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Inflammation-regulated mRNA stability and the progression of vascular inflammatory diseases

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Abstract

Cardiovascular disease remains a major medical and socioeconomic burden in developed and developing societies, and will increase with an aging and increasingly sedentary society. Vascular disease and atherosclerotic vascular syndromes are essentially inflammatory disorders, and transcriptional and post-transcriptional processes play essential roles in the ability of resident vascular and inflammatory cells to adapt to environmental stimuli. The regulation of mRNA translocation, stability, and translation are key processes of post-transcriptional regulation that permit these cells to rapidly respond to inflammatory stimuli. For the most part, these processes are controlled by elements in the 3′-UTR of labile, proinflammatory transcripts. Since proinflammatory transcripts almost exclusively contain AU-rich elements (AREs), this represents a tightly regulated and specific mechanism for initiation and maintenance of the proinflammatory phenotype. RNA-binding proteins (RBPs) recognize cis elements in 3′-UTR, and regulate each of these processes, but there is little literature exploring the concept that RBPs themselves can be directly regulated by inflammatory stimuli. Conceptually, inflammation-responsive RBPs represent an attractive target of rational therapies to combat vascular inflammatory syndromes. Herein we briefly describe the cellular and molecular etiology of atherosclerosis, and summarize our current understanding of RBPs and their specific roles in regulation of inflammatory mRNA stability. We also detail RBPs as targets of current anti-inflammatory modalities and how this may translate into better treatment for vascular inflammatory diseases.

Cardiovascular disease and vascular inflammation

Despite nutritional modification and lipid reducing medications, atherosclerotic vascular syndromes account for 50% of all mortality in the United States and is increasing in the developing world. Cardiovascular disease is a considerable medical and socioeconomic problem contributing to mortality of many conditions including myocardial infarction, stroke, renal failure, and peripheral vascular disease, and this will worsen with an increasing number of patients with comorbidity such as obesity and Type 2 diabetes. Current research points to atherosclerosis as a lipid-driven inflammatory disease which drives nearly all vascular diseases such as PAD and coronary artery disease (CAD) [1]. The early process of

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atherosclerosis is initiated by common major risk factors which include hypertension, high low-density lipoprotein (LDL) levels, smoking, obesity, and insulin resistance, which promote lipoprotein particle accumulation in the subendothelial space. The vascular endothelium is the continuous cellular lining of the cardiovascular system, and is another often targeted area for antiatherosclerotic therapies. The healthy endothelium is involved in the regulation of cholesterol and lipid transcytosis, as well as inflammation and immunity; however, under pathological conditions such as hypercholesterolemia, the endothelial cell layer can contribute to the progression of vascular disease [2]. Atherosclerosis develops at sites of disturbed blood flow and LDL retention which activate inflammatory signaling pathways such as NF-κB and c-Jun N terminal kinase-1, transactivators of tumor necrosis factor α (TNFα), and interleukin-1β (IL-1β) which up-regulate cell adhesion molecule (CAM) expression [3–6]. Atherosclerotic plaque development begins with the expression of adhesion molecules such as ICAM-1 and VCAM-1 by endothelial cells, which bind circulating leukocytes, allowing monocytes to enter the arterial intima by extravasation due in large part to expression of chemoattractants such as monocyte chemoattractant protein-1 (MCP-1) [7]. In addition, recent studies have shown that microparticles from human atherosclerotic plaques can transfer ICAM-1 to endothelial cells to recruit inflammatory cells that also contributing to the inflammatory cycle that has been initiated [8]. Therapeutics which target endothelial cells and the steps described above have been met with limited success and more investigation is required into the mechanisms by which these cells promote vascular disease.

Following changes to the endothelium that permit extravasation of leukocytes, monocytes mature into macrophages where they express lipid and scavenger receptors which recognize and engulf oxidized lipoproteins. Our understanding of the heterogeneity in macrophage populations has evolved greatly over the last few years, specifically identifying new subsets of monocyte and dendritic cells, as well as tissue-specific functions for macrophages and macrophage phenotype [7,9–11]. Recent studies have further cemented vascular smooth muscle cells (VSMCs) contribution to atherosclerotic plaques through their transition to macrophage-like cells, adding to the heterogeneity of the population [12,13]. The continual accumulation of cholesterol esters by macrophages leads to the formation of foam cells, while the macrophages perpetuate the proinflammatory response by chronic synthesis of cytokines [14]. These cytokines act in an autocrine and paracrine manner to regulate cell proliferation, migration, and extracellular matrix production, which participate in producing large, lipid-laden, proinflammatory plaques that may become obstructive and vulnerable to rupture [15]. Macrophages have long been attractive targets for therapeutics due to their major role in the pathogenesis of atherosclerosis. The most recent studies on atherosclerosis have focused on utilizing monocyte-derived cells to promote lesion regression and cholesterol efflux in order to further understand how we can treat atherosclerosis following its development [16,17].

