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Review Article

Post-Transcriptional Gene Regulation by MicroRNA and RNA-Binding Protein

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Abstract: Recent advanced studies have demonstrated that post-transcriptional gene regulation is involved in many aspects of biological processes and in the pathogenesis of various types of disorders, such as neurodegenerative diseases, autoimmune diseases, and cancer. In addition to transcriptional regulation, spatially and temporally regulated gene expression is achieved by the post-transcriptional control of transcribed RNAs, including through splicing, export, stability, localization, and translation. These processes are regulated by the formation of ribonucleoprotein complexes with RNA-binding proteins and small non-coding RNAs. Here, we will describe the findings obtained from studies on mice deficient for individual RNA-binding proteins and microRNAs involved in maintaining homeostasis or causing disease.

Keywords: Post-Transcriptional Regulation, MicroRNA, RNA-Binding Protein, Knockout Mice

1. Introduction

Accumulating evidence clearly indicates that post-transcriptional gene regulation is essential for various aspects of biological processes [1-6]. Transcription of mRNA is the first step in gene expression, and changes in transcriptional rates caused by the assembly of transcription factors are critical determinants of the majority of temporal changes in mRNA levels in cells [7]. In addition, post-transcriptional regulation such as splicing; modification with an m7G cap and poly-adenylation at the 5′- and 3′-ends, respectively; nuclear export; stabilization; and localization is also important for shaping "peaked" responses such as receiving external stimuli and extending the duration of expression [7-10]. Furthermore, mRNA stabilization and/or degradation; RNA modifications such as methylation and pseudouridylation; and associations with the translational machinery affect the efficiency of protein synthesis [11-14]. These post-transcriptional events are regulated by the formation of ribonucleoprotein complexes with RNA-binding proteins (RBPs). In addition, small non-coding RNAs such as microRNAs (miRNAs) are also involved in regulation through the formation of RNA-induced silencing complexes (RISCs).

In this review, we will highlight the roles of the post-transcriptional regulation of gene expression in development, maintaining immune homeostasis, and the pathogenesis of autoimmune diseases and cancer, with insights from studies on knockout mouse models of individual miRNAs and RBPs.

2. MicroRNAs

MiRNAs are ~22-nucleotide single-stranded small non-coding RNAs that are highly conserved among eukaryotes and play a critical role in the post-transcriptional regulation of gene expression. miRNAs have a unique synthesis process [15]. Many miRNAs have their own transcription initiation region, and some miRNAs are located on introns of other genes or exist as a cluster of transcriptional

units. First, an miRNA is transcribed by RNA polymerase II to produce a primary miRNA (pri-miRNA), which is a long primary transcript containing one or more miRNA. Drosha, a nuclear RNase III, then crops the pri-miRNA into a precursor miRNA (pre-miRNA), which has a hairpin loop structure and is then exported from the nucleus into the cytoplasm by Exportin 5 [16-18]. Finally, the pre-miRNA is cleaved by cytoplasmic RNase III Dicer to form short nucleotide duplexes, and either one of the single strands is incorporated into an Argonaute (Ago) protein complex, known as an RISC. The single-stranded RNA is now a mature miRNA and is able to bind a partial complementary sequence, mainly located on the 3′-untranslated region (3′-UTR) of the target mRNA, and impart its translation repression or degradation [19-21]. Because Dicer knockout mice display severe growth arrest at an early embryonic stage [22], miRNAs are regarded as critical factors for development. Recently, some loss-of-function studies revealed that individual miRNAs play critical roles in various biological processes such as development, homeostasis, and immune system function, and these roles are highlighted below.

2.1. MiR 17~92 Cluster

The miR-17~92 cluster contains miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, and miR-92a-1, which are promoted by MYC (v-myc avian myelocytomatosis viral oncogene homolog) and are representative miRNAs that are considered “oncogenes” [23, 24]. miR-17~92 knockout mice show perinatal lethality with heart, lung, and skeletal defects, indicating that the miR-17~92 cluster is critical for development [25]. Knockout mice lacking miR-106a~363 and miR-106b~25, which are known as the miR-17~92 family, have no obvious phenotypes; however, mice with co-deletion of miR-17~92 with miR-106a~363 and miR-106b~25 die before embryonic day 15 [25], indicating that these clusters functionally cooperate in regulating embryonic development.

