Immunity
Commentary

MicroRNAs and Immunity: Tiny Players in a Big Field

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MicroRNAs (miRNAs) are found in most metazoan organisms as well as in viruses and are implicated in an increasingly wide variety of biological processes in animals. Here, Taganov et al. discuss the role of miRNAs in the innate immune response to microbial infection.

Introduction

RNA-mediated gene silencing is used by most eukaryotic organisms to modulate expression of protein-coding genes, to fight invading viruses, and to quell the spread in the genome of "parasitic" genetic mobile elements such as transposons. RNA silencing relies on a sequence-specific interaction between the target RNA (or DNA in certain cases) molecule and a small RNA incorporated into the multisubunit RNA-induced silencing (RISC) complex. Several classes of small silencing RNAs have been identified thus far including microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), transacting siRNAs (tasiRNAs), etc. They differ in length (18–30 bp) and origin but share a common set of proteins required for their function and sometimes production. This commentary will focus exclusively on the role in immunity of miRNAs, an evolutionarily conserved and abundant class of small silencing RNAs.

miRNAs: Biogenesis, Regulation of Expression, and Molecular Targets

Functional miRNAs are excised from long endogenous transcripts by the sequential action of a pair of endonucleases (Drosha and Dicer) that reside in different compartments in the cell (see Figure 1). In the nucleus, the primary microRNA (pri-miRNA) transcript is first cleaved by Drosha, liberating an approximately 60-to-80-nucleotide-long hairpin-shaped precursor miRNA (pre-miRNA). This pre-miRNA is then exported from the nucleus to the cytoplasm, where it undergoes a further round of processing by the Dicer enzyme and the resulting ~22 nucleotide RNA is then loaded onto the RISC complex. Perfect base pairing between the RISC-bound miRNA and the target mRNA results in cleavage and degradation of the latter, whereas imperfect complementarity generally leads to translational repression of the target.

Primary microRNAs transcripts, like protein-coding mRNAs, are generally synthesized by RNA polymerase II, and therefore their expression is subject to the exquisite regulation provided by the assortment of transcriptional factors in the cell. Although some miRNAS are widely expressed, others are only expressed in limited developmental stages or in specific tissues or cells. Several recent reports suggest that amounts of mature miRNAs in the cell can be also controlled posttranscriptionally at the level of processing by Drosha or Dicer. For example, several pri-miRNA transcripts, including those of the *let-7* family, are highly expressed during early mouse development, whereas no corresponding pre-miRNAs or mature miRNAs are detected until approximately day 10.5 of gestation; these findings indicate some type of modulation of Drosha activity in the embryo (Thomson et al., 2006). Regulation at the Dicer processing step has also been observed (Ambros et al., 2003; Obernosterer et al., 2006).

There are currently 474 human miRNA sequences listed in the miRNA registry (release 9.0, October 2006 [Griffiths-Jones, 2004]), but the total number of miRNA genes according to some computer-assisted estimates might be closer to a thousand (or even higher) and thus potentially constitutes ~3% of the human genome (Bentwich et al., 2005; Berezikov et al., 2005; Miranda et al., 2006). The number of predicted conserved mRNA targets per mammalian miRNA is estimated to be roughly 200 on average, suggesting that expression of ~10,000 genes or 30% of the human genome can be regulated by miRNAs (Lewis et al., 2005). The number of target genes for particular miRNAs may be substantially increased if sequence alterations due to A-to-I RNA editing of miRNA sequences (Blow et al., 2006) and potential evolutionarily nonconserved target sites are factored in.

Given the difficulty of defining the real, in vivo targets for animal miRNAs, it is not surprising to find that our knowledge of the biological functions of miRNAs is far from comprehensive. The vital importance of RNA silencing pathways during development has been clearly demonstrated by targeted deletions of genes involved in different aspects of miRNA biogenesis and function in a number of biological models (Bernstein et al., 2003; Kanellopoulou et al., 2005; Liu et al., 2004).