Aside from an innate immune response, new evidence suggests that the innate and adaptive arms of the immune system work together in the progression of atherosclerosis. Dendritic cells, which provide a link between the two arms, accumulate both in human and mouse plaque and promote atherogenesis by expression of CCL17 directed suppression of T-regulatory cells [18]. Another study showed lesion resident CD11c+ antigen presenting cells

(APCs) can activate T cells, demonstrating a key role for dendritic cells bridging the innate and adaptive immune response in atherosclerosis [19]. The adaptive immune response is mediated by T and B lymphocytes and has been shown to promote inflammation during atherogenesis, but these cells also produce regulatory cells and antibodies that can be protective as well [20]. In the end, we need to further investigate how targeting the innate and adaptive immune responses could have beneficial effects in vascular diseases.

The injurious effects of proinflammatory cytokines resulting in VSMC activation and development of multiple vascular diseases are well described, although these cells are often overlooked as therapeutic targets compared with macrophages and endothelial cells [21,22]. VSMCs respond to and synthesize proinflammatory immune modulators, promulgate autocrine activation, and recruitment of leukocytes to the lesion, which leads to a localized vascular inflammatory response [2,23]. In more advanced atherosclerosis, VSMCs form a cap over the developing plaque; the thickness of which is tightly linked to VSMC activation and is responsible for lesion stability [24,25]. The major cause of mortality in human patients is rupture of the atherosclerotic plaque resulting in acute thrombus formation and myocardial infarction [24]. In restenosis and allograft vasculopathy (AV), which are also vascular inflammatory diseases, VSMCs play a larger role, where they migrate into the intima, proliferate, and synthesize cytokines and matrix proteins, leading to loss of lumen and subsequent tissue ischemia. Consequently, identification of proteins, pathways, and processes which modulate the VSMC response to injury is key to development of therapeutics to combat multiple vascular diseases.

The inducible inflammatory response is very dynamic, requiring the coordination of both cell-type and signal-specific programs to regulate hundreds of genes. Transcriptional control of inflammatory mediators is required to initiate the inflammatory response and is the topic of several comprehensive reviews, but post-transcriptional mechanisms are also critical in regulating the balance of inflammation [26–31]. The instability of inflammatory mediator mRNAs is essential as it permits a fine-tuned control of inflammation to ensure that it can be rapidly turned on and off as needed during disruptions to homeostasis [32]. The dysregulated expression of cytokines has been linked to cancer, inflammatory, and autoimmune diseases [33]. Post-transcriptional mRNA stability is an oft over-looked, yet key mechanism in the regulation of inflammation, particularly in the context of vascular disease, and is the focus of this review [34,35].

Cis-acting sequences regulate RNA stability

A large number of inflammatory mediators, mainly cytokines and chemokines, have unstable mRNAs due to cis-acting adenine and uridine-rich (AU-rich) elements (AREs) in the 3′-untranslated (UTR) regions [36]. The ARE provides a binding site for trans-acting RNA-binding proteins (RBPs) which regulate stability or translation of the transcript. ARE-mediated regulation of transcripts provides an exquisite and specific level of control in the processing of mRNAs. An estimated 5–8% of all human transcripts contain AREs and they consist of various large clusters of overlapping AUUUA pentamer and UUAUUUAUU nonamers [37]. Class I AREs contain multiple copies of the AUUUA pentamer motif clustered as tandem repeats, while class II AREs have one or a few AUUUA pentamers

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throughout the 3′-UTR [38]. Class III AREs lack the classic AUUUA motif but contain other cis-regulatory elements such as the nonameric UUAUUUA(U/A)(U/A) or UUAUUUAUU motifs [39]. Many transcripts contain more than one single ARE and these different cis-acting elements can act independently from one another which allows the transcript to be regulated via multiple mechanisms of post-transcriptional regulation [40]. The fate of a transcript to be stabilized or destabilized can depend on the combined actions of RBPs along the transcript [41]. Transcripts containing AREs are also capable of binding multiple RBPs, either via the same ARE sequence or at different cis-acting elements, suggesting competition and underlining the complex regulation of mRNA stability [42]. Clearly, the many cis-regulatory sequences and RBPs which recognize them underscore the complexity and opportunity to characterize the regulation of these transcripts (Figure 1). Multiple studies thus far have demonstrated that RNA stability provides a rapid level of regulation that can have major effects in maintaining global inflammation. For example, Shaw and Kamen demonstrated that inserting the ARE from the 3′-UTR of GM-CSF mRNA into the 3′-UTR of a stable transcript such as β-globin caused the β-globin transcript to be significantly unstable [43]. This finding paved the way for more studies on transcript stability, particularly of inflammatory mediators, especially TNFα, where mice lacking the TNFα ARE demonstrated increased constitutive circulating TNFα as well as sensitivity to LPS [44]. More importantly, TNFΔ ARE mice accrued more TNFα mRNA than control mice as a result of increased TNFα mRNA half-life due to loss of the unstable ARE [44]. The importance of RNA stability was further supported by Hao and Baltimore in 2009 when they found that differences in mRNA stability of TNFα had a greater influence on the temporal order of gene expression than transcriptional control elements [45].