2.2. MiR-34/449

The miR-34/449 family contains miR-34a/b/c and miR-449a/b/c, constituting a conserved family in vertebrates, and seed sequence homology among miR-34/449 miRNAs predicts robust functional redundancy. Although the miR-34 family has been proposed as critical modulators of the p 53 pathway and potential tumor suppressors, miR-34 knockout mice exhibit normal development and do not display increased susceptibility to spontaneous, irradiation-induced, or c-Myc-initiated tumorigenesis [26]. On the other hand, miR-34/449 miRNAs are highly enriched in mucociliary epithelia that contain motile cilia, and miR-34/449 deletion in mice and frogs leads to defects in ciliogenesis. miR-34/449 knockout mice exhibit frequent postnatal mortality, with only ~40% surviving to adulthood [27]. These results indicate that the miR-34/449 family is essential for development.

2.3. MiR-1/126/205

The above examples are phenotypes of the deletion of an miRNA cluster or family in mice. However, severe developmental phenotypes in single-miRNA knockout mice have also been reported. miR-1 and miR-133 are muscle-specific miRNAs [28-32]. miR-1-2 knockout mice exhibit cardiac morphogenic, electrical conduction, and cell cycle defects and 50% lethality at weaning [33]. In addition to this miRNA, other single-miRNA knockout mice showing lethal phenotypes, such as knockout mice lacking miR-205 (100% neonatal lethality with compromised epidermal and hair follicle growth; [34]) and miR-126 (40-50% embryonic/perinatal lethality with angiogenesis defects; [35]), have also been reported.

2.4. MiR-140

Our group revealed that miRNA is important for cartilage development and homeostasis. miR-140 is specifically expressed in the cartilage of mouse embryos and zebrafish [36-38], and this expression is regulated by Sox 9, a master regulator of chondrogenesis [39]. Previous studies also found reduced miR-140 expression in human osteoarthritis (OA) cartilage [40, 41]. miR-140 knockout mice show a mild skeletal phenotype with a short stature, although the structure of the articular joint cartilage appeared grossly normal in 1-month-old miR-140 mutant mice. However, interestingly, miR-140−/− mice manifest age-related OA-like changes [37]. miR-140 directly regulates Adamts-5, which degrades aggrecan and is a critical enzyme for OA pathogenesis [37, 42, 43]. These findings demonstrate that miR-140 is required for skeletal development and cartilage homeostasis and protects against OA-like pathology via Adamts-5 regulation.

2.5. MiR-155

MiRNAs are critical regulators of not only development and homeostasis but also the immune system. miR-155 maps within an exon of the noncoding RNA bic [44, 45], its primary miRNA precursor [46]. bic/miR-155 shows greatly increased expression in activated B- and T-cells, macrophages, and dendritic cells (DCs) [46-50]. Increased expression of bic/miR-155 has been reported in B-cell lymphomas and solid tumors [51], and transgenic miR-155 mice have also been shown to develop B-cell malignancies [52], indicating that the miRNA might be linked to cancer. miR-155/bic knockout mice are immunodeficient and display increased lung airway remodeling [53], indicating that miR-155 plays a key role in the function of the immune system.

2.6. MiR-146

MiR-146a was initially discovered as an miRNA that was induced upon microbial infection [49]. miR-146a is induced by NF-kB and inhibits innate immune responses by repressing TRAF6 and IRAK1 [49], and miR-146a knockout mice show several immune defects. miR-146a null mice show hyperresponsiveness of macrophages to bacterial LPSs, which leads to an exaggerated inflammatory response in LPS-challenged mice [54]. Later in life, miR-146a knockout mice develop a spontaneous autoimmune disorder,

characterized by splenomegaly, lymphadenopathy, and multiorgan inflammation; as a result, many die prematurely [54]. In addition, aged miR-146a knockout mice display an excessive production of myeloid cells and develop myeloid sarcomas and some lymphomas [54, 55], suggesting that miR-146a can function as a tumor suppressor in the context of the immune system.