Role of miRNAs in the Development of Hematopoietic Cell Lineages and Cancer

The elevated tissue-specific expression of some miRNA genes suggests that they might be involved in tissue differentiation and maintenance of cell-type identity in animals; miRNAs would share such a role with tissue-specific

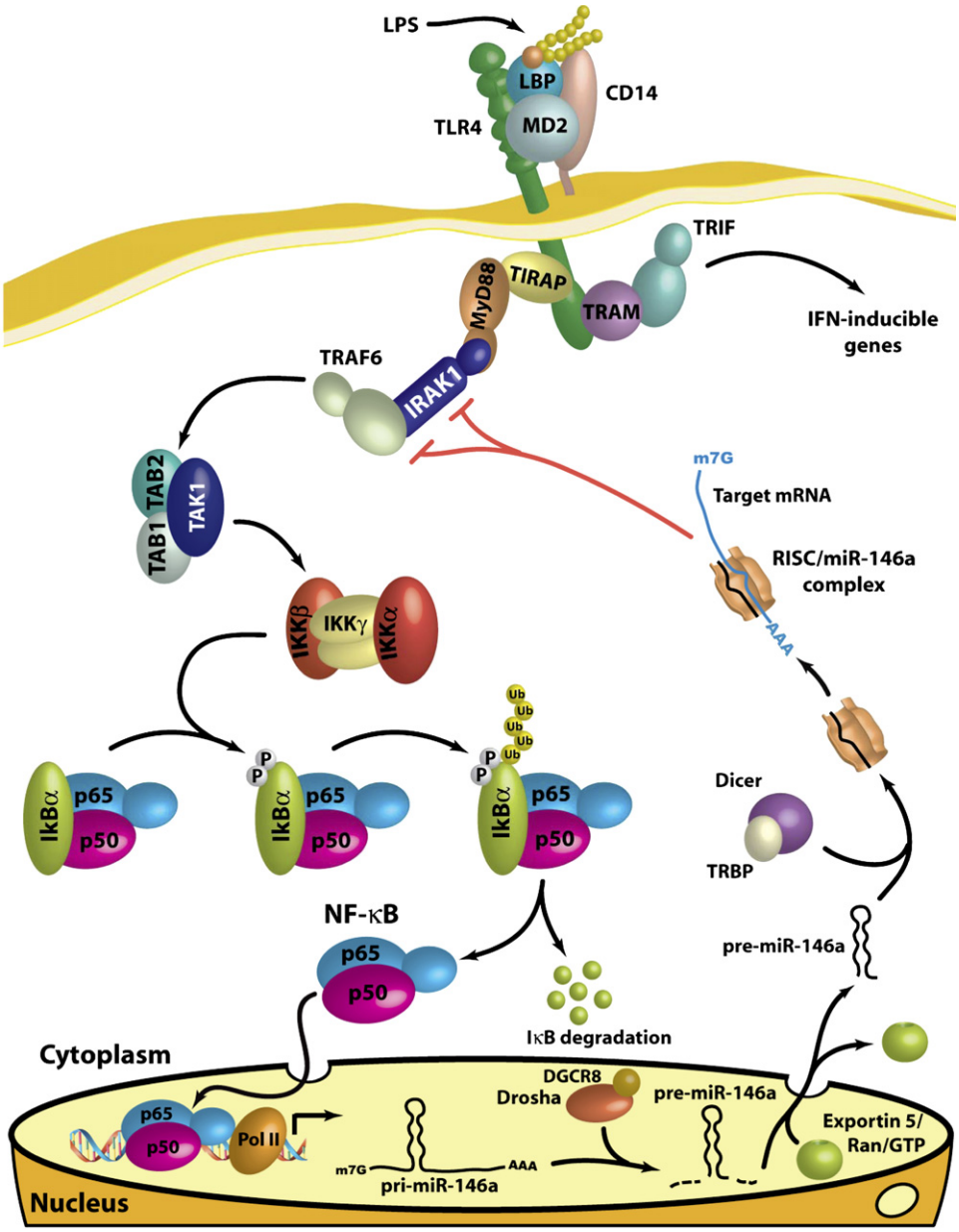

Figure 1. A Proposed Model of miR-146 Negative Feedback Regulation of TLR4 Signal Transduction Pathways
Triggering of TLR4 receptor by LPS activates downstream NF-κB signaling, leading to subsequent induction of immune-response genes, including the gene for miR-146. miR-146 primary (pri-miR-146) transcript undergoes stepwise processing by two RNase III enzymes, Drosha and Dicer. The mature miR-146 is then loaded onto the RISC complex and guides it to the target IRAK1 and TRAF6 mRNAs and thus brings about a reduction in their expression and attenuation of TLR4 signaling.

transcriptional factors. A number of expression-profiling studies found distinct patterns of miRNA expression in different hematopoietic cell lineages (Chen et al., 2004; Felli et al., 2005; Garzon et al., 2006). For example, miR-150 amounts are elevated during developmental stages of B and T cell maturation, although its expression is rapidly decreased upon differentiation of naive T cells into Th1 or Th2 subtypes; in contrast, miR-146 expression under the same conditions was differentially upregulated in the Th1 subset and abolished in Th2 cells (Monticelli et al., 2005). Regulatory T (Treg) cells were also shown to express a distinct miRNA subset, which resembles a profile of miRNAs expressed in activated but not naive T cells. Moreover, enforced expression of the Treg signature transcriptional factor FoxP3 in conventional T cells can confer a partial Treg-cell miRNA profile (Cobb et al., 2006). The most direct evidence so far for the ability of miRNAs to influence hematopoietic lineage commitment and differentiation was developed with both ectopic expression of candidate, lineage-specific miRNAs in

progenitor cells and T cell-specific ablation of the *Dicer-1* gene in mice (Chen et al., 2004; Cobb et al., 2005; Muljo et al., 2005). T cell levels were variably affected by particular manipulations used in these studies.