The cis ARE sequences are highly specific and recognized by over 20 trans-acting RBPs. Among them there are two distinct categories: those that bind and stabilize transcripts allowing for increased protein translation, and those that destabilize the transcript to induce increased transcript degradation. One mechanism used to destabilize transcripts begins with 3′–5′ exonucleolytic deadenylation of the polyA-tail. Exonucleolytic cleavage of mRNA is driven by the “exosome,” a large multiprotein complex consisted of different exonucleases to execute the exonucleolytic cleavage in ARE-mediated decay [46]. The exact mechanisms by which RBPs interact with the target ARE to induce exosomal degradation are not completely understood, but some human exosomal homologs specifically bind to AU-rich elements, which may facilitate ARE-dependent degradation by the exosomal complex [47,48]. RNA stability factors can mask endonucleolytic cleavage sites, thus protecting mRNA from endonucleolytic cleavage to increase mRNA stability [31].

As we learn more about the mechanisms behind RNA stability, we are certain that the temporal regulation of ARE-containing transcripts is crucial to many cellular processes. In the context of inflammation, fibroblasts and bone marrow-derived macrophages stimulated with TNFα were analyzed for ARE-containing transcripts and it was found that genes of class I AREs peaked mRNA expression at 0.5 h, class II AREs at 2 h, and class III ARE-containing genes at 12 h. These results also indicated a correlation between mRNA stability and the function of TNFα-induced proteins. For example, the first group of genes that were induced appeared involved in acute phase inflammation, the second group encoded cytokines and chemokines to perpetuate inflammation, and finally, the third group of genes included those for tissue remodeling, wound healing and therefore possibly chronic inflammation [49]. The post-transcriptional, temporal regulation of these inflammatory genes should be further investigated in inflammatory diseases such as atherosclerosis to determine if targeting specific classes at specific times would be beneficial.

Destabilizing RNA-binding proteins currently outnumber stabilizing RNA-binding proteins, but the roles and regulation of both during the inflammatory response and disease progression are still unclear. Based on the cis-acting sequences on inflammatory transcripts, there is a clear coexistence, or competitive balance between RNA elements that stabilize and destabilize the mRNA, and that destabilizing RBPs may be used to dampen an inflammatory response via AREs [50].

Destabilization of inflammatory transcripts by trans-acting RBPs

Tristetraprolin (TTP), the most well-characterized member of the small family of CCCH tandem zinc finger (TZF) domain proteins, is one of the first identified destabilizing RNA-binding proteins. In 1990, Lai et al. identified TTP to be rapidly transcribed in response to insulin and other mitogens [141]. DuBois et al. [51] identified a novel regulatory protein Nup475 (alias for TTP), which functioned as a nucleic acid binding protein involved in regulating the response of growth factors. TTP was found to bind directly to the ARE within the 3′-UTR of TNFα mRNA, and TTP knockout mice had chronic excess of TNFα as well as macrophage hypersecretion of TNFα, one of the first reports to implicate TTP as a possible inflammation-modulating RNA-binding protein [52]. The mechanism by which TTP is activated has been elucidated. Mitogen-activated protein kinase (MK2) directly phosphorylates TTP at serine residues 52 and 178 in mouse TTP which reduces the stability of TTP by allowing 14-3-3 adaptor proteins to bind TTP, forming a complex that inhibits TTP-dependent degradation of transcripts [53]. In addition, phosphorylation of TTP may also reduce its affinity for binding the ARE [54]. In a knockin TTP mouse in which serine 52 and 178 are mutated to nonphosphorylatable alanine residues, TTP is constitutively active, with a significant decrease in TNFα mRNA and transcript stabilization in macrophages stimulated with LPS [55]. In 2016, Patial et al. developed a TTPΔARE mouse, in which an ARE instability element was genetically removed from TTP mRNA, preventing its autoregulation, resulting in more abundant TTP [56]. Increased expression of TTP in mice resulted in protection in mouse models for human inflammatory diseases such as rheumatoid arthritis, psoriasis, and multiple sclerosis by decreasing the production or stability of proinflammatory cytokines [56]. TTP has also been shown to regulate the stability of COX-2 [57], IL-2 [58], IL-6 [57,59], IL-8 [60], Cyclin D1 [61], IL-1α [57], GM-CSF [62], IL-2 [63], IL-10 [57], and CXCL1 [64]. TTP is a negative regulator of the NLRP3 inflammasome via binding of the ARE in the 3′-UTR [65]. The inflammasome is a central regulator of many inflammatory diseases including atherosclerosis where it drives the production of proinflammatory cytokines, mainly IL-1β [65]. The synthesis of these studies implicates TTP as an important regulator of inflammation.

