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MicroRNAs and autoimmunity

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Abstract

The role of microRNAs (miRNAs) in the regulation of many physiological and pathological processes has been intensely studied in recent years. Some miRNAs, such as miR-146a and miR-182, play a dominant role in the regulation of the innate and adaptive immune responses, respectively. Many miRNAs are reportedly deregulated in autoimmune diseases, but miR-146a in particular seems to be consistently altered. The over- or underexpression of miRNAs can influence specific targets and pathways, leading to autoimmune disease phenotypes, and this is supported also by some *in vivo* studies. Targeting miRNAs could represent a valid future therapeutic option for autoimmune diseases.

New findings in microRNA gene regulation

The discovery of microRNAs (miRNAs) has represented one of the most important advances in the understanding of mechanisms in gene regulation. Since the identification of the first miRNAs in *C. elegans* in 1993, many hundreds of miRNAs have been discovered, and they can regulate various conserved biological systems including those in mammalian cells [1]. The complexity of miRNAs regulation is also linked to the redundancy of the system, as each miRNA can target and repress many different genes, and vice versa one gene can be regulated by multiple miRNAs [2]. MiRNAs are transcribed from their respective gene loci in the nucleus, and after their initial status as primary-miRNAs (pri-miRNA), they are processed by the Drosha/DGCR8 complex, which transforms them into precursor-miRNA (pre-miRNA) approximately 70 nucleotides (nt) long double-stranded RNA hairpin structure. This “immature” miRNA is exported from the nucleus to the cytoplasm through Exportin-5 and further processed by Dicer into double-stranded miRNA duplex of about 22nt long that is loaded onto the RNA-induced silencing complex (RISC) to exert repressive function on its target messenger RNA (mRNA) [3]. The double-stranded RNA duplex is composed of a guide strand and a passenger strand, also known as miRNA*. Until recently, most reports suggested that the miRNA guide strand is the main species preferentially loaded to Argonaute 2 (Ago2) in RISC. However, a recent report showed the passenger strand (miR-155*) can cooperate with the guide strand (miR-155) to regulate type

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I interferon production in human plasmacytoid dendritic cells [4]. miRNAs are generally known to require perfect pairing of their seed regions (nt 2-7) to the 3'UTR (untranslated region) of their target mRNAs [5]. In some cases the miRNA:mRNA interaction does not have a perfect pairing in the seed region, but instead contains at least 11 contiguous nucleotides that pair to the center of the miRNA at positions 4-14 or 5-15, so called "centered pairing" [5]. The binding site for miRNAs can also be located in the 5'UTR, leading in some cases to translation enhancement [6], or to protein coding regions, causing translational repression and degradation. Besides the classical miRNA:mRNA interaction leading to target mRNA decay [7], miR-328 has additional "decoy" activity by binding to RNA-binding protein hnRNP E2 and thus interrupt hnRNP E2 binding capacity to its target mRNA, leading to interference with its mRNA-regulatory function [8]. However, the important note is that centered pairing, binding sites in coding region and 5'UTR, and decoy function are not detected in most miRNA target prediction algorithms to date, and it is critical to acknowledge that there is much to learn in miRNA target analyses. One limitation of the most common algorithms is that they do not consider additional aspects involved in miRNA-mRNA interaction, such as the three-dimensional folding structure of mRNA and the RNA-binding proteins that may block the mRNA targeting by miRNAs [9]. New methods such as HITS-CLIP and PAR-CLIP have recently been developed to identify the protein-RNA interactions that could allow a better understanding of the mechanisms of control in gene expression mediated by miRNAs [10-11].

Some recent studies have focused on exosomes in carrying genetic material such as miRNAs and mRNAs [12]. Exosomes are 40-100 nm vesicles that are formed inside the secreting cells probably via multivesicular bodies [13]. Exosomes can transfer miRNAs to recipient cells, where they have been shown to retain functions as demonstrated in reporter assay. This mechanism of cell-cell communication can be important in certain conditions, for example during immune response to pathogens [13]. However, it is not clear how they can be targeted to specific cells to exert their function. Understanding how miRNAs are transported and delivered via exosomes is important for their potential therapeutic application [14].