3. RNA-Binding Proteins

RBPs play pivotal roles post-transcriptionally in the regulation of gene expression. RBPs are estimated to be encoded in over 1,500 genes in the human genome and are known to be involved in inflammation and neurodegenerative disorders [56-58]. As with miRNAs, the binding specificity between cognate RNA and RBPs is determined by the primary RNA sequence. The most prominent example is the adenine (A) and uridine (U)-rich element (ARE) that is characterized by AUUUA or UUAUUUUAUU in the U-rich context. Over three decades ago, AREs were found in the 3′-UTR of GM-CSF and TNFα and shown to destabilize mRNA [59, 60]. A later study estimated that 5-8% of all mRNAs contain functional AREs and are involved in various biological processes, including the immune response, inflammation, and development [61, 62]. The secondary structure, as well as the primary sequence, of mRNA is also a critical determinant of its specificity to cognate mRNAs. Although miRNAs and ARE-binding proteins are the most prominent examples of post-transcriptional regulation as mentioned above, a stem-loop structure that differed from the complementary sequence to any known miRNA and ARE was found in the 3′-UTR of TNFα and named the conserved decay element [63, 64]. We will describe the findings obtained from mice deficient in RBPs involved in development, reproduction, the immune system, and neural function.

3.1. Tristetraprolin Family

Tristetraprolin (TTP) is encoded by Zfp36 and composed of two CCCH-type zinc finger domains. Although TTP has been considered a DNA-binding transcription factor, later studies demonstrated that TTP binds directly to mRNA, especially to AREs [65]. Mice deficient in TTP appear normal after birth, but they show spontaneously severe myeloid hyperplasia, arthritis with bone erosion, dermatitis, conjunctivitis, glomerular mesangial thickening, high titers of anti-nuclear antibodies, and cachexia due to elevated production of TNFα by macrophages and neutrophils [66]. These inflammation-associated phenotypes are prevented by injection of a monoclonal antibody against TNFα. Consistent with this, mice lacking an ARE in the 3′-UTR of TNFα show an auto-inflammatory phenotype resembling that of TTP-deficient mice [67]. These findings strongly suggested that TTP-mediated suppression of TNFα production is crucial for maintaining immune homeostasis *in vivo*. BRF1 and BRF2 are close homologues of TTP that possess CCCH-type zinc fingers that are very similar to that of TTP but differ in their N- and C-terminal ends. Unlike TTP-deficient mice, mice lacking BRF1 die at embryonic day 11 because of failure of chorioallantoic fusion [68]. Although BRF2-deficient mice are born at the expected Mendelian ratio, cells that originate from hematopoietic stem cells, including white and red blood cells and platelets, are greatly reduced, resulting in death within 2 weeks after birth [69]. Interestingly, it was reported that mice that lacked BRF1 and BRF2 during thymopoiesis developed a T cell acute lymphoblastic leukemia dependent on the oncogenic transcription factor Notch1. Thus, tristetraprolin families prevent the pathogenesis of autoimmune diseases and leukemia [70].

3.2. AUF1

AUF1 (also known as HNRNPD) binds to mRNAs containing an ARE and suppresses translational initiation by replacing translational initiation factor eIF4G and poly (A) binding protein [71]. AUF1-deficient mice have a higher susceptibility to endotoxin shock and die earlier compared to WT mice [72]. These differences were due to the excessive production of TNFα and IL-1β by macrophages. AUF1-deficient mice were shown to have a reduced number of splenocytes and develop spontaneously pruritic inflammatory skin disease with elevated serum IgE levels and T-cell hyper-proliferation, indicating that AUF1 is also involved in adaptive immune responses as well as in innate immunity [73]. Interestingly, AUF1 is also involved in maintainance of telomere length, and decreased survival and increased markers of aging are observed in late-generation AUF1 deficient mice [74].