Given the accumulating body of research on the role of miRNAs in proliferation and differentiation of hematopoietic cells, it is not surprising to find that changes in miRNA expression may contribute to cancer predisposition and progression in the cells of the immune system and may serve as a novel tumor prognostic marker. For example, a majority of chronic lymphocytic leukemia cells are characterized by deletion or misregulation of expression of the MIRN15A/MIRN16-1 gene cluster (reviewed in [Calin and Croce, 2006]). Cimmino et al. reported that these miRNAs promote apoptosis by targeting Bcl2 expression and therefore act as "tumor suppressor" RNAs (reviewed in [Calin and Croce, 2006]). In contrast, high expression of "oncogene-like" miRNAs, particularly miR-155 and those from the MIRN17-92 cluster, is characteristic of several human cancers, including some lymphomas. Strikingly, transgenic mice expressing mouse miR-155 in a B cell-specific manner showed, in the spleen and bone marrow, accelerated rates of pre-B cell proliferation that resulted in the development of a B cell lymphoproliferative disorder by 6 months (reviewed in [Calin and Croce, 2006]).

miRNAs and Virus-Host Interaction: In the Middle of a Raging War

Although there is an ongoing debate as to whether virus-derived siRNAs (thought to arise from long double-stranded RNAs through the biogenesis pathway shared with miRNAs) have an effect on antiviral immunity in vertebrates (reviewed in [Cullen, 2006a]), there is evidence that miRNAs of the host may impinge on the viral life cycle, viral tropism, and the pathogenesis of viral diseases. For example, human miR-32 has a direct negative effect on the replication of retrovirus primate foamy virus type 1 (PFV-1), which is mediated through the downregulation of replication-essential viral proteins encoded by open reading frame 2 (ORF2) (reviewed in [Cullen, 2006b]). Intriguingly, in a typical "punch-counterpunch" style of virus-host interaction, PFV encodes a protein called Tas, which appears to be a strong, global suppressor of RNA silencing. Many other viruses employ a similar strategy to deal with the antiviral effects of RNAi but implement it in different ways (reviewed in [Cullen, 2006b]).