RISC as autoantigenic target

Another interesting area of investigation is the study of the localization of miRNA activity in the cell cytoplasm. In fact, miRNAs biogenesis starts in the nucleus but it is completed in the cytosol, and initial reports on the miRNA repression machinery showed that the RISC proteins Ago2 and GW182 interact with miRNAs in cytoplasmic foci called GW bodies (GWBs) or Processing bodies [15]. Accumulation of target mRNAs can occur in GWBs, and knockdown of some components of the miRNA machinery can disrupt the formation of these bodies in the cytoplasm [16]. It is of interest that human autoantibodies to AGO proteins are common in systemic lupus erythematosus (SLE) and other systemic autoimmune diseases [17], while anti-GW182 autoantibodies are detected in Sjögren's syndrome, neurological diseases, SLE and other conditions [18]. Since AGO and GW182 are currently considered the core components of RISC, it seems appropriate to suggest that the RISC is one of the main targets of human autoantibodies.

Aberrant expression of miRNAs in autoimmune diseases

Many miRNAs have been identified as overexpressed or underexpressed in autoimmune diseases such as SLE [19-20] and rheumatoid arthritis (RA) [21], and miR-146a in particular has been reported by different investigators to be altered in these diseases. The importance of miR-146a is evident in SLE, where its decreased expression leads to hyperactivation of type I interferon (I-IFN) pathway that is a hallmark of SLE (Figure 1) [22]. Recent genome-

wide studies have identified specific genetic variants in the promoter or intergenic region of miR-146a that could be responsible for SLE genetic susceptibility. In the Asian population, the variant rs57095329 in the promoter region of miR-146a gene accounts for miR-146a downregulation and higher risk to develop SLE [23]. Within the intergenic region of miR-146a, a different variant (rs2431697) in SLE patients of European ancestry is also associated with lower expression of miR-146a and a possible link to SLE etiology [24]. In contrast, miR-146a is reported consistently up-regulated in various cell types in RA [21], where it is supposed to negatively regulate proinflammatory cytokines such as TNF- α [25] and IL-17 [26]. It is unclear whether the upregulation of miR-146a in RA is actively blocking the expression of certain yet to be determined targets or is simply a consequence of overexpression of TNF- α inducing the production of this miRNA.

Different miRNAs have been found to be altered in autoimmune diseases, and this could depend on many factors, such as variations in cell population, biological specimens analyzed, technology employed, and expertise of the investigators. Some of the earlier studies not cited here included preliminary reports of up- and down-regulated miRNAs in relatively few patients compared to controls and without identifying significant targets. Ideally it is best that confirmatory data are provided from independent studies and potentially with support from murine models to determine whether the candidate miRNA plays a role also in the diseases. This is the case of miR-155 and miR-15a, for example; their increased expression has been mainly reported in lupus mouse models, where they can influence Treg activity and production of anti-dsDNA antibodies by B cells, respectively [27-29]. However, miR-155 is overexpressed in RA patients (Figure 2), where it regulates the production of inflammatory cytokines [25]. Different mechanisms and pathways have been reported to be regulated by miRNAs that are aberrantly expressed in SLE (Figure 1) or RA (Figure 2), thus leading to different disease manifestations. For example, in SLE miR-126, miR-148a, and miR-21 can alter the expression of autoimmune-associated genes through epigenetic modifications [30-31]. In the case of miR-125a, miR-31, miR-23b, and miR-142-3p/5p, their decrease leads to the altered expression of pro- and anti-inflammatory cytokines [32-35]. Studies show that increased expression of miR-221/222, miR-323-3p, and miR-203 leads to arthritic phenotype and activated synovial fibroblasts in RA (Figure 2) [36-37]. On the contrary, reduced expression of miRNAs such as miR-34a*, miR-124a and miR-23b is related to synovial fibroblast resistance to apoptosis, and to increased cell proliferation, chemotaxis, angiogenesis and inflammatory cytokine production [34,38-39].