HNRNP or heterogeneous nuclear ribonucleic protein is a family of RBPs with a wide range of functions, but notable for this discussion is its ability to act as a trans-factor for regulating gene expression [66]. hnRNP I was found to function as a decay-promoting factor for the low-density lipoprotein receptor (LDLR) mRNA transcript through binding to the 3′-UTR [67]. AUF1 (hnRNP D) is a destabilizing RBP that has been implicated in regulating inflammatory transcripts. AUF1, similar to TTP, acts as negative feedback facilitator in response to inflammatory stimuli such as LPS and endotoxin. AUF1 exists in several isoforms, most notably as 37- and 40-kDa polypeptides [68]. AUF1 significantly increased the *in vitro* decay of the GM-CSF 3′-UTR through binding of the ARE [69]. An AUF1 isoform, p40 isoform, can bind the 3′-UTR of anti-inflammatory cytokine IL-10 and induce its synthesis in THP-1 cells following inflammatory stimulation [70]. One study focusing on the fatal progression of endotoxemia to endotoxic shock, macrophages from AUF1$^{-/-}$ mice stimulated with LPS had abnormal stabilization of TNFα and IL-1β mRNAs, leading to the conclusion that AUF1 had protective effects by attenuating proinflammatory cytokine expression [71]. An AUF1 knockout mouse model was used to study atopic dermatitis, another inflammatory disease. This study corroborated that mice lacking AUF1 display enhanced contact hypersensitivity characterized by a substantial increase in T lymphocytes and macrophage infiltration, as well as a significant increase in proinflammatory cytokines [72]. The roles of TTP and AUF1 are critical and yet understudied in regulating inflammation in specifically inflammatory vascular diseases where the resolution or significant reduction of inflammation could have potential therapeutic benefits.

Another ARE-binding protein known for its negative regulation of transcripts is T-cell intracellular antigen 1, also known as TIA1. TIA-1 and T-cell intracellular antigen related (TIAR) do not function as typical RBPs. Although TIA-1 functions through ARE binding of 3′-UTR of mRNAs, the mechanism by which it reduces proinflammatory transcripts differs from TTP and AUF1. These proteins bind AREs in mRNA 3′-UTR without affecting the mRNA stability, instead, they divert mRNA from the polysomes to untranslated messenger ribonucleoproteins [73]. TIA-1 knockout macrophages had significantly increased LPS-induced expression of TNF-α compared with the WT control, due to lack of TIA-1 translational silencing [74]. TIA-1 also regulates COX-2 expression through an ARE-dependent mechanism; again through translational silencing and not by directly decreasing RNA stability [75]. The role of TIA-1 as an anti-inflammatory RBP is not well studied or understood; however, in Phillips et al. [76] the authors examined the role of both RBPs TTP and TIA-1 in the context of arthritis. Mice lacking either TIA-1 or TTP develop inflammatory arthritis, while mice deficient in both TIA-1 and TTP develop more severe arthritis. The results of this interesting study suggest TTP and TIA-1 may cooperate to determine the severity of inflammatory arthritis, but more importantly, that these two RBPs could be considered suppressors of arthritis. At last, under hypoxic conditions, TIAR and TIA1 were found to coaggregate in stress granules to destabilize hypoxia-inducible factor-1 α (HIF-1α) mRNA, a key proangiogenic transcription factor, demonstrating the potential of these RBPs to respond to other inflammatory stressors [77]. Further studies on TIAR and TIA1 in the context of vascular inflammation are warranted.

Butyrate response factor-1 (BRF1), a zinc finger protein homologous to TTP, also contains a CCCH tandem zinc-finger motif that binds mRNA. BRF1 binds AU-rich elements in the 3′-UTR of mRNA to promote deadenylation and degradation of a target transcript. Similar to TTP, BRF1 phosphorylation is critical to controlling its function as a stability factor. BRF1 features two overlapping consensus sequences for protein kinase B (PKB) phosphorylation, and phosphorylation of BRF1 by PKB stabilizes ARE-mRNA [78]. Phosphorylation of BRF1, analogous to TTP, prevents the destabilizing function of this RBP. BRF1 shares many mRNA targets with TTP, but to distinguish the role of BRF1, a targeted disruption of BRF1 *in vivo* resulted in mid-gestation death with extra-embryonic and intraembryonic vascular abnormalities and heart defects [79]. The embryos and embryonic fibroblasts cultured under both normoxia and hypoxic conditions had elevated VEGF-A; however, this was not a result of increased stability but rather due to translational regulation [79].