3.3. TIA-1/TIAL-1

TIA-1 and TIAL-1 have well-defined RNA-recognition motifs that bind to AREs with high affinity and suppress mRNA translation through the formation of a 48S* pre-initiation complex, which is a translationally stalled complex, in processing body [11]. TIA-1-deficient mice spontaneously develop arthritis and die earlier of endotoxin shock compared to WT mice [75]. Macrophages derived from TIA-1-deficient mice produce higher amounts of TNFα and IL-1β, but not IL-6. Sucrose gradient analysis revealed that TNFα mRNAs had a higher degree of association with polysomes, which represent actively translating ribosomes, in TIA-1-deficient macrophages compared to normal macrophages. These results indicated that the translational suppression of inflammatory cytokines such as TNFα by TIA-1 is crucial for maintaining immune homeostasis *in vivo*. Similar to TIA-1 deficiency, partial lethality was also observed in mice lacking TIAL-1. In contrast to TIA-1-deficient mice, both male and female TIAL-1-deficient mice are sterile because they lack spermatogonia and oogonia, resulting from a primordial germ cell development defect [76]. These results indicated that the two related proteins, TIA-1 and TIAL-1, are involved in both the immune system and reproduction.

3.4. CUGBP1

CUGBP1, also known as CELF1, is a multifaceted RBP involved in the regulation of alternative splicing, stimulation of translation via the 5′-UTR of cognate mRNAs, and mRNA decay by binding to guanine- and uridine-rich elements found in the 3′-UTR of mRNA [11, 77, 78]. Mice deficient in CUGBP-1 are viable, but they display growth retardation [79]. Like TIAL-1 deficiency in mice, CUGBP-1 deficiency leads to impaired fertility in both male and female mice.

3.5. Hu Family

The Hu family is composed of four related genes: HuB, HuC, HuD, and HuR. The Hu family has three RRM domains and stimulates translation through binding to both AREs and polyA tails in the 3′-UTR of mRNA [80-82]. On the other hand, cross-linked RNA immunoprecipitation with high-throughput sequence analyses revealed that Hu proteins also bind to intronic sequences of RNA and regulate alternative splicing [83, 84]. The expression of HuB, HuC, and HuD is restricted to neural cells, whereas HuR is expressed ubiquitously. It was reported that HuC- or HuD-deficient mice showed poor rotarod performance and seizure, suggesting that neural Hu proteins are indispensable for normal neuron functioning [84, 85]. In contrast to neural Hu proteins, HuR-deficient embryos exhibit a stage retardation phenotype and fail to survive beyond mid-gestation [86]. T-cell-specific deletion of HuR leads to resistance to experimental autoimmune encephalomyelitis (EAE), an experimental model of human multiple sclerosis, via lower levels of T-cell proliferation and IL-17 production [87]. Floxed HuR mice harboring lysozyme-driven Cre expression show higher susceptibility to endotoxin shock, inflammatory bowel disease, and colitis-associated cancer with excessive production of inflammatory cytokines and chemokines, indicating that HuR expression in myeloid cells, including macrophages and neutrophils, is indispensable for maintaining immune homeostasis [88]. These results indicated that the Hu family of proteins is required during embryonic and adult stages of life.