Considering how adept viruses can be at the "hijacking" of host genes, utilizing them in their own counter-defense measures, it is no surprise that such small, but powerful "weapons" as host miRNAs have found their way into the viral genomes. A growing list of human viruses, including herpes viruses, polyomaviruses, and retroviruses, have been shown to encode miRNAs' targeting expression of a wide range of cellular genes, among them genes for cytokines and signaling proteins (reviewed in [Cullen, 2006b]).

miRNAs and Regulation of Signal Transduction in Immune Cells

Innate immunity is a phylogenetically ancient biological system that multicellular organisms have evolved to defend themselves from invading pathogens. The job of recognition of the "nonself" from the "self" in this system belongs to pattern recognition receptors (PRRs). Acting as molecular sentinels, PRRs sense highly conserved microbial molecules (often called pathogen-associated molecular patterns [PAMPs]) and elicit pathogen-specific cellular responses that result in elimination of intruders as well as, in higher vertebrates, mobilization of the adaptive immune system.

Toll-like receptor (TLR) signaling can be exemplified by the signal transduction pathways of the family-founding member, TLR4. Its signaling cascade starts with recruitment of a number of adaptor molecules, which include TNF receptor-associated factor 6 (TRAF6), a ubiquitin E3 ligase that activates effector kinase cascades leading to the mobilization of downstream transcriptional factors (e.g., AP-1 and NF-κB) that in turn orchestrate regulation of immune-response genes. NF-κB is a key transcriptional factor that regulates all aspects of the innate immunity response from synthesis of proinflammatory cytokines, such as IL-1β and TNFα, to regulation of immune cell migration and remodeling of tissues after the successful termination of the inflammatory response.

Recently, our laboratory has carried out a systematic effort to identify miRNAs that play a role in the mammalian response to microbial infection (Taganov et al., 2006). We identified three miRNAs (miR-146, miR-132, and miR-155) that are sharply upregulated in response to lipopolysaccharide (LPS) in human monocytic cells. A detailed survey of miR-146 expression in response to various microbial components revealed that an increase in miR-146 levels is induced by certain members of the Toll receptor family: It is triggered by those TLRs that (1) can sense bacterial rather than viral PAMPs and (2) reside on the cell surface. It is worth noting that miR-146 expression is also weakly inducible by the proinflamatory cytokines, IL-1 and TNF, whose cognate receptors share some of their signaling machinery with TLRs.

Mammals encode two copies of miR146, namely miR146a and miR146b; the genes are located on separate chromosomes in the context of quite unrelated genes but differ in their mature sequence only by two nucleotides at the 3′ end. We have shown that both loci respond to LPS and determined that induction of expression of *MIRN146A* is NF-κB dependent. Interestingly, analysis of expression of mature miRNA products of these two genes led us to a surprising finding: Whereas amounts of mature miR-146a were increased by LPS, miR-146b expression remained unchanged, suggesting that this miRNA family has a rather sophisticated mode of regulation of expression involving more than one point of control.

A biological role for the miR-146 family is not difficult to suggest because the list of its conserved predicted targets is topped by two genes, IL-1 receptor-associated kinase 1 (IRAK1) and TRAF6, whose involvement in TLR and proinflammatory cytokine (i.e., IL-1) signaling is well established. Indeed, we were able to show that these two adapters can potentially be subjects of regulation by miR-146 through the use of 3′

untranslated region (UTR) luciferase reporter assays; we demonstrated a substantial drop in luciferase activity when the 3′ UTRs of either the IRAK1 or TRAF6 genes was fused downstream of the luciferase ORF and miR-146 was present. Because these two molecules work in a linear signaling cascade, a cumulative effect of a drop in their protein expression should have a substantial impact on IL-1 and TLR receptor signaling.

Because overactivation of the innate immunity system can be seriously detrimental to the organism and in extreme cases can lead to systemic effects such as septic shock or lead to local chronic effects such as rheumatoid arthritis or inflammatory bowel disease, the signaling cascades activating innate immunity are subject to layers of regulation. We have proposed that miR-146 can serve as a regulator of TLR and cytokine signaling by acting in a negative feedback loop to attenuate activation of downstream genes through posttranscriptional regulation of expression of the IRAK1 and TRAF6 proteins.