The fragile-X family of proteins includes Fragile-X mental retardation protein (FMRP) as well as homologs Fragile-X related proteins (FXR1-) 1 and 2. FMRP is an RBP widely expressed in mammalian tissues but is most notable for a trinucleotide repeat expansion, inactivating the X-linked FMR1 gene causing the mental retardation Fragile X syndrome [80,81]. FMRP and its homolog FXR1 contain two KH domains and an RGG box which are classic domains of RBPs. Like FMRP, FXR1 is also expressed widely in tissue, but is enriched in certain tissue types, specifically in heart, liver, muscle, and testis [82]. Garnon et al. [82] demonstrated the ability of FXR1 to bind TNFα mRNA and showed that macrophages deficient in FXR1 had increased TNFα expression. However, despite FXR1 binding to TNFα mRNA, these authors concluded that FXR1 has no effect on the mRNA stability. Khera et al. [83] found that anti-inflammatory transforming growth factor-β1 (TGF-β1) can induce FXR1 expression in macrophages. In this study, TGF-β1 regulation of TNFα was dependent on FXR1, further confirming the FXR1's role in the regulation of inflammatory mediators. Evidence suggests phosphorylation of FMR at Ser 144 plays a role in translational repression, but the effects of phosphorylation are not known. Khera et al. demonstrated that inhibition of the p38/MAPK pathway up-regulates FXR1 expression through an unknown mechanism; however, the role of FXR1 as an inflammatory repressor requires further investigation, and phosphorylation could be a critical signaling event in this activity.

KSRP, the KH-type splicing regulatory protein (KSRP) is an RBP first found to be involved in regulated splicing of c-src, but has been implicated in destabilization of other transcripts which contain AREs [48]. KSRP has been associated with chronic inflammation of the central nervous system (CNS). KSRP$^{-/-}$ astrocytes expressed increased levels of inflammatory factors such as TNFα and IL-1β [84]. KSRP was identified as an RBP, binding distinct regions of the IL-8 mRNA 3′-UTR to destabilize and degrade the transcript in cultured breast cancer cells [42]. Chen et. al. [47] confirmed that KSRP is a key mediator of mRNA decay, functioning as a bridge between AREs within the 3′-UTR and the degradation machinery, mainly exosomes. Proteins involved in the cellular inflammatory response, such as iNOS and COX-2 are regulated by KSRP through binding of the ARE and interacting with the exosome [85]. Overall, there is a lack of evidence that RBPs are up-regulated or down-regulated in vascular inflammatory diseases; therefore, more investigation is needed to determine if these inflammation-regulating proteins have potential to be targets for therapeutic development.

Stabilization of inflammatory transcripts

HuR is a ubiquitously expressed member of the Hu (ELAV) family of RBPs, while family members HuB, C, and D are primarily neuronal [86]. HuR is one of the best characterized RBPs as it is known to regulate a comprehensive collection of ARE-containing mRNAs including those coding for cytokines, cell-cycle regulators, growth factors, tumor suppressors, protocooncogenes, inflammatory enzymes, and apoptosis regulatory proteins [87]. An initial study by Fan and Steitz demonstrated that overexpression of HuR enhanced the stability of a B-globin reporter mRNA *in vitro,* which was supported by a second study [88, 142]. HuR has the ability to shuttle between the nucleus and the cytoplasm to regulate transcript stability; however, the mechanisms by which HuR translocates between the nucleus and the cytoplasm are not clearly understood. Phosphorylation of HuR by various kinases including the protein-kinase C family regulates HuR activity by potentially affecting HuR's shuttling capacity [89]. AMP-activated kinase (AMPK), the p38 MAPK and MAPKAPK-2 (MK2) pathway, as well as post-transcriptional regulation by methylation, may also play a role in HuR translocation and activation [90]. HuR was identified to bind and stabilize the TNFα mRNA transcript via the ARE in the 3′-UTR, both in cell-free and *in vitro* systems [91,92]. Mice lacking HuR in myeloid-lineage cells showed enhanced sensitivity to endotoxemia and an exacerbated inflammatory cytokine profile, citing HuR as a homeostatic coordinator of mRNAs that are essential for the innate immune response [93]. HuR is also autoregulatory as it contains ARE elements in its 3′-UTR, which can potentially interact with HuR itself [94].

HuR was found to be an important regulator of the inflammatory response of endothelial cells under mechanical and biochemical stress [95]. In addition, HuR expression and cytoplasmic abundance are increased in the intima and neointima layers in vascular pathologies including atherosclerosis and intimal hyperplasia where smooth muscle cells play a critical role in progression of disease [96]. Reduced HuR expression also attenuated proliferation of hVSMCs, consistent with the recognized ability of HuR to stabilize cell-cycle promoting transcripts, including cyclin A, B1, c-Fos, c-Myc, and cyclin D1 [97]. Because proliferation and inflammation are tightly coupled in vascular disease, HuR function may represent an attractive target to develop interventions to reduce or block vascular diseases.