3.6. Regnase-1

Regnase-1 is encoded by Zc3h12a and induced immediately after exposure to ligands for Toll-like receptors. Regnase-1 has a CCCH-type zinc finger domain that has ribonuclease activity and binds to stem-loop structures found in a set of inflammatory genes, including IL-6 and IL-12p40 [89]. Interestingly, Regnase-1 specifically cleaves and degrades translationally active mRNAs and is dependent on UPF1, an essential component of nonsense-mediated decay [90]. Regnase-1-deficient mice spontaneously suffer from severe anemia and fetal autoimmune diseases and die within 12 weeks [89]. An increased number of activated and/or memory phenotype T-cells, which are CD44 positive and CD62L negative, were observed in the spleen and lymph nodes of mice lacking Regnase-1. Plasma cells, which are known to be antibody-secreting cells, were dramatically increased in the spleen, leading to the excessive production of antibodies against nuclear contents and dsDNA. T-cell-specific deletion of Regnase-1 also results in fetal autoimmune diseases and autoantibody production, as seen in Regnase-1-deficient mice [91]. $CD4^+$ T-cells lacking Regnase-1 produce large amount of IFNγ, IL-4, and IL-17, which are representative cytokines for Th1, Th2, and Th17 cells, respectively. Severe autoimmune phenotypes observed in T-cell-specific Regnase-1 deficiency are partially dependent on the expression of c-Rel, a transcription factor composed of NF-κB and a target of Regnase-1, because mice deficient in both Regnase-1 and c-Rel exhibit a reduced number of plasma cells and activated T-cells. Therefore, Regnase-1 expression is indispensable for the maintenance of immune homeostasis.

3.7. Roquin

The sanroque mouse strain, which exhibits a severe autoimmune disease, was established by ethylnitrosourea-induced mutagenesis [92]. This mutant strain harbors a point mutation in which Met 199 is replaced with Arg within the ROQ domain of Roquin, and the mice exhibit a lupus-like pathology with higher amounts of anti-nuclear and dsDNA autoantibodies due to elevated expression of ICOS, which is a co-stimulatory receptor that facilitates follicular helper T-cell differentiation [93, 94]. This mutant strain also has increased susceptibility to endotoxin shock and autoantibody-induced arthritis [95]. Roquin (Roquin-1) and its homologue Roquin-2 have a ROQ domain that binds to the stem-loop structure of mRNAs found in the 3′-UTR of ICOS, TNFα, and IL-6 and degrades target mRNA via localization to P-bodies, followed by Caf-1-dependent deadenylation and degradation [64, 90, 93, 95, 96]. Although the complete loss of Roquin-1 or Roquin-2 in mice leads to perinatal lethality, lupus-like symptoms as seen in sanroque mutants are not observed in Roquin-1- or Roquin-2-deficient mice [97, 98]. However, mice lacking both Roquin-1 and Roquin-2 in T-cells exhibit an increased number of follicular helper T-cells and germinal center B cells, and develop lung inflammation and gastritis, indicating that Roquin-1 and Roquin-2 have redundant roles in the regulation of autoantibody production in a T-cell autonomous manner [98, 99].

3.8. ARID5a

The AT-rich interaction domain (ARID) is an ancient DNA-binding domain that is well conserved throughout evolution [100]. Interestingly, ARID5a also binds to single-strand RNAs and stabilizes a set of mRNAs such as IL-6 and STAT3 to facilitate their expression. As in the case of Regnase-1, ARID5a recognizes stem-loop structures via the ARID domain and prevents the binding of Regnase-1 to protect against the degradation of cognate mRNAs [101]. ARID5a is highly expressed in LPS-activated macrophages and Th17 cells, which are IL-17-secreting $CD4^+$ T-cells involved in autoimmune diseases. Mice deficient in ARID5a show resistance to EAE, indicating that ARID5a is crucial for

Th17-mediated immunopathology to stabilize IL-6 and STAT3 [102].

4. Conclusion

The findings described above highlight the importance of post-transcriptional regulation by miRNAs and RBPs in the pathogenesis of autoimmune diseases and cancer as well as in normal development. miR-16 is required for the rapid degradation of TNFα mRNA dependent on TTP, indicating that miRNAs and RBPs cooperatively regulate identical target mRNAs [103]. Recently, small compounds that regulate the alternative splicing of SMN2, the gene responsible for spinal muscular atrophy (SMA), were developed, and administrating them to Δ7 mice, a model of severe SMA, improved motor neuron function by increasing the expression of SMN protein and extending its life span [104]. Therefore, elucidating the mechanisms underlying post-transcriptional gene expression will provide new insights into novel therapeutic approaches for treating congenital diseases, autoimmune diseases, and cancer.

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