One can also propose a role for miR-146 in endotoxin tolerance. It has been known for decades that exposure to bacterial products, such as LPS, can cause desensitization of immune cells and reduce production of proinflammatory cytokines in response to subsequent bacterial challenge. Although there are multiple theories to explain this phenomenon, one of the major mechanisms seems to be blockade of signaling. A few publications in this field suggest that reduction in expression of IRAK1 protein in response to the first bacterial challenge might be a reason for the cell's becoming refractory to the next LPS treatment (Li et al., 2000; Siedlar et al., 2004). These authors also noted that although IRAK1 protein levels fell dramatically during the induction of endotoxin tolerance, its mRNA amounts were not affected, and such a scenario fits the miRNA mode of action. Of note, miR-146 is also predicted to exhibit an antiviral role because its binding site has been observed in the genome of the PFV-1 virus, Dengue virus, Hepatitis C virus, Influenza B virus, and several others (Hsu et al., 2007).

A second LPS-inducible miRNA, miR-132, identified through our microarray studies, has been recently shown to be a CREB-responsive gene that regulates neuronal outgrowth in the rat by controlling the expression of the GTPase-activating protein p250GAP (Vo et al., 2005). Because members of the CREB and ATF family were reported to play a role in LPS signaling (Gilchrist et al., 2006), it will be interesting to see whether it also mediates the upregulation of miR-132 expression in response to endotoxin in monocytes or whether the activation goes through the classical inflammation mediator, NF-κB. Of note, we observed differences in expression between miR-132 and miR-146 in response to other immunity-related stimuli, suggesting different transcriptional or posttranscriptional regulation. For example, miR-132 is highly induced in response to phorbol-12-myristate-13-acetate, whereas no response is seen for the level of mature miR-146 (unpublished data).

Our latest "fishing expedition" to identify miRNAs upregulated during the macrophage antiviral response netted us a single catch, miR-155. Upregulation of miR-155 seems to be under the control of TLR3 and, to a lesser extent, the IFNαβ receptor in mouse bone-marrow-derived macrophages (O'Connell et al., 2007). Although TLR3 induces miR-155 as an immediate early gene, upregulation of miR-155 by IFNs is indirect and requires TNFα autocrine or paracrine signaling. Thus, we found that miR-155 is induced by both bacterial (i.e., LPS) and viral (i.e., double-stranded RNA) ligands, suggesting a role for miR-155 in the regulation of antimicrobial defense. Although miR-155 function under physiological conditions awaits discovery, its involvement in the development of B cell malignancies is well documented (reviewed in [Calin and Croce, 2006]). Therefore, miR-155 may provide a potential link between the inflammatory response and cancer.

The complete scope of miRNA involvement in immunity will probably take some time to emerge, but it is already clear that these tiny players can have a big impact on this complicated and life-sustaining system. Because miRNAs appear to provide quantitative regulation of genes, rather than on-off decisions, they can be seen as fine-tuning a cell's responses to external influences. Although the immune system developed early in metazoan evolution, each organism has its own constellation of pathogens and each immune system is tuned to that particular set of challenges. We can speculate that the shaping of immune reactions, both in terms of the repertoire of immune cells and the magnitude and duration of response to particular pathogens, is an important function of miRNAs. Because they regulate through very short interaction sites where one or a few base pairs can play a critical role, they should be easily mutable over evolutionary time and provide for the organism a reservoir of genetic regulators that can be brought into play on the same time scale as the evolution of pathogens. Also, as seen for miR-146 family, the same regulator can appear in quite different genomic contexts and therefore show variance in expression patterns. As a result, it might be involved in shaping the response to different stimuli.

Although the discovery of miRNAs has provided us with a key, previously unappreciated player in orchestrating the immune response, it is perhaps not too optimistic to suggest that in the future, novel strategies for therapeutic intervention in immune-related diseases could be based on manipulation of miRNA levels.

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