Quaking (QKI) is an RBP primarily expressed in the brain, heart, lung, and testis [98]. QKI is a member of the highly conserved signal transduction and activator of RNA (STAR) family of RBPs [99]. Various QKI isoforms regulate RNA metabolism of cellular processes such as myelination, cell fate determination, embryogenesis, and blood vessel development [100]. Most of the functions of QKI have been identified in neurobiology, such as QKI's role as a stabilizing RBP for Sirtuin 2 (SIRT2) during differentiation of oligodendrocytes [101]. Li et al. [102] identified QKI as a critical regulator of smooth muscle development, as qki null mice showed that the vitelline vessel, which connects the artery to the yolk sac, was deficient in smooth muscle cells. In adult vascular smooth muscle cells, QKI regulates Myocardin (Myocd), a driver of smooth muscle cell redifferentiation and regulator of VSMC phenotype, through expression and alternative splicing [103]. The same study also found QKI poorly expressed in quiescent VSMC but strongly induced in response to vascular injury, suggesting QKI is responsible for dedifferentiation of VSMC as part of a vascular repair process [103]. Using femoral cuff placement to induce injury, Veer et al. [103] demonstrated that isolated primary VSMCs from the Qk^V (hypomorphic) mouse had significantly reduced cellular proliferation, migration, and collagen production, further supporting QKIs role in regulating VSMC phenotype and dedifferentiation. QKI is also expressed in the endothelium of healthy human arteries where it binds the 3′-UTR of VE-cadherin and β-catenin to enhance protein expression and maintain endothelial barrier function [104]. Overall, it is clear that although QKI has multiple functions, it plays a critical role in regulating smooth muscle and endothelial cell response to inflammatory stimuli which may contribute to inflammatory and proliferative vascular diseases.

Inflammatory signals and regulation of RBPs

Most regulatory processes in a cell occur through modified responses rather than large overarching changes. RBPs provide a fine-tuned response to inflammation, functioning to create a balance of transcripts readily available for cellular needs (Table 1). Because RNA-binding proteins play such a key role in regulating inflammatory transcripts, it is intuitive then that RBPs themselves are regulated by inflammatory stimuli, but evidence that they actually are is scant (Figure 2). Although both TTP and BRF1 are negative regulators of inflammation, both are induced by LPS in macrophages, likely as a compensatory response [105]. AUF1 is also induced in macrophages following LPS stimulation [70]. The expression of QKI was found to be initially down-regulated by 4 h of LPS stimulation in RAW-264.7 macrophages, but returned to baseline beyond 12 h [106]. HuR expression is tightly coupled with AKT phosphorylation levels, and HuR gene transcription is up-regulated via PI3K/AKT/NF-κB signaling [107]. For a more detailed summary of proinflammatory stimuli altering RBPs see Table 1 [108–110].

P38 regulation of RBPs: a counterregulatory mechanism?

Numerous studies have shown LPS-induced induction of RBPs, which begs the question; which other inflammatory or anti-inflammatory stimuli can modulate RBPs and therefore regulate the repertoire of ARE-containing inflammatory transcripts? p38 MAPK activation drives atherosclerosis, playing many roles including stimulating the secretion of MCP-1 and IL-8 to attract monocytes to vascular endothelial cells; promotes differentiation of monocytes into macrophages; inhibits proliferation while also inducing apoptosis in endothelial cells; mediates MCP-1 dependent transendothelial cell migrations; and activates integrins and chemotaxis [111]. Many RBPs are substrates of the p38 MAPK pathway, implying these proteins are part of signaling pathways responding to proinflammatory stimuli [112,113]. A strong link has been established between the p38 pathway and inflammation, including known roles in rheumatoid arthritis, Alzheimer’s disease, inflammatory bowel disease, as well as endotoxin-induced shock, collagen-induced arthritis, and acute lung inflammation [114–118]. In addition, inflammatory cytokines such as TNFα and IL-1 as well as LPS and environmental stresses are known catalysts of initiating p38 signaling. In macrophages, p38a plays a critical role in regulating the expression of proinflammatory mediators including IL-1β, TNFα, COX2, IL-6, and many others [113,119,120]. The activation of p38 MAPK is not restricted to monocytes/macrophages, but also vascular endothelial and vascular smooth muscle cells [111]. P38 can also regulate endothelial cell proliferation and differentiation through VCAM-1 [121]. Various stimuli can activate the p38 pathway in all of these cell types including ROS [122], high levels of glucose [123], free fatty acids [124], cholesterol [125], as well as proinflammatory cytokines [126], placing RBP expression downstream from all of these cardiovascular risk factors. Despite this, selective p38 MAPK inhibitors so far have yielded little or no clinical benefit in vascular diseases. It is well documented that p38 promotes expression of proinflammatory mediators but is also activated by them, creating a feed-forward amplifying inflammatory response; therefore, it is important to note p38 has an anti-inflammatory mechanism to prevent uncontrolled inflammation. As a result, a negative feedback loop via intracellular signaling leads to the up-regulation of anti-inflammatory cytokines such as IL-10 [127]. Long-term blockades of p38 by selective inhibitors may also prevent the function of anti-inflammatory factors needed to limit and resolve inflammation, which in some cases exacerbates more inflammation and defeats the purpose of the therapeutic, which may explain the disappointing p38 clinical trials. Therefore, it is critical we look for more specific, down-stream targets of p38, such as RBPs, to attempt to effectively limit inflammation and chronic inflammatory diseases.