Developments in therapeutics

The importance of studying miRNAs also relies on the possibility to target them for therapeutic purpose. Clearly there are still many concerns for the successful delivery of small interfering RNA or miRNA to the affected tissues/organs. Some advances include a recent report in *in vitro* systems using small interfering RNA conjugated to carbachol, a ligand specific for muscarinic receptor, to target human salivary gland cells in Sjögren's syndrome to prevent apoptosis [40]. Animal models are now also used to test the efficacy and the selectivity of miRNA targeting [41]. For example, in collagen-induced arthritis mouse models, the silencing of miRNA(s) by lentiviral vectors can reduce the severity of arthritis [42], while silencing of miR-21 using the "tiny" locked nucleic acids anti-miRNA [43] in the SLE mouse model B6.Sle123 leads to amelioration of splenomegaly [44]. Another interesting example in the efficacy of anti-miRNA strategy performed on non-human primates has shown that inhibition of miR-33a and miR-33b can increase plasma HDL and lower VLDL triglycerides, with cardiovascular protective role and improvement of dyslipidemia [45]. It is not clear what delivery system is the best for miRNAs, in order to be tissue-specific, non-immunogenic, and survive degradation. One possibility is the use of exosomes [13], as demonstrated for brain-delivery of specific miRNAs in mouse models

[14]. Beside their therapeutic use, miRNAs could also be used as biomarkers of specific conditions without the need of invasive procedures, such as blood drawing or tissue biopsy. For this reason, studies are currently focusing also on saliva or urine specimens for miRNA analysis and correlation with physiological or pathological conditions [46-47].

Dominant miRNAs in immunity

MiRNAs are important in controlling gene expression supposedly serving as “rheostats” to make fine-scale adjustments to protein output [48-49]. These investigators used quantitative mass spectrometry to analyze the effects on thousands of proteins after overexpression or inhibition of single specific miRNA in cultured cells *in vitro* or *in vivo* using cells from specific miRNA knockout mouse, and they show that changes in individual miRNA can affect directly and indirectly the level of hundreds of proteins [48-49]. MiRNAs have been extensively studied for their capability to regulate one mechanism of innate immunity called endotoxin tolerance, characterized by the refractory response of neutrophils and macrophages to subsequent lipopolysaccharide (LPS) stimuli, leading to the reduced production of proinflammatory cytokines [50]. These studies have shown that there is clearly a subset of miRNAs with dominant functions (Figure 3). The definition of “dominant miRNA” is based on our extensive study of miR-146a as prototype, by identifying time-dependent sequence of events during LPS stimulation and challenge [51-52]. miR-146a has such a significant increase (10-200 folds) after LPS stimulation of THP-1 cells, underlying its key role in the regulation of the LPS/Toll-Like Receptor (TLR)4 signaling pathway for the production of proinflammatory cytokines [52-53]. The negative correlation of miR-146a with TNF- α is important for maintaining the control of the inflammatory response against pathogens, and its deregulation is responsible for abnormal cytokine production and pathological manifestations (Figure 3, panel A). Recent studies on mice deficient in miR-146a expression show that they are hyper-responsive to LPS, they produce excessive quantity of proinflammatory cytokines and they are highly susceptible to endotoxin shock [41]. This mouse model develops signs of autoimmune disease and premature death while aging, further suggesting that miR-146a is required for the control of the immune response [41]. Another study performed in aged BALB/c mice shows that miR-146a deregulation is responsible for age-related dysfunction in macrophages and increased susceptibility to infections [54]. Beside miR-146a, miR-182 is another example of dominant miRNAs. After stimulation of T helper cells by IL-2, a cytokine important for the regulation of clonal expansion of activated T cells, miR-182 expression increases by 50-200 folds from day 1-2, and reaches the peak expression 3 days after stimulation (Figure 3, panel B) [55]. *In vitro* and *in vivo* studies using mouse models show that IL-2 induces the expression of miR-182 with dose-dependent effect, and miR-182 consequently promotes the clonal expansion of T helper cells by regulating its target Foxo1. Blocking miR-182 can lead to the improvement of arthritis in the ovalbumin-induced arthritis mouse model [55]. What is peculiar about these dominant miRNAs is that they can be induced to >100 fold when stimulated by specific ligands. These features are not typical for other miRNAs, as in most cases their stimulation induces changes of just a few folds [48-49]. Because of their dominant role, these miRNAs may affect many target genes in a dose-dependent manner but this still needs further study. The concept of dominant miRNA is important because they could be responsible for specific clinical features observed in autoimmune diseases and thus they could be used as targets for possible therapeutic interventions.

