs and the immune response.* Trends Immunol, 2008. **29**(7): p. 343-51.

55. Oglesby, I.K., N.G. McElvaney, and C.M. Greene, *MicroRNAs in inflammatory lung disease--master regulators or target practice?* Respir Res, 2010. **11**: p. 148.

56. Taganov, K.D., M.P. Boldin, and D. Baltimore, *MicroRNAs and immunity: tiny players in a big field.* Immunity, 2007. **26**(2): p. 133-7.

57. Rodriguez, A., et al., *Requirement of bic/microRNA-155 for normal immune function.* Science, 2007. **316**(5824): p. 608-11.

58. Grosshans, H. and W. Filipowicz, *Molecular biology: the expanding world of small RNAs.* Nature, 2008. **451**(7177): p. 414-6.

59. Lu, T.X., A. Munitz, and M.E. Rothenberg, *MicroRNA-21 Is Up-Regulated in Allergic Airway Inflammation and Regulates IL-12p35 Expression.* J Immunol, 2009. **182**(8): p. 4994-5002.

60. Mattes, J., et al., *Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease.* Proceedings of the National Academy of Sciences, 2009. **106**(44): p. 18704-18709.

61. Love, T.M., H.F. Moffett, and C.D. Novina, *Not miR-ly small RNAs: big potential for microRNAs in therapy.* J Allergy Clin Immunol, 2008. **121**(2): p. 309-19.

62. Mattes, J., A. Collison, and P.S. Foster, *Emerging role of microRNAs in disease pathogenesis and strategies for therapeutic modulation.* Curr Opin Mol Ther, 2008. **10**(2): p. 150-7.

63. Chang, N., et al., *HuR uses AUF1 as a cofactor to promote p16INK4 mRNA decay.* Mol Cell Biol, 2010. **30**(15): p. 3875-86.

64. von Roretz, C. and I.E. Gallouzi, *Decoding ARE-mediated decay: is microRNA part of the equation?* J Cell Biol, 2008. **181**(2): p. 189-94.

65. Clark, A.R., J.L. Dean, and J. Saklatvala, *Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38.* FEBS Lett, 2003. **546**(1): p. 37-44.

66. Bhavsar, P., et al., *Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma.* Thorax, 2008. **63**(9): p. 784-90.

67. Liu, W., et al., *Cell-specific activation profile of extracellular signal-regulated kinase 1/2, Jun N-terminal kinase, and p38 mitogen-activated protein kinases in asthmatic airways.* Journal of Allergy and Clinical Immunology, 2008. **121**(4): p. 893-902.e2.

68. Doller, A., J. Pfeilschifter, and W. Eberhardt, *Signalling pathways regulating nucleo-cytoplasmic shuttling of the mRNA-binding protein HuR.* Cell Signal, 2008. **20**(12): p. 2165-73.

72

69.   Sandler, H. and G. Stoecklin, *Control of mRNA decay by phosphorylation of tristetraprolin.* Biochem Soc Trans, 2008. **36**(Pt 3): p. 491-6.

70.   Rhen, T. and J.A. Cidlowski, *Antiinflammatory Action of Glucocorticoids -- New Mechanisms for Old Drugs.* N Engl J Med, 2005. **353**(16): p. 1711-1723.

71.   Stellato, C., *Post-transcriptional and nongenomic effects of glucocorticoids.* Proceedings of the American Thoracic Society, 2004. **1**: p. 255-263.

72.   Stellato, C., *Glucocorticoid actions on airway epithelial responses in immunity: functional outcomes and molecular targets.* J Allergy Clin Immunol, 2007. **120**(6): p. 1247-63; quiz 1264-5.

73.   Clark, A.R., J.R. Martins, and C.R. Tchen, *Role of dual specificity phosphatases in biological responses to glucocorticoids.* J Biol Chem, 2008. **283**(38): p. 25765-9.

74.   Abraham, S.M., et al., *Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1.* J Exp Med, 2006. **203**(8): p. 1883-9.

75.   Smoak, K. and J.A. Cidlowski, *Glucocorticoids Regulate Tristetraprolin Synthesis and Posttranscriptionally Regulate Tumor Necrosis Factor Alpha Inflammatory Signaling.* Mol. Cell. Biol., 2006. **26**(23): p. 9126-9135.

76.   Patil, C.S., et al., *Targeting mRNA Stability Arrests Inflammatory Bone Loss.* Mol Ther, 2008. **16**(10): p. 1657-1664.

77.   Stoecklin, G., et al., *MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay.* EMBO J, 2004. **23**(6): p. 1313-24.

78.   Brook, M., et al., *Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways.* Mol Cell Biol, 2006. **26**(6): p. 2408-18.

79.   Hitti, E., et al., *Mitogen-activated protein kinase-activated protein kinase 2 regulates tumor necrosis factor mRNA stability and translation mainly by altering tristetraprolin expression, stability, and binding to adenine/uridine-rich element.* Mol Cell Biol, 2006. **26**(6): p. 2399-407.

80.   Dhawan, L., et al., *A Novel Role for the Glucocorticoid Receptor in the Regulation of Monocyte Chemoattractant Protein-1 mRNA Stability 10.1074/jbc.M605925200.* J. Biol. Chem., 2007. **282**(14): p. 10146-10152.

81.   Ishmael, F.T., et al., *The Human Glucocorticoid Receptor as an RNA-Binding Protein: Global Analysis of Glucocorticoid Receptor-Associated Transcripts and Identification of a Target RNA Motif.* J Immunol, 2010.

82.   Shi, L., et al., *NF90 regulates inducible IL-2 gene expression in T cells.* J Exp Med, 2007. **204**(5): p. 971-7.

83.   Kim, H.S., et al., *Elucidation of a C-rich signature motif in target mRNAs of RNA-binding protein TIAR.* Mol Cell Biol, 2007. **27**(19): p. 6806-17.

84.   Ginisty, H., et al., *Structure and functions of nucleolin.* J Cell Sci, 1999. **112 (Pt 6)**: p. 761-72.

85.   Shi, L., et al., *NF90 regulates cell cycle exit and terminal myogenic differentiation by direct binding to the 3'-untranslated region of MyoD and p21WAF1/CIP1 mRNAs.* J Biol Chem, 2005. **280**(19): p. 18981-9.

86.   Rainer, J., et al., *Glucocorticoid-regulated microRNAs and mirtrons in acute lymphoblastic leukemia.* Leukemia, 2009. **23**(4): p. 746-52.

87.   Williams, A.E., et al., *MicroRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy.* PLoS One, 2009. **4**(6): p. e5889.

88.   Zhu, Q.Y., et al., *MicroRNA-101 targets MAPK phosphatase-1 to regulate the activation of MAPKs in macrophages.* J Immunol, 2010. **185**(12): p. 7435-42.

89.   Smith, L.K., R.R. Shah, and J.A. Cidlowski, *Glucocorticoids modulate microRNA expression and processing during lymphocyte apoptosis.* J Biol Chem, 2010. **285**(47): p. 36698-708.