In terms of anti-inflammatory cytokine effects on RBPs expression or activity, IL-10, the prototypical anti-inflammatory cytokine, is able to destabilize TNFα mRNA through suppression of p38 MAPK and therefore inhibition of HuR [128,129]. In addition, Cuneo et al. [130] demonstrated that IL-19, a member of the IL-10 superfamily of anti-inflammatory cytokines, was able to reduce the stability of proinflammatory and proliferative ARE-containing transcripts by modifying the nucleocytoplasmic translocation of HuR. TGF-β, another anti-inflammatory cytokine, up-regulates TTP, increased FXR1 mRNA levels, and induced HuR translocation from the nucleus [83,131–133].

Many anti-inflammatory compounds owe their efficacy to effects on RBPs. For example, the common NSAID aspirin has an inhibitory effect on COX-2 activity, but can also paradoxically increase the stability of COX-2 via the p38 MAPK pathway [134]. Glucocorticoids are also capable of destabilizing the mRNA of numerous cytokines and inflammatory genes including TNFα, IL-1β, IL-6, MCP-1, and iNOS. Destabilization of TNFα via glucocorticoid therapy is a direct result of increased TTP expression induced by glucocorticoids and could be blocked with a transcriptional inhibitor such as actinomycin D [135,136]. Immunosupressive drugs cyclosporin A (CsA) and rapamycin have both been found to destabilize IL-3 mRNA by ARE-dependent mechanisms [137,138]. The antirejection drug, rapamycin, alters cyclin D1 and c-myc mRNA stability via TTP and its phosphorylation states [61]. Statins, which are most recognized for their ability to inhibit HMG-CoA reductase to lower cholesterol levels, also have vascular protective effects independent of its effects on cholesterol. A beneficial anti-inflammatory side-effect of statins is a result of stabilizing endothelial nitric oxide synthesis (eNOS) mRNA through a PI3K/PKB pathway. It is known from a separate study that hnRNP E1 can stabilize eNOS, both resulting in beneficial vascular dilation [139,140].

Together, these studies suggest anti-inflammatory treatments can alter RNA stability, in many cases by destabilizing inflammatory mediators; however, very little is known about the exact mechanisms by which RBPs are involved in the ARE-dependent regulation of these transcripts. More work is needed to characterize the pathways and processes in which pro- and anti-inflammatory stimuli affect the landscape of protein-RNA interactions which mediate ARE-dependent stabilization of transcripts.

Summary and future directions

RBPs are cytokine responsive proteins and are activated distally of key inflammatory pathways such as p38 MAPK and NF-kB signaling events. In response to inflammatory perturbation, they fine-tune the cellular response to inflammatory stimuli, and multiple studies demonstrate the ability of RBPs to directly regulate critical inflammatory mediators that specifically contribute to vascular inflammatory diseases. The mixed results of many anti-inflammatory therapies point to the need for more specific, downstream targets to reduce or resolve inflammation. Emerging data suggest RBPs, and regulation of inflammatory mRNA stability in particular, are attractive targets for more specific vascular disease therapeutics. The role of RBPs in regulating inflammation is far from solidified; however, the evidence thus far suggests they play a critical role in the cellular response to inflammatory signals. A better understanding of these processes will undoubtedly enable us to develop better therapeutics to combat vascular disease.

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Abbreviations

ARE	AU-rich element
AV	allograft vasculopathy
CAD	coronary artery disease
CAM	cell adhesion molecule
COX-2	cyclooxygenase-2
ICAM-1	intracellular adhesion molecule 1
IL-1β	interleukin-1β
MCP-1	monocyte chemoattractant protein-1
PAD	peripheral artery disease
PI3K	phosphoinositide 3-kinase
RBP	RNA-binding protein

TNFα	tumor necrosis factor α
VCAM-1	vascular cell adhesion molecule 1
VSMC	vascular smooth muscle cell