Conclusions

The extensive study of miRNAs performed in the last decade has allowed the better understanding of the mechanisms of miRNAs biogenesis and their regulation of gene expression. One of the most interesting aspects linked to miRNAs as master gene regulators

is the possibility to target them for therapeutic purpose, because their up- or down regulation can be responsible for pathological conditions in autoimmune diseases. A lot still needs to be done, in particular to translate the knowledge on miRNAs from the animal models to humans, to allow the accomplishment of clinical trials with miRNAs in autoimmune diseases.

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HIGHLIGHTS

- miRNAs regulate target mRNA stability in normal and pathological cellular processes
- Alterations in miRNAs expression are common in systemic autoimmune diseases
- “Dominant miRNAs” such as miR-146a strongly influence innate immune response
- miRNAs can be used as biomarkers and therapeutic targets in autoimmune diseases

Figure 1. Aberrantly expressed miRNAs in SLE

Different cell types in SLE patients and lupus murine models are targeted by miRNAs with increased (left) or decreased (right) expression. MiR-126, miR-148a, and miR-21 modulate autoimmune-associated methylation sensitive genes in T cells, and miR-21 also stimulates the production of IL-10. MiR-155 and miR-15a are overexpressed in murine lupus models, causing Treg alterations and anti-dsDNA antibodies production by B cells, respectively. Decreased expression of miR-125a, miR-31, miR-142-3p/5p, and miR-23b induces the production of pro-inflammatory cytokines. MiR-146a is downregulated in SLE and it induces the hyperactivation of I-IFN pathway in peripheral blood mononuclear cells. Solid lines, overexpressed miRNAs (left); dotted lines, underexpressed miRNAs (right). References are shown in brackets.

Figure 2. Aberrantly expressed miRNAs in RA

The increased expression of miR-221/222, miR-323-3p, miR-203 (left) leads to arthritic/activated phenotype of synovial fibroblasts, while overexpression of miR-155 and miR-146a is associated with overproduction of inflammatory cytokines. Decreased expression of miR-34a*, miR-124a, miR-23b (right) causes different pro-inflammatory effects in joints of RA patients. Solid lines, overexpressed miRNAs (left); dotted lines, underexpressed miRNAs (right).

Figure 3. Examples of dominant miRNAs in immunity

Examples of dominant miRNAs that, after a specific stimulation, increase of >100 fold and induce a specific effect in the immune response. **Panel A.** miR-146a as a dominant miRNA in endotoxin tolerance. THP-1 monocytes stimulated with high-dose LPS at 1 μ g/ml at time 0 produce a prominent TNF- α response that peaks at ~4-6 h. There are respective increases in both MyD88 pathway adaptors IRAK1 and TRAF6, which peak at 2-4 h and are documented targets of miR-146a. The miR-146a expression progressively increases about 100 fold starting at 2 h to down-regulate IRAK1/TRAF6 and continues to increase in the presence of LPS over a 3-day period. The high level of miR-146a maintains the LPS tolerance stage such that, when challenged with LPS again, it leads to a hypo-responsiveness state. **Panel B.** miR-182 as a dominant miRNA for adaptive immune response. The stimulation of different T cell populations (T_H1 , T_H2 and T_H17) by IL-2 causes the strong overexpression (50-200 fold) of miR-182, with a peak at day 3. This miRNA downregulates its target Foxo1, causing the consequent T cell expansion.