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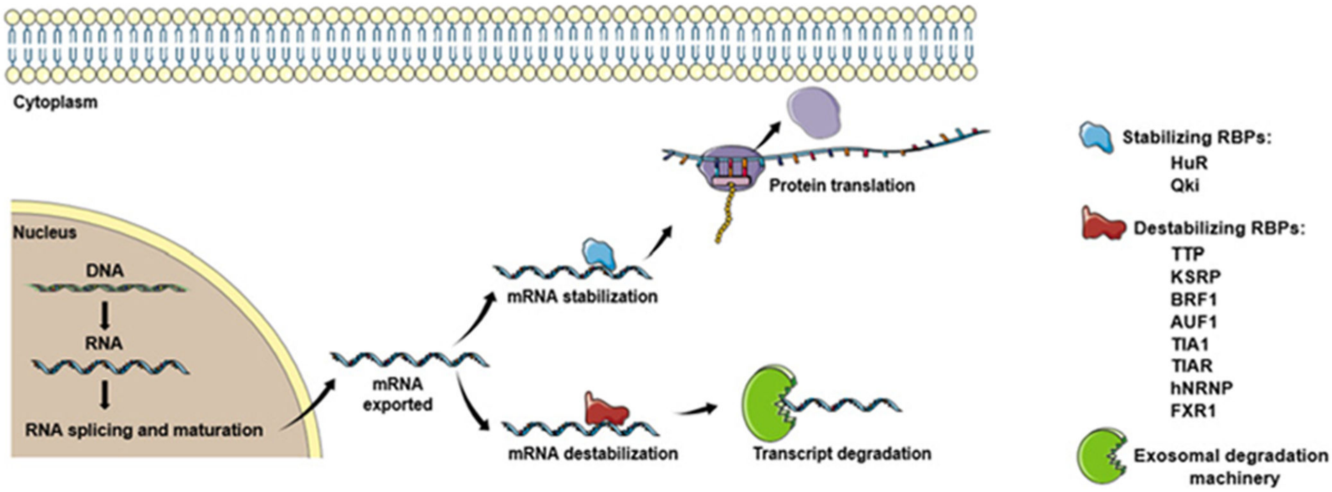

Figure 1. Stability of mRNAs regulated by RNA-binding proteins

Most inflammatory mRNA transcripts contain AU-rich elements in their 3′-UTR. Once exported to the cytoplasm, trans-acting RBPs bind and regulate transcript stability. Stabilizing RBPs can promote translation preventing its degradation. Destabilizing RBPs can promote transcript degradation by binding and recruiting exosomal degradation machinery.

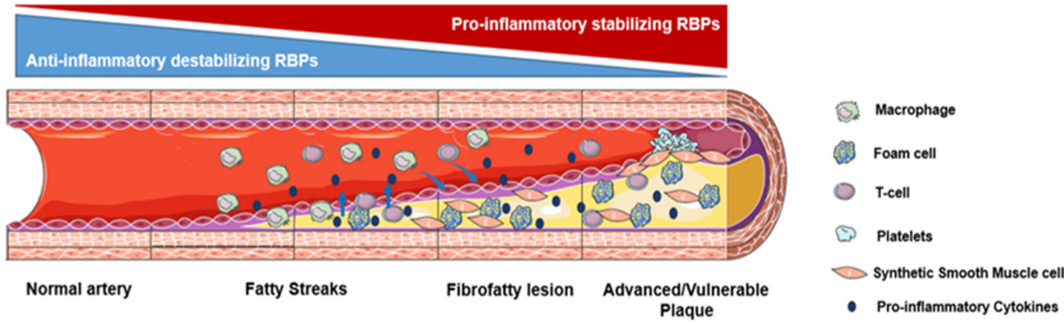

Figure 2. RNA-binding proteins as pro- and anti-inflammatory regulators of atherosclerosis

Proinflammatory signaling and cytokine expression drive the progression of atherosclerosis. Regulated expression and activation of RNA-binding proteins in these critical areas further promotes localized inflammation. The competitive balance of stabilizing RBPs over destabilizing RBPs could induce atherosclerosis progression, and the reverse could in theory promote plaque regression.

Table 1

Inflammatory effects on RNA-binding proteins (RBPs) and the resulting changes in RNA stability

Proinflammatory	RBP	Effect	Inflammatory transcripts
LPS	TTP	Increased expression	Decrease stability [91]
	BRF1	Increased expression	Decrease stability [91]
	AUF1 isoform	Increased expression	Increase stability [56]
	HuR	Increased expression	Increase stability [94]
Hypoxia	HuR	(Predicted) alter HuR phosphorylation	Increase stability [95]
	TTP	Increased expression	Decrease stability [96]
	TIA1/TIAR	Aggregate within granules	Decrease stability [63]
Anti-inflammatory	**RBP**	**Effect**	**Inflammatory transcripts**
IL-10	HuR	Decrease HuR activation	Decrease stability [114,115]
IL-19	HuR	Decrease HuR translocation	Decrease stability [116]
TGF-β	TTP	Increased expression	Decrease stability [117]
	FXR1	Increased expression	No effect [69]
	HuR	Increased expression	Increase stability [119]
Glucocorticoids	TTP	Increased expression	Decrease stability [121]
NSAIDS	Unknown	Unknown	Unknown [120]
Rapamycin	(Predicted) HuR	Unknown	Unknown [36]
	TTP	Phosphorylation, accumulation	Decrease stability [47]
Statins	hnRNP E1	Unknown	Decrease stability [125,126]